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DABRAFENIB IN BRAF V600E-POSITIVE SOLID TUMORS: RESISTANCE, CLINICAL APPLICATIONS, AND DRUG-DELIVERY STRATEGIES

Fatemeh Dodangeh¹, Sara Azizi², Maryam Ebrahimi³, Mahsa Javan⁴, Aziz Eftekhari^{5*}

Abstract

Dabrafenib, particularly in combination with trametinib, is an established targeted therapy for several BRAF V600E-positive solid tumors, although the magnitude and durability of response vary considerably across tumor types. This review summarizes the molecular rationale, clinical applications, resistance mechanisms, pharmacokinetic limitations, and emerging delivery strategies of dabrafenib-based therapy. Treatment outcome is influenced not only by BRAF V600E status but also by tumor lineage, co-occurring genomic alterations, receptor tyrosine kinase feedback, microenvironmental signaling, and lesion-level drug exposure. Resistance develops through MAPK reactivation, PI3K/AKT bypass signaling, cellular plasticity, tumor heterogeneity, stromal protection, and pharmacological underexposure. Dabrafenib efficacy is further constrained by pH-dependent solubility, food-sensitive absorption, CYP-mediated metabolism, autoinduction, drug interactions, limited brain distribution, and heterogeneous intratumoral penetration. Solid-state modification, nanostructured formulations, targeted nanocarriers, locoregional administration, and coordinated dabrafenib–trametinib delivery have shown preclinical promise, but evidence of superiority over standard oral therapy remains limited. Clinical findings across melanoma, lung, thyroid, biliary tract, colorectal, and central nervous system tumors indicate that BRAF V600E is highly actionable but insufficient as a standalone predictor of benefit. Treatment optimization therefore requires a tumor-specific approach integrating molecular selection, resistance profiling, and lesion-level drug exposure.

Keywords: *Dabrafenib, BRAF V600E, Solid tumor, Drug resistance, Nano drug-delivery*

1. Introduction

BRAF V600E is one of the most clinically actionable oncogenic alterations in solid tumors [1]. This mutation constitutively activates the RAS-RAF-MEK-ERK signaling pathway, leading to sustained proliferative and survival signaling [2]. Although BRAF V600E was initially established as a major therapeutic target in

melanoma, its clinical relevance has expanded to several non-melanoma solid tumors, including non-small-cell lung cancer, anaplastic thyroid cancer, pediatric low-grade glioma, biliary tract cancer, and other rare BRAF V600E-positive malignancies [3-6]. Dabrafenib is a selective small-molecule inhibitor of mutant BRAF, with particular activity against BRAF



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V600-driven signaling [4]. In combination with the MEK inhibitor trametinib, dabrafenib provides vertical inhibition of the MAPK pathway and has become an important targeted therapeutic strategy for BRAF V600E-positive tumors [7]. The clinical value of this combination is reflected by its expansion from melanoma to tissue-agnostic treatment of selected BRAF V600E-positive advanced solid tumors [4, 5]. However, the presence of BRAF V600E alone does not guarantee uniform therapeutic benefit across tumor types. Increasing evidence indicates that response to dabrafenib-based therapy is shaped by tumor lineage, BRAF mutation class, co-occurring genomic alterations, receptor tyrosine kinase feedback, tumor microenvironment, and tissue-specific pharmacological barriers [3, 8]. Therefore, BRAF V600E should be considered a powerful but incomplete predictor of response. A major limitation of dabrafenib-based therapy is the emergence of intrinsic or acquired resistance [9]. Tumors may escape treatment through MAPK pathway reactivation [10], PI3K/AKT-mediated bypass signaling [11], EGFR feedback activation [12], cell-cycle deregulation [13], tumor heterogeneity [14],

immune-microenvironmental remodeling [15], and insufficient drug exposure at specific tumor sites.

In addition, pharmacokinetic and formulation-related challenges, including pH-dependent solubility, variable oral absorption, drug-drug interactions, limited tumor penetration, and blood-brain barrier restrictions, may further limit the durability of response in solid tumors [16, 17].

This review aims to integrate the molecular pharmacology, clinical relevance, resistance mechanisms, pharmacokinetic limitations, and emerging drug-delivery strategies of dabrafenib-based therapy in BRAF V600E-positive solid tumors. Unlike reviews focused mainly on melanoma or BRAF/MEK inhibition alone, this article emphasizes the concept that effective dabrafenib therapy requires a context-guided approach in which tumor biology, resistance evolution, tissue-delivery barriers, and formulation design are considered together. This perspective may help define future strategies for improving the therapeutic performance of dabrafenib beyond conventional targeted therapy.

2. Molecular Basis of BRAF Signaling and Dabrafenib-Based Therapy

2.1. The RAS-RAF-MEK-ERK Signaling Pathway

The RAS–RAF–MEK–ERK cascade, commonly referred to as the canonical MAPK/ERK pathway, is a central signaling network that regulates cell proliferation, differentiation, survival, and migration [18]. Under physiological conditions, extracellular growth factors activate receptor tyrosine kinases, leading to the recruitment of adaptor proteins and conversion of RAS to its active GTP-bound state [19]. RAS-GTP subsequently recruits RAF kinases, including ARAF, BRAF, and CRAF, to the plasma membrane, where

Yazışma üçün əlaqə:

Fatemeh Dodangeh¹, Sara Azizi², Maryam Ebrahimi³,
Mahsa Javan⁴, Aziz Eftekhari⁵*

1 Institute for Advanced Studies in Basic Sciences,
Faculty of Biological Sciences, Zanjan, Iran

2 Institute for Advanced Studies in Basic Sciences,
Faculty of Biological Sciences, Zanjan, Iran

3 Kharazmi University, Faculty of Biological Sciences,
Tehran, Iran

4 Tabriz University of Medical Sciences, Faculty of
Pharmacy, Tabriz, Iran

5 Ege University, Department of Biochemistry, Faculty of
Science, Izmir, Türkiye

* For correspondence E-mail: eftekhari.aziz@gmail.com

conformational rearrangement and RAF dimerization promote kinase activation [20]. Activated RAF phosphorylates MEK1/2, which in turn activates ERK1/2. ERK then translocates to the nucleus and regulates gene-expression programs involved in cell-cycle progression, differentiation, and survival. Persistent dysregulation of this cascade can promote malignant transformation [21, 22]. Among RAF-family members, BRAF exhibits the highest intrinsic kinase activity and is the RAF isoform most frequently affected by oncogenic mutations [23]. Oncogenic BRAF activation can uncouple MAPK signaling from normal upstream control, producing sustained pathway output that supports uncontrolled tumor-cell proliferation and survival. BRAF V600E is the most prevalent activating BRAF alteration and can function as a dominant oncogenic driver across multiple solid-tumor lineages [3, 8, 24].

RAF proteins contain three conserved regions: CR1, which includes the RAS-binding and cysteine-rich domains; CR2, which contains regulatory phosphorylation sites; and CR3, which comprises the catalytic kinase domain, including the glycine-rich P-loop and activation segment [21]. RAF signaling is strongly influenced by kinase-domain conformation and homo- or heterodimerization, including formation of BRAF–CRAF dimers [21, 23, 25]. In the autoinhibited state, a 14-3-3 dimer interacts with phosphorylated regulatory sites in BRAF and stabilizes a closed conformation [26]. Structural studies further indicate that coordinated interactions among the BRAF cysteine-rich domain, kinase domain, MEK1, and 14-3-3 contribute to maintenance of this inactive state [21, 27]. These structural features help explain why RAF dimerization, conformational state,

and cellular context strongly influence sensitivity to RAF inhibitors.

2.2. BRAF Mutation Classes: Class I, Class II, and Class III

BRAF mutations are commonly classified into three functional classes according to their intrinsic kinase activity, dependence on upstream RAS signaling, and requirement for RAF dimerization. This classification has important therapeutic implications because the three classes differ substantially in their signaling mechanisms and sensitivity to RAF-targeted therapies [3, 8, 25, 28]. Class I mutations comprise substitutions at codon V600, particularly V600E and V600K. These alterations generate highly active BRAF monomers that signal independently of upstream RAS activation and do not require RAF dimerization. Sustained ERK activation imposes negative feedback on RAS, resulting in relatively low RAS-GTP activity in class I BRAF-mutant tumors [29]. Their monomeric and RAS-independent signaling explains why class I mutants are the most sensitive to approved BRAF inhibitors such as dabrafenib, particularly when BRAF inhibition is combined with downstream MEK inhibition [3, 8, 25].

Class II mutations are activating non-V600 alterations that signal as constitutively active, RAS-independent RAF dimers. They include selected substitutions involving G469, L597, and K601, as well as many BRAF fusions [29]. Because conventional BRAF V600-selective inhibitors more effectively inhibit monomeric BRAF than RAF dimers, class II alterations generally show limited sensitivity to these agents and may require strategies targeting RAF dimers or downstream MEK–ERK signaling [3, 8, 25, 30]. In contrast, class III mutations have impaired or absent intrinsic kinase activity and depend on

upstream RAS activation [31]. These mutants enhance signaling through interactions with RAS-GTP and CRAF-containing dimers, with RAS activation arising from receptor tyrosine kinase signaling, activating RAS alterations, NF1 loss, or other upstream events [32]. Consequently, class III tumors are generally unsuitable for conventional BRAF V600-selective inhibitor monotherapy, and their treatment must be guided by the upstream driver, tumor lineage, and broader genomic context [3, 8, 28].

2.3. Mechanistic Basis of Dabrafenib and Trametinib Combination Therapy

Dabrafenib is primarily active against class I BRAF V600 alterations, particularly V600E and V600K, which generate constitutively active, RAS-independent BRAF monomers [3, 8, 24, 33]. In vitro, dabrafenib inhibits BRAF V600E, BRAF V600K, and BRAF V600D with IC_{50} values of 0.65, 0.50, and 1.84 nM, respectively, while inhibiting wild-type BRAF and CRAF at higher concentrations, with corresponding IC_{50} values of 3.2 and 5.0 nM [34]. In BRAF V600-mutant cells, inhibition of mutant BRAF reduces downstream MEK–ERK signaling and suppresses MAPK-dependent tumor-cell proliferation. Consistently, dabrafenib inhibits the growth of BRAF V600 mutation-positive tumors in both in vitro and in vivo models [3, 8, 24, 34].

The pharmacological effects of RAF inhibitors depend strongly on mutation status and RAF dimerization. Whereas dabrafenib suppresses MAPK signaling in BRAF V600-mutant cells, RAF inhibition in BRAF wild-type cells with elevated RAS activity may promote RAF dimerization and transactivation, resulting in paradoxical MEK–ERK pathway activation [8, 21]. This context-dependent activity reinforces the

requirement for accurate identification of the BRAF variant and explains why dabrafenib should not be used in molecularly unselected BRAF wild-type or RAS-activated tumors.

Trametinib is a reversible allosteric inhibitor of MEK1 and MEK2 activation and kinase activity, thereby limiting downstream ERK signaling [35–38]. Combining trametinib with dabrafenib therefore provides vertical inhibition of two sequential nodes of the RAS–RAF–MEK–ERK cascade. This dual blockade suppresses residual or recovering MEK–ERK signaling more effectively than BRAF inhibition alone and delays MAPK-pathway reactivation. Preclinical studies demonstrated greater growth inhibition in BRAF V600 mutation-positive tumor-cell lines and more prolonged tumor-growth suppression in xenograft models with the combination than with either drug alone [34, 38]. Clinical studies in BRAF V600-mutant melanoma subsequently showed that dabrafenib plus trametinib improved disease control and delayed resistance compared with BRAF inhibitor monotherapy, while reducing cutaneous hyperproliferative toxicities associated with paradoxical MAPK activation [36, 37].

The mechanistic rationale for this combination is strongest in class I BRAF V600-driven tumors and should not be generalized to all BRAF alterations [39]. Class II mutations signal through RAF dimers, whereas class III mutations depend on upstream RAS or receptor tyrosine kinase activity and CRAF-containing dimers [40]. Accordingly, the therapeutic relevance of dabrafenib–trametinib therapy must be interpreted according to the exact BRAF variant, functional mutation class, and tumor context [3, 8, 25, 28]. The molecular organization of BRAF signaling, the functional classification of BRAF

alterations, and the mechanistic rationale for combined dabrafenib–trametinib therapy are summarized in Figure 1.

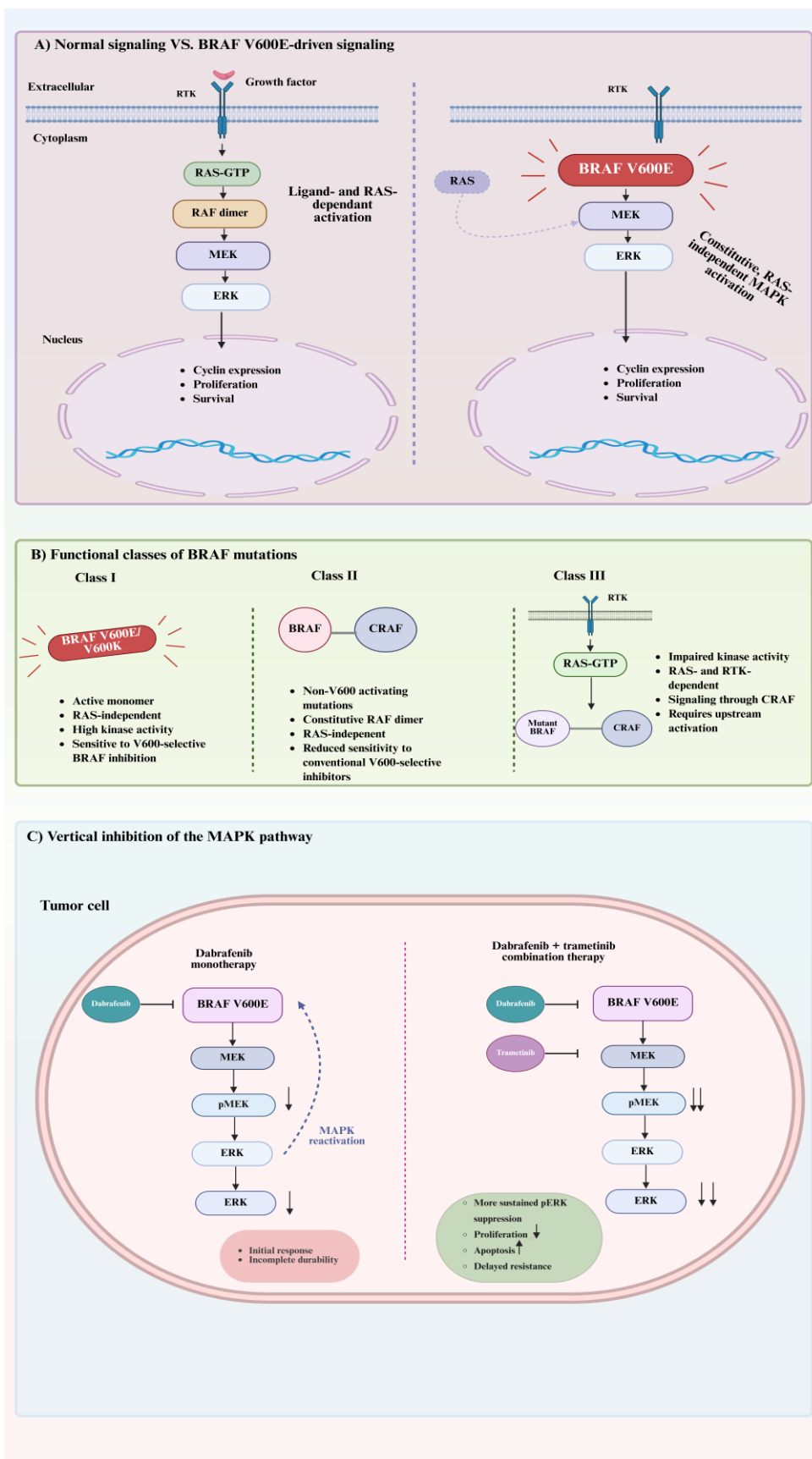


Figure 1. Molecular basis of BRAF signaling and the rationale for combined dabrafenib–trametinib therapy. (A) Comparison of physiological RTK–RAS–RAF–MEK–ERK signaling with constitutive, RAS-independent MAPK activation driven by BRAF V600E. (B) Functional classification of BRAF alterations into class I monomers, class II RAF dimers, and class III RAS-dependent mutants. (C) Dabrafenib inhibits mutant BRAF, whereas combined dabrafenib–trametinib therapy provides vertical BRAF/MEK blockade, more sustained MAPK suppression, and delayed pathway reactivation. Abbreviations: ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; RTK, receptor tyrosine kinase. This figure was created using BioRender. (BioRender.com, accessed on 17 July, 2026).

3. Clinical Applications of Dabrafenib-Based Therapy Across Solid Tumors

Dabrafenib-based therapy has expanded from melanoma to several BRAF V600E-positive solid tumors, particularly when combined with trametinib [3, 8, 41]. However, clinical benefit varies across tumor types because response is influenced by tumor lineage, co-occurring alterations, feedback signaling, the tumor

microenvironment, and tissue-specific drug accessibility [3, 5]. As illustrated in Figure 2, the clinical performance of dabrafenib-based therapy is determined by the interaction between BRAF dependency and tumor-specific factors, including lineage, mutation class, co-occurring alterations, feedback signaling, and drug accessibility.

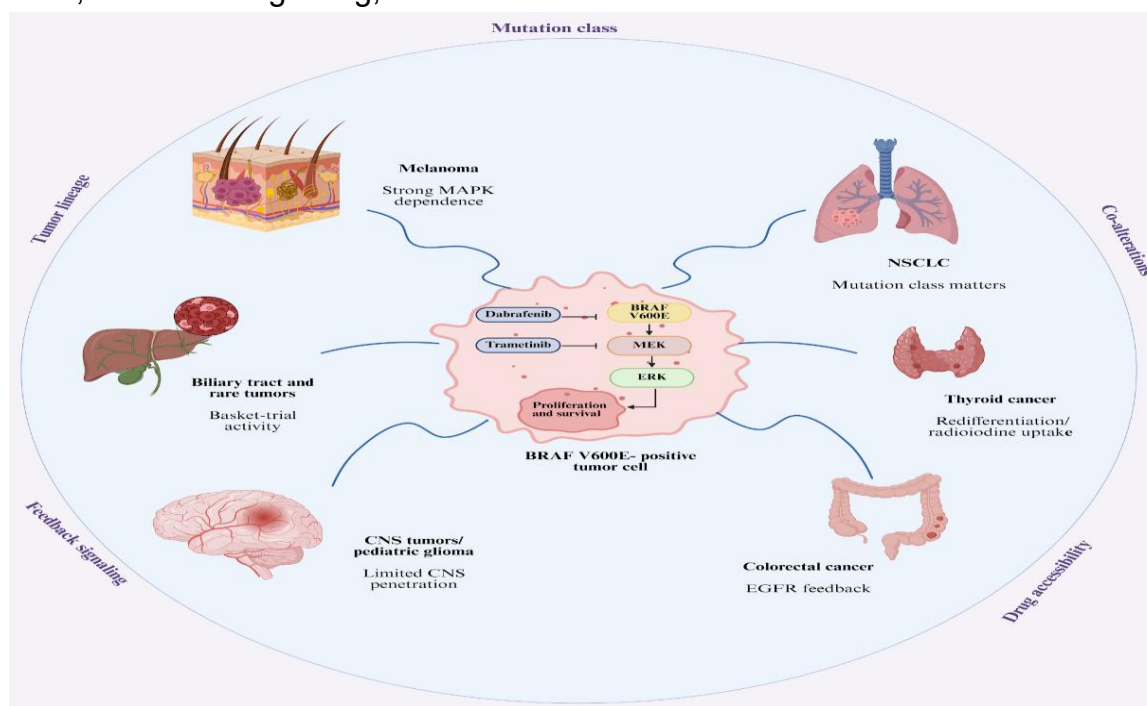


Figure 2. Tumor-context-dependent determinants of dabrafenib-based therapy across BRAF V600E-positive solid tumors. The therapeutic performance of BRAF/MEK inhibition is shaped by tumor lineage, BRAF mutation class, co-occurring molecular alterations, feedback signaling, and tissue-specific drug accessibility. The illustrated clinical contexts include strong MAPK dependence in melanoma, mutation-class-dependent sensitivity in NSCLC, redifferentiation potential in selected differentiated thyroid cancers, EGFR-mediated feedback in colorectal cancer, restricted CNS penetration in brain tumors, and clinical activity observed in basket trials of biliary tract and other rare tumors. Abbreviations: CNS, central nervous system; EGFR, epidermal growth factor receptor; MAPK, mitogen-activated protein kinase; NSCLC, non-small-cell lung cancer. This figure was created using BioRender. (BioRender.com, accessed on 17 July, 2026).

3.1. Established Clinical Applications: Melanoma and Non-Small-Cell Lung Cancer

Melanoma provided the initial clinical evidence supporting BRAF-targeted therapy [42]. Dabrafenib monotherapy improved outcomes compared with dacarbazine in patients with previously untreated BRAF V600E-mutant metastatic melanoma [43]. However, the rapid emergence of MAPK-pathway reactivation limited the durability of monotherapy. Subsequent phase III studies demonstrated that combining dabrafenib with trametinib improved disease control and clinical outcomes compared with single-agent BRAF inhibition, establishing dual BRAF/MEK inhibition as a standard treatment for BRAF V600-mutant melanoma [44, 45].

In non-small-cell lung cancer, BRAF V600E represents a clinically actionable but uncommon molecular subgroup [46]. Phase II studies showed that dabrafenib plus trametinib produced objective response rates of approximately 63–64% in both previously treated and treatment-naïve patients with metastatic BRAF V600E-positive NSCLC [3, 47]. These findings established dabrafenib plus trametinib as an important targeted option for BRAF V600E-mutant NSCLC. However, clinical evidence in NSCLC is best established for class I BRAF V600E disease, because non-V600 BRAF alterations exhibit distinct signaling mechanisms and variable sensitivity to conventional BRAF inhibitor [3, 6, 48, 49].

3.2. Thyroid, Biliary Tract, and Other Rare Solid Tumors

BRAF V600E has important therapeutic implications in thyroid cancer. In differentiated thyroid cancer, sustained MAPK activation suppresses thyroid-

specific genes involved in iodine uptake and contributes to radioactive iodine resistance [1, 50]. BRAF/MEK inhibition may partially restore iodine avidity and enable redifferentiation in selected patients with BRAF-mutant radioactive iodine-refractory disease [51].

Dabrafenib plus trametinib has also demonstrated clinically meaningful activity in BRAF V600E-mutant anaplastic thyroid carcinoma, an aggressive malignancy with historically limited treatment options [5, 52]. These findings support routine molecular testing in advanced thyroid tumors and illustrate how the clinical role of BRAF inhibition may differ according to histological subtype [53].

Among other rare solid tumors, biliary tract cancer represents an important application of dabrafenib-based therapy [54]. In the ROAR basket trial, dabrafenib plus trametinib produced meaningful responses in patients with BRAF V600E-mutant biliary tract cancer [5]. Its activity has also been reported across selected rare BRAF V600E-positive tumors, although interpretation remains limited by small and heterogeneous patient cohorts [55]. These data support broad molecular profiling in rare cancers for which standard therapeutic options are limited [5].

3.3. Colorectal Cancer as a Model of Tumor-Context Dependence

BRAF V600E-mutant colorectal cancer illustrates why the presence of an actionable mutation does not necessarily predict uniform sensitivity to BRAF inhibition across tumor types [55]. Unlike melanoma, BRAF inhibitor monotherapy has limited activity in colorectal cancer because suppression of mutant BRAF rapidly relieves MAPK-dependent negative feedback and promotes EGFR-mediated reactivation of RAS–RAF–MEK–ERK

signaling [3, 56]. Consequently, effective treatment requires simultaneous blockade of BRAF and EGFR. The BEACON CRC trial demonstrated that encorafenib plus cetuximab improved clinical outcomes in previously treated BRAF V600E-mutant metastatic colorectal cancer, establishing combined BRAF/EGFR inhibition as a standard therapeutic strategy [56-58].

3.4. CNS Tumors and Pediatric Low-Grade Glioma

BRAF alterations occur in several pediatric and adult central nervous system tumors, including pediatric low-grade glioma, ganglioglioma, pleomorphic xanthoastrocytoma, and selected high-grade gliomas [55]. However, therapeutic sensitivity depends on the exact alteration because BRAF V600 mutations, BRAF fusions, and other non-V600 variants differ substantially in their signaling biology and response to RAF-targeted therapy [3, 59]. Dabrafenib plus trametinib has demonstrated clinically meaningful activity in BRAF V600E-positive low- and high-grade gliomas, supporting its use in molecularly selected CNS tumors [5]. The CNS setting nevertheless presents an important pharmacological challenge. Blood–brain barrier integrity, transporter-

mediated drug efflux, tumor location, and heterogeneous vascular permeability may restrict intracranial and intratumoral exposure despite adequate systemic concentrations [60, 61]. These limitations help explain the growing development of brain-penetrant RAF inhibitors and highlight the need for delivery strategies capable of improving drug distribution within CNS tumors [3, 59]. Thus, CNS tumors provide a direct link between molecular sensitivity to dabrafenib and the tissue-specific barriers that may limit durable therapeutic response [4]. The clinical relevance of dabrafenib-based therapy and the major tumor-context-specific factors that shape or limit its use across selected BRAF V600E-positive solid tumors are summarized in Table 1. Collectively, these clinical experiences demonstrate that BRAF V600E is a broadly actionable molecular target, but the therapeutic performance of dabrafenib-based therapy remains strongly influenced by tumor lineage, feedback signaling, co-occurring alterations, and tissue-specific drug accessibility. Accordingly, treatment selection should integrate BRAF mutation status with the biological and pharmacological context of each tumor.

Table 1. Clinical applications and tumor-context-specific limitations of dabrafenib-based therapy in selected BRAF V600E-positive solid tumors.

Tumor context	Clinical role of BRAF targeting	Main context-specific issue	Reference
Melanoma	Dabrafenib plus trametinib is an established treatment for BRAF V600-mutant advanced melanoma	Acquired resistance and CNS progression may limit durability.	[62, 63]
NSCLC	The combination produces meaningful responses in metastatic BRAF V600E-positive NSCLC	Benefit is mainly established for class I V600E tumors	[47, 64]
Thyroid cancer	It is active in anaplastic thyroid cancer and may restore iodine	Treatment goals differ by histological subtype.	[65, 66]

	avidity in selected differentiated tumor		
Colorectal cancer	BRAF V600E is actionable, but dabrafenib-based therapy is not a standard regimen	EGFR-mediated reactivation requires combined BRAF/EGFR inhibition.	[67, 68]
Biliary tract cancer and rare tumors	Dabrafenib plus trametinib has shown activity in BRAF V600E-positive biliary and rare tumors	Evidence is limited by small, heterogeneous cohorts.	[4, 5]
CNS tumors/pediatric glioma	The combination is active in selected BRAF V600E-positive gliomas	BBB restriction, efflux transporters, and variant heterogeneity may reduce response.	[4, 69]
Abbreviations: BBB: blood–brain barrier, CNS: central nervous system, EGFR: epidermal growth factor receptor, MAPK: mitogen-activated protein kinase, NSCLC: non-small-cell lung cancer.			

4. Pharmacokinetic and Formulation Challenges

Although dabrafenib has high molecular potency and substantial oral bioavailability after a single dose, its clinical exposure is influenced by pH-dependent solubility, formulation and food effects, time-dependent pharmacokinetics, drug–drug interactions, and restricted distribution into selected tumor compartments. These factors may produce variable systemic exposure or inadequate drug concentrations at pharmacologically protected sites, particularly within the central nervous system and poorly penetrated tumor regions [70–75]. Accordingly, the principal formulation challenge is not simply to increase total exposure, but to achieve reproducible and site-appropriate drug availability.

4.1. Oral Biopharmaceutical Limitations: Solubility, Formulation, and Food Effects

Dabrafenib mesylate is a weakly basic compound with strongly pH-dependent aqueous solubility. According to the prescribing information, it is very slightly soluble at pH 1 and practically insoluble above pH 4. Passage from the acidic

stomach to the higher-pH intestinal environment may therefore reduce drug solubility and promote precipitation from a transiently supersaturated state, limiting the fraction that remains available for absorption [34, 76, 77]. Dabrafenib also exhibits a complex solid-state landscape comprising polymorphs, hydrates, perhydrates, and multiple solvates. Consequently, crystal form and solid-state stability may substantially influence dissolution behavior and formulation performance [70, 71].

Clinical formulation studies confirm that oral exposure is sensitive to apparently conventional formulation variables. In patients with BRAF mutation-positive tumors, particle size, capsule-shell composition, and prandial state altered the relative bioavailability of dabrafenib. Hypromellose capsules produced higher exposure than gelatin capsules, whereas the nonmicronized drug substance unexpectedly showed greater bioavailability than the micronized form [72]. These findings indicate that particle-size reduction does not necessarily improve in vivo exposure when dissolution, supersaturation, capsule disintegration,

and precipitation interact dynamically. They also demonstrate that conventional in vitro dissolution results may not always predict clinical performance. Food significantly reduces and delays dabrafenib absorption. Administration with a high-fat meal decreases C_{max} by approximately 51% and AUC by 31% and delays T_{max} relative to fasting conditions; consequently, dabrafenib should be administered at least 1 h before or 2 h after food [34, 78, 79]. These findings support oral formulations that maintain dabrafenib in a dissolved or supersaturated state and reduce precipitation. Solid-form engineering, polymers, amorphous systems, and nanocarriers may improve exposure, but their clinical value requires in vivo validation [70, 71, 80].

4.2. Metabolism, Autoinduction, and Drug–Drug Interactions

Dabrafenib undergoes extensive oxidative metabolism, primarily through CYP2C8 and CYP3A4. Hydroxy-dabrafenib is subsequently converted to carboxy-dabrafenib, which can undergo decarboxylation to form desmethyl-dabrafenib [73, 74]. Fecal excretion is the major elimination route, accounting for approximately 71% of the administered radioactive dose, whereas urinary recovery accounts for approximately 23% and consists of metabolites rather than unchanged dabrafenib. Hydroxy-dabrafenib and desmethyl-dabrafenib retain pharmacological activity and may contribute to the overall clinical effects of treatment [34, 73, 74].

A distinctive pharmacokinetic property of dabrafenib is time-dependent autoinduction. Repeated administration increases apparent clearance, resulting in lower-than-expected accumulation and less-than-dose-proportional exposure at

steady state. Population pharmacokinetic analyses estimate an induction half-life of approximately 67 h, with steady state reached within about 14 days [73, 81]. Consequently, exposure measured after an initial dose may not represent the concentrations achieved during chronic twice-daily therapy.

Dabrafenib acts as both a substrate and an inducer of drug-metabolizing enzymes. Ketoconazole, a strong CYP3A4 inhibitor, increased dabrafenib AUC by 71%, whereas the strong CYP2C8 inhibitor gemfibrozil increased AUC by 47% [82]. Conversely, repeated dabrafenib administration reduced midazolam AUC by 65% and decreased the AUCs of S-warfarin and R-warfarin by 37% and 33%, respectively, reflecting induction of CYP3A4, CYP2C9, and related pathways [82, 83]. These interactions may increase dabrafenib toxicity when its metabolism is inhibited or reduce the effectiveness of concomitant medications when enzyme induction accelerates their clearance.

4.3. Tissue Distribution and Penetration Barriers

Adequate plasma exposure does not necessarily ensure sufficient drug concentrations within every tumor compartment. This distinction is particularly important in the central nervous system, where the blood–brain barrier and active efflux transporters restrict drug entry. Preclinical studies demonstrated that dabrafenib is a substrate of both P-glycoprotein and breast cancer resistance protein and that simultaneous activity of these transporters markedly limits its brain distribution when the blood–brain barrier is intact [75]. Trametinib is also subject to transporter-mediated restriction at the blood–brain barrier, which may further limit

coordinated intracranial exposure during combination therapy [75, 84].

Distribution heterogeneity is not limited to the CNS. Experimental imaging and computational analyses have shown that BRAF inhibitors differ in their penetration across tumors and metastatic sites. High albumin binding, lipophilicity, systemic clearance, and target-dissociation kinetics were identified as factors that may restrict the depth and duration of dabrafenib exposure within visceral lesions [85]. Consequently, some tumor regions may receive subtherapeutic concentrations despite apparently adequate plasma levels, creating pharmacological niches in which residual or drug-tolerant cells can persist. The development of brain-penetrant and efflux-evasive RAF inhibitors demonstrates that molecular and physicochemical optimization can improve intracranial

distribution[86-89]. However, these agents are distinct from dabrafenib and should not be presented as evidence that current dabrafenib formulations overcome the blood–brain barrier. For dabrafenib itself, the available evidence instead supports investigation of brain-targeted carriers, efflux-evasive delivery systems, and platforms capable of producing more uniform intratumoral distribution. Hence, Dabrafenib delivery strategies should be tailored to the specific pharmacokinetic barrier, including poor oral absorption, metabolic interactions, or limited tissue penetration. The sequential pharmacokinetic and anatomical barriers that shape dabrafenib exposure from oral administration to tumor and central nervous system delivery are summarized in Figure 3.

Table 2. Key pharmacokinetic and formulation barriers limiting dabrafenib delivery.

Barrier	Main mechanism	Clinical relevance	Potential delivery response
Poor/pH-dependent solubility	Dissolution-limited absorption; supersaturation followed by precipitation	Variable oral exposure and formulation sensitivity	Solid-form engineering, precipitation inhibitors, nanocarriers
Food/formulation effects	Meal-dependent changes in dissolution, gastric emptying, and precipitation	Need for fasted administration and reduced exposure consistency	Food-effect-resistant oral formulations and controlled-release systems
CYP metabolism and autoinduction	CYP2C8/CYP3A4 metabolism; time-dependent increase in clearance	Changing exposure during chronic therapy and potential DDIs	Exposure-stabilizing formulations; careful co-medication management
BBB and efflux transporters	P-gp/BCRP-mediated restriction of CNS entry	Reduced intracranial exposure, especially in intact BBB regions	Brain-targeted or efflux-evading delivery strategies
Heterogeneous tumor penetration	Spatial differences in drug distribution across lesions and tumor regions	Site-specific response variability	Tumor-penetrating, stimuli-responsive, or locally triggered delivery platforms

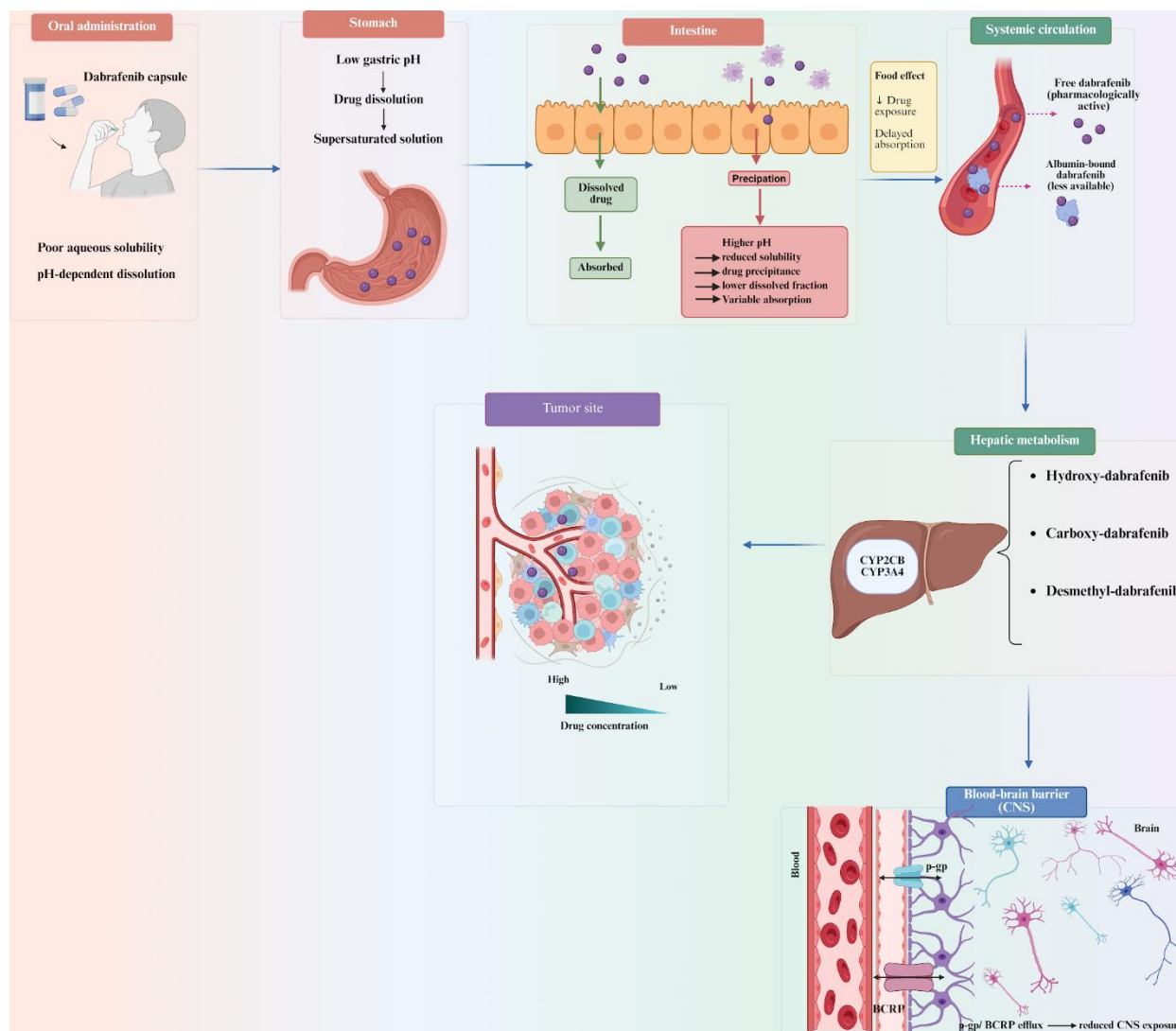


Figure 3. Pharmacokinetic and anatomical barriers affecting dabrafenib exposure. This figure was created using BioRender (BioRender.com, accessed on 17 July, 2026).

5. Resistance to Dabrafenib-Based Therapy

Resistance remains the principal limitation to durable dabrafenib-based therapy in BRAF V600E-positive solid tumors. It may arise from pre-existing insensitivity, acquired molecular alterations, adaptive signaling, tumor-cell plasticity, microenvironmental protection, or insufficient drug exposure within specific tumor compartments [3, 8, 21, 90]. Although many mechanisms were initially characterized in melanoma, their relevance extends across BRAF V600E-positive tumors, with their relative importance

determined by tumor lineage and biological context [3-6, 91].

5.1. Intrinsic and Acquired Resistance, Tumor Heterogeneity, and Adaptive Plasticity

Intrinsic resistance occurs when a BRAF V600E-positive tumor shows limited initial sensitivity despite the presence of the therapeutic target. Pre-existing receptor tyrosine kinase activity, compensatory survival pathways, co-occurring genomic alterations, stromal support, and inadequate drug accessibility may reduce dependence on BRAF signaling and weaken the response to dabrafenib-based

therapy [3, 8]. These factors vary among tumor lineages, explaining why BRAF V600E does not predict equivalent therapeutic sensitivity across all solid tumors.

Acquired resistance develops under continued treatment pressure as residual tumor cells adapt and repopulate the tumor. It may result from selection of pre-existing resistant subclones or from initially reversible, non-genetic states that allow cells to tolerate BRAF/MEK inhibition before stable resistance mechanisms emerge [8, 21, 90]. Genetic and phenotypic heterogeneity facilitates this process: drug-sensitive populations regress, whereas resistant subclones and drug-tolerant persister-like cells survive through transcriptional, epigenetic, metabolic, or differentiation-state plasticity [21, 90]. Resistance should therefore be viewed as an evolutionary process that progresses from baseline insensitivity or transient drug tolerance to stable molecular and phenotypic escape, rather than as the consequence of a single newly acquired mutation.

Cell-cycle deregulation and adaptive transcriptional rewiring may further stabilize resistant states. CDK4 alterations, CCND1 amplification, and CDKN2A loss weaken G1/S checkpoint control and may permit continued proliferation despite incomplete MAPK suppression [3, 92]. Co-occurring alterations in PTEN, TP53, the TERT promoter, and lineage-specific pathways may also modify baseline sensitivity and the route through which resistance develops [3, 8, 92]. In parallel, YAP and androgen receptor signaling can support transcriptional adaptation and reduced dependence on BRAF/MEK signaling, although the available evidence remains

largely preclinical and melanoma-derived [3, 8, 92].

5.2. MAPK Reactivation and Bypass Signaling

Reactivation of MAPK signaling is a major mechanism of resistance to dabrafenib-based therapy. Tumor cells may restore ERK activity through RAS activation, RAF dimerization, BRAF amplification or alternative isoforms, MEK alterations, and compensatory activation of receptor tyrosine kinases [8, 90]. Although trametinib delays pathway recovery by inhibiting MEK1/2, dual BRAF/MEK blockade may still be overcome by downstream alterations or reduced dependence on mutant BRAF. Non-canonical signaling may also contribute; ERK5 activation has been associated with acquired resistance to dabrafenib in BRAF V600E-mutant melanoma cells, and combined ERK5 and MEK inhibition reduced resistant-cell growth [93].

Resistance may also arise through bypass pathways that maintain survival without fully restoring canonical MAPK signaling. Activation of EGFR, PDGFR, IGF-1R, MET, and related receptors can stimulate PI3K–AKT–mTOR signaling and reduce dependence on BRAF–MEK–ERK activity [8, 90]. Tumor- or stromal-derived ligands may further promote this rescue: HGF can activate both MAPK and PI3K signaling, whereas IGF and NRG1 predominantly support PI3K-dependent survival and EGF or FGF can restore MAPK output [3]. The importance of these mechanisms varies according to receptor expression, stromal composition, and tumor lineage. ALDH1A1-associated stem-like states provide another form of bypass survival by maintaining PI3K/AKT signaling without necessarily restoring canonical MAPK activity [94].

BRAF V600E-mutant colorectal cancer provides the clearest clinical example of lineage-specific feedback resistance. BRAF inhibition relieves ERK-dependent negative feedback, leading to rapid EGFR activation and renewed RAS–RAF–MEK–ERK signaling [3, 8, 95]. Consequently, BRAF inhibitor monotherapy has limited activity in this setting, and effective pathway suppression requires concurrent EGFR blockade. This example demonstrates that resistance is shaped not only by the BRAF alteration itself but also by the signaling network of the tissue in which it occurs.

5.3. Tumor-Microenvironmental and Pharmacological Resistance

The tumor microenvironment can protect residual tumor cells during dabrafenib-based therapy. Cancer-associated fibroblasts, immune cells, extracellular matrix components, hypoxia, abnormal vasculature, and soluble mediators may activate RTK-dependent survival pathways, reduce effective drug penetration, and create protective niches for cells exposed to BRAF/MEK inhibition [3, 8, 21, 90]. Immune remodeling, including regulatory T-cell expansion, M2-like macrophage polarization, and CD73-mediated adenosine signaling, may further suppress antitumor immunity and support tumor-cell persistence under therapeutic pressure [21, 96].

Pharmacological resistance may also occur when dabrafenib exposure is insufficient or uneven across the tumor, even when cancer cells remain molecularly sensitive to BRAF inhibition. Heterogeneous perfusion, elevated interstitial fluid pressure, hypoxia, and variable vascular permeability can create underexposed regions in which residual or drug-tolerant cells survive [3, 85]. Experimental and computational analyses have further shown that

lipophilicity, albumin binding, systemic clearance, and target-dissociation kinetics may influence the depth and duration of dabrafenib penetration into visceral lesions [85].

This problem is particularly relevant in CNS tumors and brain metastases, where the blood–brain and blood–tumor barriers, active efflux transporters, and heterogeneous vascular permeability may create pharmacological sanctuary sites [3, 57]. Accordingly, delivery strategies should aim to reduce spatial underexposure, improve tumor- and brain-specific accumulation, and coordinate dabrafenib–trametinib exposure rather than merely increasing plasma drug concentrations [3, 57, 85].

6. Emerging Drug-Delivery Strategies for Dabrafenib in Solid Tumors

The pharmacokinetic, anatomical, and microenvironmental barriers that limit dabrafenib exposure provide a rationale for delivery strategies aimed at improving drug solubilization, tumor-site accumulation, and coordination with trametinib. Current approaches include oral formulation and solid-state engineering, nanocarrier-based delivery, and locoregional or co-delivery platforms; however, most remain preclinical and require comparison with standard oral therapy.

6.1. Oral Formulation and Solid-State Engineering

Oral formulation development for dabrafenib should primarily aim to improve dissolution robustness and maintain supersaturation rather than simply increase overall absorption. Clinical formulation studies have shown that capsule composition, particle properties, and precipitation behavior can substantially influence systemic exposure, despite the

high absolute bioavailability of dabrafenib under fasting conditions [97, 98].

Crystal engineering offers one strategy for modifying dabrafenib dissolution. Rai et al. (2020) developed several salts and cocrystals, among which a dabrafenib–ethylenediamine hydrate salt showed an approximately 11-fold higher dissolution rate than the free drug and dissolved more rapidly than dabrafenib mesylate at pH 1.2. However, these findings were limited to in vitro solid-state and dissolution analyses, and the pharmacokinetic, stability, and therapeutic advantages of this alternative form remain unconfirmed [70].

Nanostructured oral formulations have also been investigated. β -Cyclodextrin nanosponges and PLGA-based particles described as dabrafenib nanobubbles produced several-fold increases in systemic exposure after oral administration in rats [99, 100]. Nevertheless, neither platform demonstrated improved tumor accumulation, MAPK-pathway inhibition, or antitumor efficacy in a genetically confirmed BRAF V600E-positive model. These systems should therefore be regarded as preliminary approaches for modifying oral exposure rather than established improvements over conventional dabrafenib formulations.

6.2. Nanocarrier-Based and Tumor-Targeted Delivery

Nanocarrier-based formulations have been explored to improve dabrafenib solubilization and release while enhancing cellular uptake and, in selected preclinical models, tumor-site accumulation. Russi et al. (2023) encapsulated dabrafenib in approximately 10-nm self-assembled nanomicelles formed from an amphiphilic dendrimer, achieving an encapsulation efficiency of approximately 70% and a drug-loading capacity of 27%. The formulation

showed greater drug release at pH 5.0 than at pH 7.4 and enhanced antiproliferative activity compared with free dabrafenib in four melanoma cell lines [101]. However, the study was limited to in vitro experiments and did not assess pharmacokinetics, biodistribution, safety, or antitumor efficacy in vivo.

Pham et al. (2021) developed an acid-responsive, CD47-functionalized human serum albumin nanoconstruct for systemic dabrafenib delivery. The approximately 220-nm formulation increased cellular uptake, tumor accumulation, apoptosis, and tumor-growth inhibition compared with free dabrafenib or non-targeted nanoconstructs in a murine melanoma model. Nevertheless, the B16F10 model was not genetically validated as BRAF V600E-positive; therefore, the findings demonstrate the carrier's delivery and immunomodulatory potential but do not directly establish improved dabrafenib efficacy in molecularly appropriate BRAF V600E-driven disease [102].

Overall, these studies support the potential of nanomicellar and albumin-based platforms to modify dabrafenib release and tumor delivery. However, evidence remains limited to in vitro or melanoma-focused preclinical models, and future studies should quantify intratumoral drug exposure and MAPK inhibition in genetically confirmed BRAF V600E-positive tumors.

6.3. Locoregional and Dabrafenib–Trametinib Co-Delivery

Locoregional delivery and drug co-encapsulation offer complementary strategies for improving dabrafenib-based therapy. Locoregional administration may increase drug deposition in accessible cutaneous tumors, whereas co-delivery systems aim to coordinate the distribution of dabrafenib and trametinib within the

same tumor compartments. However, evidence for both approaches remains limited to preclinical melanoma models. Tham et al. (2018) developed photodynamically active mesoporous organosilica nanoparticles loaded with dabrafenib and trametinib. Microneedle pretreatment facilitated nanoparticle transport across the epidermal barrier and increased penetration into deeper porcine skin. The integrated platform also inhibited melanoma spheroids and improved tumor control in an A375 xenograft model. Nevertheless, it was not compared directly with standard oral dabrafenib–trametinib therapy, and its application is largely restricted to accessible cutaneous or subcutaneous lesions [103]. Koshrovski-Michael et al. (2024) developed sulfate-functionalized PLGA–PEG nanoparticles that co-encapsulated dabrafenib and trametinib at a preselected synergistic ratio and targeted P-selectin expressed by melanoma cells and tumor-associated vasculature. In murine melanoma models, the targeted nanoparticles increased tumor

accumulation, preserved the drug ratio within tumor tissue, and improved MAPK-pathway suppression, tumor-growth inhibition, and survival compared with the corresponding free-drug combination at matched low doses. However, their performance may depend on tumor P-selectin expression, and clinical superiority over conventional oral therapy remains unproven [104]. Overall, these studies support locoregional delivery and coordinated BRAF/MEK inhibitor co-delivery as promising strategies, but further evaluation requires pharmacokinetic comparisons, systemic safety assessment, and validation in genetically confirmed BRAF V600E-positive tumors beyond melanoma. As summarized in Table 3, dabrafenib-specific delivery research encompasses oral formulation optimization, nanocarrier-based solubilization, locoregional administration, and coordinated dabrafenib–trametinib delivery; however, the supporting evidence remains predominantly preclinical and heterogeneous.

Table 3. Dabrafenib-specific oral formulation, nanocarrier, locoregional, and combination-delivery strategies

Delivery approach	Formulation/platform	Administration route	Experimental model	Main findings	Key limitations	Reference
Oral formulation optimization	Hypromellose versus gelatin capsules; micronized versus nonmicronized drug substance	Oral	Randomized single-dose study in patients with BRAF mutation-positive tumors	Fasting, capsule-shell composition, and particle size altered systemic exposure. Hypromellose prolonged supersaturation	Single-dose formulation study; no assessment of lesion-level tumor exposure or clinical efficacy.	[97]

				tion and reduced precipitation of dabrafenib free base.		
Crystal engineering	Dabrafenib salts and cocrystals, including an ethylenediamine hydrate salt	Proposed oral use	In vitro solid-state and dissolution studies	The ethylenediamine hydrate salt showed an approximately 11-fold faster dissolution rate than dabrafenib free base and dissolved faster than dabrafenib mesylate at pH 1.2.	No pharmacokinetic, biodistribution, safety, or efficacy evaluation.	[70]
Nanosponge formulation	Diphenyl-carbonate-crosslinked beta-cyclodextrin nanosponges	Oral	Formulation studies and rat pharmacokinetics	The optimized formulation had a mean size of approximately 158 nm and increased Cmax and systemic exposure relative to free dabrafenib.	No BRAF V600E-positive tumor model; no tumor accumulation, pathway-inhibition, or antitumor-efficacy data.	[99]
Nanobubble formulation	PLGA-based dabrafenib nanobubbles	Oral; ultrasound-responsive release evaluated in vitro	Formulation studies and rat pharmacokinetics	The optimized particles were approximately 191 nm and increased Cmax and	No genetically confirmed BRAF V600E-positive tumor model; no demonstrati	[100]

				systemic exposure after oral administration in rats.	on improved tumor delivery or therapeutic efficacy.	
Nanomicellar delivery	Self-assembled amphiphilic dendrimer nanomicelles	In vitro	Four melanoma cell lines	Approximately 10-nm micelles improved dabrafenib solubilization, showed greater release at pH 5.0 than at pH 7.4, and enhanced antiproliferative activity relative to free drug.	No in vivo pharmacokinetic, biodistribution, safety, or efficacy assessment.	[101]
Protein-based nanocarrier	Acid-responsive, CD47-conjugated human-serum-albumin nanoconstruct	Systemic	B16F10 murine melanoma model	The carrier increased cellular uptake, tumor accumulation, and tumor-growth inhibition relative to free dabrafenib.	B16F10 tumors express wild-type rather than V600E-mutant BRAF; therefore, the study does not directly validate dabrafenib delivery in BRAF V600E-positive disease.	[102]
Locoregional multimodal delivery	Microneedle-assisted topical delivery of photodynamically active mesoporous	Topical/locoregional with near-infrared irradiation	Porcine skin, melanoma spheroids, and A375 xenografts	Microneedle pretreatment increased penetration into deeper	No direct comparison with standard oral dabrafenib-	[103]

	organosilica nanoparticles loaded with dabrafenib and trametinib			tissue, while the integrated platform combined BRAF/MEK inhibition with photodynamic cytotoxicity and improved tumor control.	trametinib therapy; applicable mainly to accessible cutaneous or subcutaneous lesions; individual contribution of enhanced dabrafenib delivery was not isolated.	
Targeted combination co-delivery	Sulfated, P-selectin-targeted PLGA-PEG nanoparticles co-encapsulating dabrafenib and trametinib	Intravenous	Murine and human melanoma cell lines and D4M.3A murine melanoma models	The nanoparticles increased tumor accumulation, maintained the initial drug ratio in tumor tissue, and improved MAPK suppression, tumor-growth inhibition, and survival relative to free drugs or nontargeted particles at matched low doses.	Preclinical evidence only; performance may depend on tumor P-selectin expression and vascular distribution; clinical superiority over oral therapy is unproven.	[104]

7. Future Directions and Translational Priorities

Clinical translation of dabrafenib delivery systems requires evidence of improved tumor-site exposure and MAPK-pathway

inhibition compared with standard oral therapy. Future studies should also address formulation reproducibility, scalable manufacturing, biodistribution, clearance, chronic toxicity, immunological effects, and

off-target accumulation, because preclinical antitumor activity alone does not ensure clinical feasibility or safety [105].

Next-generation RAF inhibitors may address resistance mechanisms or molecular contexts that are poorly controlled by conventional BRAF V600-selective inhibition. Tovorafenib, a type II RAF inhibitor, demonstrated clinically meaningful activity in relapsed or refractory BRAF-altered pediatric low-grade glioma in the FIREFLY-1 trial [59]. The investigational brain-penetrant pan-mutant BRAF inhibitor PF-07799933 also produced systemic and intracranial responses in heavily pretreated patients with BRAF-mutant cancers, including tumors with dimerization-associated resistance [39]. However, these agents should not be considered universal replacements for dabrafenib; their relevance depends on the exact BRAF alteration, resistance mechanism, tumor lineage, treatment history, and anatomical site of disease.

Future combination strategies should likewise be selected according to tumor biology. In BRAF V600E-mutant colorectal cancer, combined PD-1, BRAF, and MEK inhibition with spartalizumab, dabrafenib, and trametinib demonstrated proof-of-concept activity but remains investigational and should not replace established BRAF–EGFR-based treatment strategies [67]. More broadly, treatment selection should integrate BRAF mutation class, fusions, co-occurring alterations, resistance pathways, and tissue-specific drug accessibility. Next-generation sequencing and, where sufficiently validated, longitudinal circulating tumor DNA analysis may support treatment selection and early resistance monitoring [67]. Thus, future improvement of dabrafenib-based therapy will require coordinated consideration of molecular

context, resistance biology, delivery performance, and tumor-site accessibility.

8. Conclusion

The clinical activity of dabrafenib, particularly in combination with trametinib, confirms BRAF V600E as an important therapeutic target across multiple solid tumors. Nevertheless, mutation status alone does not determine the magnitude or durability of response. Tumor lineage, co-occurring alterations, adaptive signaling, microenvironmental protection, and lesion-level drug exposure collectively shape therapeutic outcome. Pharmacokinetic and tissue-distribution barriers may further generate poorly exposed tumor compartments in which residual or drug-tolerant cells persist. Emerging approaches, including solid-state engineering, nanocarriers, locoregional administration, and coordinated dabrafenib–trametinib co-delivery, offer potential means of improving drug availability, but their clinical advantage over standard oral therapy remains unproven. Future studies should therefore employ molecularly appropriate tumor models, directly quantify intratumoral drug concentrations and MAPK inhibition, and compare new formulations with current treatment standards. Ultimately, durable improvement in dabrafenib-based therapy will require an integrated framework that aligns molecular selection and resistance profiling with tumor-specific pharmacology and drug-delivery performance.

References

1. Riesco-Eizaguirre, G., BRAF V600E thyroid cancer: navigating prognostic uncertainty and therapeutic opportunity. *European Thyroid Journal*, 2025. 14(6).

2. Jafari, M., et al., Prevalence and patterns of mutations in RAS/RAF/MEK/ERK/MAPK signaling pathway in colorectal cancer in North Africa. *Bmc Cancer*, 2022. 22(1): p. 1142.
3. Mechahougui, H., et al., BRAF targeting across solid tumors: molecular aspects and clinical applications. *International Journal of Molecular Sciences*, 2025. 26(8): p. 3757.
4. Gouda, M.A. and V. Subbiah, Expanding the benefit: dabrafenib/trametinib as tissue-agnostic therapy for BRAF V600E–positive adult and pediatric solid tumors. *American Society of Clinical Oncology Educational Book*, 2023. 43: p. e404770.
5. Subbiah, V., et al., Dabrafenib plus trametinib in BRAFV600E-mutated rare cancers: the phase 2 ROAR trial. *Nature medicine*, 2023. 29(5): p. 1103–1112.
6. Shimoj, T., et al., Dabrafenib and trametinib administration in patients with BRAF V600E/R or non-V600 BRAF mutated advanced solid tumours (BELIEVE, NCCH1901): a multicentre, open-label, and single-arm phase II trial. *EClinicalMedicine*, 2024. 69.
7. Wang, X., et al., Decabromodiphenyl ether exposure reduces dabrafenib sensitivity of papillary thyroid carcinoma harboring BRAFV600E mutation through the EGFR-CRAF-MAPK pathway: An in vitro study. *Toxicology*, 2024. 504: p. 153807.
8. Hanrahan, A.J., et al., BRAF—a tumour-agnostic drug target with lineage-specific dependencies. *Nature Reviews Clinical Oncology*, 2024. 21(3): p. 224–247.
9. Eller, S., et al., Exploiting metabolic adaptations to overcome dabrafenib treatment resistance in melanoma cells. *Molecular Oncology*, 2026. 20(5): p. 1202–1219.
10. Xu, H., et al., Abnormal activation of the Ras/MAPK signaling pathway in oncogenesis and progression. *Cancer Adv*, 2025. 8: p. e25002.
11. Nian, H., et al., Targeting the Osteopontin-regulated PI3K/AKT signaling pathway: A molecular approach to overcome drug resistance and metastasis in gastrointestinal tumors. *World Journal of Gastrointestinal Oncology*, 2025. 17(11): p. 109923.
12. Gu, Y., et al., EGFR: new insights on its activation and mutation in tumor and tumor immunotherapy. *Advanced Science*, 2025. 12(36): p. e05785.
13. Matthews, H.K., C. Bertoli, and R.A. De Bruin, Cell cycle control in cancer. *Nature reviews Molecular cell biology*, 2022. 23(1): p. 74–88.
14. Mubin, N., et al., Multidimensional tumor heterogeneity and its role in therapeutic resistance. *Frontiers in Immunology*, 2026. 17: p. 1794130.
15. Zhang, H., et al., Immune niche-associated and niche-regulated plasticity in glioblastoma: state transitions, immune escape, and therapeutic vulnerabilities. *Frontiers in Immunology*, 2026. 17: p. 1857659.
16. Balakirouchenane, D., et al., Pharmacokinetics/pharmacodynamics of dabrafenib and trametinib for redifferentiation and treatment of radioactive iodine-resistant mutated advanced differentiated thyroid cancer. *Thyroid*, 2023. 33(11): p. 1327–1338.
17. Isberner, N., et al., Monitoring of dabrafenib and trametinib in serum and self-sampled capillary blood in patients with BRAFV600-mutant melanoma. *Cancers*, 2022. 14(19): p. 4566.
18. Rocca, A., et al., The predictive and prognostic role of RAS–RAF–MEK–ERK pathway alterations in breast cancer: revision of the literature and comparison

with the analysis of cancer genomic datasets. *Cancers*, 2022. 14(21): p. 5306.

19. Nair, A. and B. Saha, Regulation of Ras-GTPase signaling and localization by post-translational modifications. *Kinases and Phosphatases*, 2023. 1(2): p. 97–116.

20. Jeon, H., E. Tkacik, and M.J. Eck, Signaling from RAS to RAF: the molecules and their mechanisms. *Annual review of biochemistry*, 2024. 93.

21. Imani, S., et al., The evolution of BRAF-targeted therapies in melanoma: overcoming hurdles and unleashing novel strategies. *Frontiers in Oncology*, 2024. 14: p. 1504142.

22. Dhillon, A.S., et al., MAP kinase signalling pathways in cancer. *Oncogene*, 2007. 26(22): p. 3279–3290.

23. Spencer-Smith, R. and D.K. Morrison, Regulation of RAF family kinases: new insights from recent structural and biochemical studies. *Biochemical Society Transactions*, 2024. 52(3): p. 1061–1069.

24. Wan, P.T., et al., Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell*, 2004. 116(6): p. 855–867.

25. Yao, Z., et al., BRAF mutants evade ERK-dependent feedback by different mechanisms that determine their sensitivity to pharmacologic inhibition. *Cancer cell*, 2015. 28(3): p. 370–383.

26. Yu, A., et al., A novel phosphorylation site involved in dissociating RAF kinase from the scaffolding protein 14-3-3 and disrupting RAF dimerization. *Journal of Biological Chemistry*, 2023. 299(10): p. 105188.

27. Park, E., et al., Architecture of autoinhibited and active BRAF–MEK1–14-3-3 complexes. *Nature*, 2019. 575(7783): p. 545–550.

28. Yao, Z., et al., Tumours with class 3 BRAF mutants are sensitive to the inhibition of activated RAS. *Nature*, 2017. 548(7666): p. 234–238.

29. Arter, Z.L., et al., Comprehensive Survey of AACR GENIE Database of Tumor Mutation Burden (TMB) Among All Three Classes (I, II, III) of BRAF Mutated (BRAF+) NSCLC. *Lung Cancer: Targets and Therapy*, 2025: p. 1–9.

30. Gunderwala, A., N. Cope, and Z. Wang, Mechanism and inhibition of BRAF kinase. *Current opinion in chemical biology*, 2022. 71: p. 102205.

31. Özgü, E., et al., Therapeutic vulnerabilities and pan-cancer landscape of BRAF class III mutations in epithelial solid tumors. *BJC reports*, 2024. 2(1): p. 77.

32. Singh, A.K., et al., Challenges and Opportunities in the Crusade of BRAF Inhibitors: From 2002 to 2022. *ACS omega*, 2023. 8(31): p. 27819–27844.

33. Fasano, M., et al., Dramatic therapeutic response to dabrafenib plus trametinib in BRAF V600E mutated papillary craniopharyngiomas: a case report and literature review. *Frontiers in Medicine*, 2022. 8: p. 652005.

34. Corporation, N.P., TAFINLAR® (dabrafenib) prescribing information. 2025, Novartis Pharmaceuticals Corporation: East Hanover, NJ.

35. Gonzalez-Del Pino, G.L., et al., Allosteric MEK inhibitors act on BRAF/MEK complexes to block MEK activation. *Proceedings of the National Academy of Sciences*, 2021. 118(36): p. e2107207118.

36. Flaherty, K.T., et al., Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. *New England Journal of Medicine*, 2012. 367(18): p. 1694–1703.

37. Long, G.V., et al., Combined BRAF and MEK inhibition versus BRAF inhibition

alone in melanoma. *New England Journal of Medicine*, 2014. 371(20): p. 1877–1888.

38. Corporation, N.P., MEKINIST® (trametinib) prescribing information. 2026, Novartis Pharmaceuticals Corporation: East Hanover, NJ.

39. Yaeger, R., et al., A next-generation BRAF inhibitor overcomes resistance to BRAF inhibition in patients with BRAF-mutant cancers using pharmacokinetics-informed dose escalation. *Cancer Discovery*, 2024. 14(9): p. 1599–1611.

40. Tkacik, E., et al., Structure and RAF family kinase isoform selectivity of type II RAF inhibitors tovorafenib and naporafenib. *Journal of Biological Chemistry*, 2023. 299(5): p. 104634.

41. Kossakowski, K., et al., FDA-approved kinase inhibitors in PROTAC design, development and synthesis. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 2025. 40(1): p. 2542357.

42. Zhang, S., et al., Targeted therapeutic strategies for melanoma. *Chinese medical journal*, 2023. 136(24): p. 2923–2930.

43. Hauschild, A., et al., Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. *The Lancet*, 2012. 380(9839): p. 358–365.

44. Robert, C., et al., Improved overall survival in melanoma with combined dabrafenib and trametinib. *New England Journal of Medicine*, 2015. 372(1): p. 30–39.

45. Robert, C., et al., Five-year outcomes with dabrafenib plus trametinib in metastatic melanoma. *New England Journal of Medicine*, 2019. 381(7): p. 626–636.

46. Tabbò, F., et al., How far we have come targeting BRAF-mutant non-small cell

lung cancer (NSCLC). *Cancer Treatment Reviews*, 2022. 103: p. 102335.

47. Planchard, D., et al., Phase 2 study of dabrafenib plus trametinib in patients with BRAF V600E-mutant metastatic NSCLC: updated 5-year survival rates and genomic analysis. *Journal of thoracic oncology*, 2022. 17(1): p. 103–115.

48. Planchard, D., et al., Phase 2 Study of Dabrafenib Plus Trametinib in Patients With *BRAF* V600E-Mutant Metastatic NSCLC: Updated 5-Year Survival Rates and Genomic Analysis. *Journal of Thoracic Oncology*, 2022. 17(1): p. 103–115.

49. Shimoi, T., et al., Dabrafenib and trametinib administration in patients with *BRAF* V600E/R or non-V600 *BRAF* mutated advanced solid tumours (BELIEVE, NCCH1901): a multicentre, open-label, and single-arm phase II trial. *eClinicalMedicine*, 2024. 69.

50. Chen, D., et al., Papillary thyroid cancer organoids harboring BRAFV600E mutation reveal potentially beneficial effects of BRAF inhibitor-based combination therapies. *Journal of Translational Medicine*, 2023. 21(1): p. 9.

51. Leboulleux, S., et al., MERAIODE: a redifferentiation phase II trial with trametinib and dabrafenib followed by radioactive iodine administration for metastatic radioactive iodine refractory differentiated thyroid cancer patients with a BRAFV600E mutation (NCT 03244956). *Journal of the Endocrine Society*, 2021. 5(Supplement_1): p. A876–A876.

52. Subbiah, V., et al., Dabrafenib plus trametinib in patients with BRAF V600E-mutant anaplastic thyroid cancer: updated analysis from the phase II ROAR basket study. *Annals of oncology*, 2022. 33(4): p. 406–415.

53. Macerola, E., et al., Predictive biomarkers in thyroid cancer. *Frontiers in Oncology*, 2022. 12: p. 901004.
54. Min, K.-W., et al., Cancer-associated Fibroblasts Are Associated With Poor Prognosis in Solid Type of Lung Adenocarcinoma: Machine Learning Approach. 2021.
55. Di Nunno, V., et al., Implications of BRAF V600E mutation in gliomas: molecular considerations, prognostic value and treatment evolution. *Frontiers in Oncology*, 2023. 12: p. 1067252.
56. Tabernero Caturla, J., et al. Encorafenib Plus Cetuximab as a New Standard of Care for Previously Treated BRAF V600E–Mutant Metastatic Colorectal Cancer: Updated Survival Results and Subgroup Analyses from the BEACON Study. *American Society of Clinical Oncology*.
57. Capogiri, M., et al., Response and resistance to BRAFV600E inhibition in gliomas: Roadblocks ahead? *Frontiers in oncology*, 2023. 12: p. 1074726.
58. Tabernero, J., et al., Encorafenib Plus Cetuximab as a New Standard of Care for Previously Treated BRAF V600E–Mutant Metastatic Colorectal Cancer: Updated Survival Results and Subgroup Analyses from the BEACON Study. *Journal of Clinical Oncology*, 2021. 39(4): p. 273–284.
59. Kilburn, L.B., et al., The type II RAF inhibitor tovorafenib in relapsed/refractory pediatric low-grade glioma: the phase 2 FIREFLY-1 trial. *Nature medicine*, 2024. 30(1): p. 207–217.
60. Ding, L., et al., Crossing the blood–brain barrier: innovations in receptor-and transporter-mediated transcytosis strategies. *Pharmaceutics*, 2025. 17(6): p. 706.
61. Upton, D.H., et al., Challenges and opportunities to penetrate the blood-brain barrier for brain cancer therapy. *Theranostics*, 2022. 12(10): p. 4734.
62. Long, G.V., et al., Final results for adjuvant dabrafenib plus trametinib in stage III melanoma. *New England Journal of Medicine*, 2024. 391(18): p. 1709–1720.
63. Menzies, A., et al., Five-year analysis of neoadjuvant dabrafenib and trametinib for stage III melanoma. *Annals of Oncology*, 2024. 35(8): p. 739–746.
64. Swalduz, A., et al., Real-world efficacy of the dabrafenib-trametinib (DT) combination in BRAF V600E-mutated metastatic non-small cell lung cancer (NSCLC): Results from the IFCT-2004 BLADE cohort. *Lung Cancer*, 2025. 199: p. 108038.
65. Leboulleux, S., et al., A phase II redifferentiation trial with dabrafenib-trametinib and 131I in metastatic radioactive iodine refractory BRAF p. V600E-mutated differentiated thyroid cancer. *Clinical Cancer Research*, 2023. 29(13): p. 2401–2409.
66. Gao, M., et al., Efficacy and safety of dabrafenib plus trametinib in adults with differentiated thyroid cancer: a randomised, double-blind, placebo-controlled, phase 3 trial. *The Lancet Oncology*, 2026.
67. Tian, J., et al., Combined PD-1, BRAF and MEK inhibition in BRAFV600E colorectal cancer: a phase 2 trial. *Nature Medicine*, 2023. 29(2): p. 458–466.
68. Chapdelaine, A.G., et al., The targeted degradation of BRAF V600E reveals the mechanisms of resistance to BRAF-targeted treatments in colorectal cancer cells. *Cancers*, 2023. 15(24): p. 5805.
69. Talloa, D., et al., BRAF and MEK targeted therapies in pediatric central

nervous system tumors. *Cancers*, 2022. 14(17): p. 4264.

70. Rai, S.K., et al., Improving the dissolution rate of the anticancer drug dabrafenib. *Crystal Growth & Design*, 2020. 20(2): p. 1035–1046.

71. Rai, S.K., et al., Polymorphs, solvatomorphs, hydrate, and perhydrate of dabrafenib. *Crystal Growth & Design*, 2023. 23(2): p. 1179–1188.

72. Ouellet, D., et al., Effects of particle size, food, and capsule shell composition on the oral bioavailability of dabrafenib, a BRAF inhibitor, in patients with BRAF mutation-positive tumors. *Journal of pharmaceutical sciences*, 2013. 102(9): p. 3100–3109.

73. Puszkiel, A., et al., Clinical pharmacokinetics and pharmacodynamics of dabrafenib. *Clinical Pharmacokinetics*, 2019. 58(4): p. 451–467.

74. Bershas, D.A., et al., Metabolism and disposition of oral dabrafenib in cancer patients: proposed participation of aryl nitrogen in carbon-carbon bond cleavage via decarboxylation following enzymatic oxidation. *Drug Metabolism and Disposition*, 2013. 41(12): p. 2215–2224.

75. Mittapalli, R.K., et al., Mechanisms limiting distribution of the threonine-protein kinase B-RaFV600E inhibitor dabrafenib to the brain: implications for the treatment of melanoma brain metastases. *The Journal of pharmacology and experimental therapeutics*, 2013. 344(3): p. 655–664.

76. Indulkar, A.S., et al., pH-dependent liquid–liquid phase separation of highly supersaturated solutions of weakly basic drugs. *Molecular pharmaceutics*, 2015. 12(7): p. 2365–2377.

77. Kumari, L., et al., Advancement in solubilization approaches: a step towards bioavailability enhancement of poorly soluble drugs. *Life*, 2023. 13(5): p. 1099.

78. Cheng, L. and H. Wong, Food effects on oral drug absorption: application of physiologically-based pharmacokinetic modeling as a predictive tool. *Pharmaceutics*, 2020. 12(7): p. 672.

79. Tan, E.Y., et al., Evaluation of a Low-Fat Low-Calorie Meal on the Relative Bioavailability of Trametinib and Dabrafenib: Results From a Randomized, Open-Label, 2-Part Study in Healthy Participants. *Clinical Pharmacology in Drug Development*, 2023. 12(3): p. 333–342.

80. Onoue, S., K. Yamada, and H. Sato, Advanced oral drug delivery systems: Current challenges and emerging technologies. *Acta Pharmaceutica Sinica B*, 2025.

81. Ouellet, D., et al., Population pharmacokinetics of dabrafenib, a BRAF inhibitor: effect of dose, time, covariates, and relationship with its metabolites. *The Journal of Clinical Pharmacology*, 2014. 54(6): p. 696–706.

82. Suttle, A.B., et al., Assessment of the drug interaction potential and single-and repeat-dose pharmacokinetics of the BRAF inhibitor dabrafenib. *The Journal of Clinical Pharmacology*, 2015. 55(4): p. 392–400.

83. Nebot, N., et al., Evaluation of the Effects of Repeat-Dose Dabrafenib on the Single-Dose Pharmacokinetics of Rosuvastatin (OATP1B1/1B3 Substrate) and Midazolam (CYP3A4 Substrate). *Clinical Pharmacology in Drug Development*, 2021. 10(9): p. 1054–1063.

84. Vaidhyanathan, S., et al., Factors influencing the CNS distribution of a novel MEK-1/2 inhibitor: implications for combination therapy for melanoma brain metastases. *Drug Metabolism and Disposition*, 2014. 42(8): p. 1292–1300.

85. Ng, T.S., et al., Overcoming differential tumor penetration of BRAF inhibitors using computationally guided

combination therapy. *Science Advances*, 2022. 8(17): p. eabl6339.

86. Ren, L., et al., Identification of the clinical candidate PF-07284890 (ARRY-461), a highly potent and brain penetrant BRAF inhibitor for the treatment of cancer. *Journal of Medicinal Chemistry*, 2024. 67(15): p. 13019–13032.

87. Kelly, A.M., et al., Target-agnostic p-glycoprotein assessment yields strategies to evade efflux, leading to a BRAF inhibitor with intracranial efficacy. *Journal of the American Chemical Society*, 2022. 144(27): p. 12367–12380.

88. Wichmann, J., et al., Preclinical characterization of a next-generation brain permeable, paradox breaker BRAF inhibitor. *Clinical Cancer Research*, 2022. 28(4): p. 770–780.

89. Kim, Y.-Y., et al., Belvarafenib penetrates the BBB and shows potent antitumor activity in a murine melanoma brain metastasis model. *Clinical & Experimental Metastasis*, 2023. 40(2): p. 137–148.

90. Cosci, I., et al., Molecular basis of BRAF inhibitor resistance in melanoma: a systematic review. *Pharmaceuticals*, 2025. 18(8): p. 1235.

91. Planchard, D., et al., BRAFV600E-mutant metastatic NSCLC: disease overview and treatment landscape. *NPJ precision oncology*, 2024. 8(1): p. 90.

92. Kreuger, I.Z., et al., Therapeutic strategies for targeting CDKN2A loss in melanoma. *Journal of Investigative Dermatology*, 2023. 143(1): p. 18–25. e1.

93. Mondru, A.K., et al., The ERK5 pathway in BRAFV600E melanoma cells plays a role in development of acquired resistance to dabrafenib but not vemurafenib. *FEBS letters*, 2024. 598(16): p. 2011–2027.

94. Ciccone, V., et al., ALDH1A1 confers resistance to RAF/MEK inhibitors in melanoma cells by maintaining stemness phenotype and activating PI3K/AKT signaling. *Biochemical pharmacology*, 2024. 224: p. 116252.

95. Xu, T., et al., Molecular mechanisms underlying the resistance of BRAF V600E-mutant metastatic colorectal cancer to EGFR/BRAF inhibitors. *Therapeutic advances in medical oncology*, 2022. 14: p. 17588359221105022.

96. Giraulo, C., et al., High levels of soluble CD73 unveil resistance to BRAF inhibitors in melanoma cells. *Biomedicine & Pharmacotherapy*, 2024. 177: p. 117033.

97. Ouellet, D., et al., Effects of particle size, food, and capsule shell composition on the oral bioavailability of dabrafenib, a BRAF inhibitor, in patients with BRAF mutation-positive tumors. *J Pharm Sci*, 2013. 102(9): p. 3100–9.

98. Puszkiel, A., et al., Clinical Pharmacokinetics and Pharmacodynamics of Dabrafenib. *Clin Pharmacokinet*, 2019. 58(4): p. 451–467.

99. Reddy, K.S.C., D. Bhikshapathi, and J.P. Kumar, Unlocking dabrafenib's potential: A quality by design (QbD) journey to enhance permeation and oral bioavailability through nanosponge formulation. *Brazilian Journal of Pharmaceutical Sciences*, 2025. 61: p. e24209.

100. Laxmi, B.V., D. Bhikshapathi, and P.S. Rao, Optimization and enhancement of oral bioavailability of dabrafenib as nanobubbles using quality by design approach. 2025.

101. Russi, M., et al., Some things old, new and borrowed: Delivery of dabrafenib and vemurafenib to melanoma cells via self-assembled nanomicelles based on an amphiphilic dendrimer. *European Journal of*

Pharmaceutical Sciences, 2023. 180: p. 106311.

102. Pham, L.M., et al., Preparation and evaluation of dabrafenib-loaded, CD47-conjugated human serum albumin-based nanoconstructs for chemoimmunomodulation. Colloids and Surfaces B: Biointerfaces, 2021. 208: p. 112093.

103. Tham, H.P., et al., Microneedle-Assisted Topical Delivery of Photodynamically Active Mesoporous Formulation for Combination Therapy of

Deep-Seated Melanoma. ACS Nano, 2018. 12(12): p. 11936–11948.

104. Koshrovski-Michael, S., et al., Two-in-one nanoparticle platform induces a strong therapeutic effect of targeted therapies in P-selectin-expressing cancers. Science advances, 2024. 10(50): p. eadr4762.

105. Joyce, P., et al., A translational framework to DELIVER nanomedicines to the clinic. Nature nanotechnology, 2024. 19(11): p. 1597–1611.

BRAF V600E-POZITIV SOLİD ŞİŞLƏRDƏ DABRAFENİB: REZİSTENTLİK, KLİNİK TƏTBİQLƏR VƏ DƏRMAN ÇATDIRILMA STRATEGİYALARI

*Fatemeh Dodangeh¹, Sara Azizi², Maryam Ebrahimi³, Mahsa Javan⁴, Aziz Eftekhari⁵ **

¹ *Fundamental Elmlər üzrə Qabaqcıl Tədqiqatlar İnstitutu, Biologiya Elmləri Fakültəsi, Zəncan, İran*

² *Fundamental Elmlər üzrə Qabaqcıl Tədqiqatlar İnstitutu, Biologiya Elmləri Fakültəsi, Zəncan, İran*

³ *Xarezmı Universiteti, Biologiya Elmləri Fakültəsi, Tehran, İran*

⁴ *Təbriz Tibb Elmləri Universiteti, Əczaçılıq Fakültəsi, Təbriz, İran*

⁵ *Ege Universiteti, Biokimya kafedrası, Təbiət Elmləri Fakültəsi, İzmir, 35040, Türkiyə*

**Yazışma üçün əlaqə E-mail: eftekhari.aziz@gmail.com*

Xülasə

Dabrafenib, xüsusilə trametinib ilə kombinasiyada, BRAF V600E-pozitiv bir sıra solid şişlərin müalicəsində artıq təsdiqlənmiş hədəflənmiş terapiyadır. Bununla belə, müalicəyə cavabın dərəcəsi və davamlılığı şiş növündən asılı olaraq əhəmiyyətli dərəcədə dəyişir. Bu icmalda dabrafenib əsaslı terapiyanın molekulyar əsasları, klinik tətbiqləri, rezistentlik mexanizmləri, farmakokinetik məhdudiyyətləri və inkişaf etməkdə olan dərman çatdırılma strategiyaları ümumiləşdirilir.

Müalicənin nəticəsinə yalnız BRAF V600E statusu deyil, həmçinin şişin mənşəyi, yanaşı mövcud olan genomik dəyişikliklər, reseptor tirozin kinazalarının əks-əlaqə mexanizmləri, mikroətraf mühit siqnalları və dərmanın ayrı-ayrı lezyonlarda ekspozisiyası təsir göstərir. Rezistentlik MAPK yolunun yenidən aktivləşməsi, PI3K/AKT alternativ siqnal ötürülməsi, hüceyrə plastikliyi, şiş heterogenliyi, stromal qoruyucu təsirlər və farmakoloji cəhətdən kifayət qədər ekspozisiyanın olmaması vasitəsilə inkişaf edir. Dabrafenibin effektivliyi pH-dan asılı həllolma, qida qəbuluna həssas sorulma, CYP vasitəçiliyi ilə metabolizm, autoinduksiya, dərman qarşılıqlı təsirləri, beyinə məhdud paylanma və şiş daxili toxumaya qeyri-bərabər nüfuzetmə ilə daha da məhdudlaşır. Bərk hal modifikasiyası, nanostruktur formulları, hədəflənmiş nanodaşıyıcılar, lokoregional tətbiq və dabrafenib–trametinibin koordinasiya şəkildə çatdırılması prekliniki tədqiqatlarda ümidverici nəticələr göstərmişdir. Lakin onların standart oral terapiyadan üstünlüyünü təsdiqləyən sübutlar hələ də məhduddur.

Melanoma, ağciyər, qalxanabənzer vəz, öd yolları, kolorektal və mərkəzi sinir sistemi şişləri üzrə klinik nəticələr göstərir ki, BRAF V600E mühüm terapevtik hədəf olsa da, müalicədən faydanı müstəqil şəkildə proqnozlaşdırmaq üçün kifayət etmir. Buna görə də müalicənin optimallaşdırılması molekulyar

seçim, rezistentlik profilinin müəyyənləşdirilməsi və dərmanın ayrı-ayrı lezyonlarda ekspozisiyasını birləşdirən şişə spesifik yanaşma tələb edir.

Açar sözlər: Dabrafenib, BRAF V600E, solid şiş, dərmana rezistentlik, nanodərman çatdırılması

ДАБРАФЕНИБ ПРИ СОЛИДНЫХ ОПУХОЛЯХ С BRAF V600E-ПОЛОЖИТЕЛЬНЫМ СТАТУСОМ: РЕЗИСТЕНТНОСТЬ, КЛИНИЧЕСКОЕ ПРИМЕНЕНИЕ И СТРАТЕГИИ ДОСТАВКИ ЛЕКАРСТВЕННЫХ ПРЕПАРАТОВ

Fatemeh Dodangeh¹, Sara Azizi², Maryam Ebrahimi³, Mahsa Javan⁴, Aziz Eftekhari^{5*}

¹ Факультет биологических наук, Институт перспективных исследований в области фундаментальных наук, Зенджан, Иран

² Факультет биологических наук, Институт перспективных исследований в области фундаментальных наук, Зенджан, Иран

³ Факультет биологических наук, Университет Харазми, Тегеран, Иран

⁴ Факультет фармации, Тебризский университет медицинских наук, Тебриз, Иран

⁵ Кафедра биохимии, факультет естественных наук, Университет Эге, Измир, 35040, Турция

* Для корреспонденции E-mail: eftekhari.aziz@gmail.com

Резюме

Дабрафениб, особенно в комбинации с траметинибом, является признанной таргетной терапией для лечения ряда **BRAF V600E**-положительных солидных опухолей, хотя выраженность и продолжительность ответа на лечение значительно различаются в зависимости от типа опухоли. В настоящем обзоре обобщены молекулярное обоснование применения дабрафениба, его клиническое использование, механизмы резистентности, фармакокинетические ограничения и перспективные стратегии доставки препаратов. Результаты лечения определяются не только статусом **BRAF V600E**, но и тканевым происхождением опухоли, сопутствующими геномными изменениями, механизмами обратной регуляции рецепторных тирозинкиназ, сигнальными процессами в микроокружении опухоли и уровнем воздействия препарата непосредственно в очаге поражения. Резистентность развивается вследствие реактивации сигнального пути **MAPK**, обходной активации сигнального пути **PI3K/AKT**, клеточной пластичности, внутриопухолевой гетерогенности, защитного воздействия стромы и недостаточной фармакологической экспозиции. Эффективность дабрафениба дополнительно ограничивается зависимой от pH растворимостью, зависящим от приема пищи всасыванием, метаболизмом, опосредованным системой **CYP**, аутоиндукцией, лекарственными взаимодействиями, ограниченным проникновением в головной мозг и неоднородным распределением препарата в ткани опухоли.

Модификация твердой лекарственной формы, наноструктурированные лекарственные формы, таргетные нанопереносчики, локально-регионарное введение и согласованная доставка дабрафениба и траметиниба продемонстрировали перспективные доклинические результаты, однако доказательства их превосходства над стандартной пероральной терапией остаются ограниченными. Клинические данные, полученные при лечении меланомы, рака лёгкого, рака щитовидной железы, опухолей желчных путей, колоректального рака и опухолей центральной нервной системы, свидетельствуют о том, что **BRAF V600E** представляет собой важную терапевтическую мишень, однако его наличие само по себе недостаточно для прогнозирования эффективности лечения.

Таким образом, оптимизация терапии требует индивидуализированного подхода с учётом типа опухоли, объединяющего молекулярный отбор пациентов, профилирование механизмов резистентности и оценку уровня воздействия препарата непосредственно в опухолевом очаге.

Ключевые слова: *дабрафениб, BRAF V600E, солидная опухоль, лекарственная резистентность, нанодоставка лекарственных препаратов*

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MACROPHAGE MIGRATION INHIBITORY FACTOR: CHEMICAL STRUCTURE, FUNCTIONAL PROPERTIES AND ITS ROLE AS A CYTOKINE IN DISEASES

Aliyarbayova A. A.^{1*}, Hasanov İ.A.², Unal G.³, Cinar-Asa S.⁴

Abstract

Macrophage Migration Inhibitory Factor (MIF) is a multifunctional cytokine that plays a pivotal role in immune regulation, inflammation and disease pathogenesis. Unlike classical cytokines, MIF exhibits unique structural and biochemical properties, including enzymatic activity and non-classical secretion, enabling rapid immune responses. This review aims to provide a detailed overview of the molecular structure, biosynthesis, signaling pathways, functional properties and pathophysiological roles of MIF, with a focus on its therapeutic potential. A narrative synthesis of current literature conducted integrating findings on MIF structure, receptor-mediated signaling, immune functions and involvement in various diseases, as well as also analyzed recent advances in therapeutic targeting and biomarker applications. MIF regulates key signaling pathways, including MAPK/ERK, PI3K/Akt, and NF-κB, influencing inflammation, cell survival and immune responses. It plays critical roles in physiological processes such as host defense, tissue repair and immune homeostasis. Dysregulation of MIF contributes to inflammatory, autoimmune, metabolic, cardiovascular, infectious diseases and cancer. Emerging therapies targeting MIF, including small molecule inhibitors and monoclonal antibodies, demonstrate promising clinical potential. MIF serves as a central mediator linking immune regulation with disease progression. Despite its therapeutic promise, challenges related to its multifunctionality and complex signaling networks remain. Future research should focus on targeted interventions and precision medicine approaches to optimize MIF-based therapies.

Keywords: *Macrophage migration inhibitory factor, cytokine signaling, inflammation, immune regulation, therapeutic targeting*

INTRODUCTION

Cytokines are an eclectic family of miniature signal proteins that are central to the regulation of immune actions, as well as to physiological homeostasis. They are engaged in numerous biological processes, such as cell proliferation, differentiation,

inflammation and apoptosis. The cytokines are significant in the process of interaction between immune or non-immune cells in order to synchronize the reply to infection, harm and stress. However, the asymmetry in the generation or release of cytokines significantly attributed to the development



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of other pathological disorders, including chronic inflammatory diseases, cardiovascular issues and cancer [1]. Because of the intricacy of the cytokine networks and their pleiotropicity, cytokines are the focus of both the health and the diseases and accumulating evidence is linking the existence of circulating inflammatory mediators to the risk and progression of cancers [2].

Macrophages are one of the greatest providers of cytokines in the body and they are crucial to the immune system control. These are very plastic cells that respond to the environmental cues and can take different functional phenotypes depending on the physiological or pathological circumstances. Macrophages are involved in the host defense, repair process and homeostasis of inflammatory processes in tissues. They can coordinate the recruitment or activation of immune cells due to cytokines and chemokine. One of the major pathogenesis of such chronic respiratory illnesses as chronic obstructive pulmonary disease is a macrophage functional alteration [3]. Furthermore, such chemokine as CXCL8 and CXCL12 show the complexity of the cytokine signaling pathways as they regulate the circulatory leukocytes and define the pathogenesis of some diseases [4].

Among the cytokines that initially identified is the so-called Macrophage Migration Inhibitory Factor (MIF) that holds a niche in the field of immunology. In 1960, MIF was initially defined as the soluble factor that was released by activated T lymphocytes and inhibited the macrophage migration, initially associated with delayed-type hypersensitivity reactions [5, 6]. Over time, it has been determined that MIF is developed by tremendous masses of cells incorporating macrophages, T cells, epithelial cells and endocrine cells. Compared to a number of classical cytokines, MIF constitutively expressed, accumulated within cells, extremely rapidly released in response to stress or inflammatory stimuli. MIF has structural and functional differences with the majority of other cytokines. It is also multifunctional because it possesses an enzymatic activity along with cytokine-like activities [7]. MIF can regulate immune response by binding to such receptors as CD74 receptor and chemokine receptor that consequently causes vital intracellular signaling pathways to become active. The pathways participate in the control of inflammation, cell division and survival, therefore, the primary role of MIF in the immune system regulation. Moreover, MIF is the only one that has the capacity to prevent glucocorticoids' immunosuppressive effects while maintaining inflammatory responses even on the condition of regulation [8].

The biological significance of MIF also supported by the fact that MIF plays a role in numerous diseases. MIF levels observed to be high in inflammatory and autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus, where it identified in the cause of chronic inflammation and tissue destruction [9]. Additionally, macrophages mediate

Yazışma üçün əlaqə:

Aliyarbayova A. A.^{1*}, Hasanov İ.A.², Unal G.³, Cinar-Asa S.⁴

^{1*} Azerbaijan Medical University, Department of Cytology, Embryology and Histology, Baku, Azerbaijan

² "Ozone" medical Centre, Ganja, Azerbaijan

³ Erciyes University, Department of Pharmacology, Kayseri, Türkiye

⁴ Bursa Uludag University, Department of Biology, Bursa, Türkiye

*For correspondence E-mail: aeliyarbeyova@amu.edu.az

inflammatory processes that are significant in cardiovascular diseases and cytokine-mediated signaling pathways influence the emergence of the disease and therapeutic target treatment [10]. Because of its multifunctional character and its fundamental position in the pathogenesis of diseases, MIF has become a possible treatment target. Additional research that is underway is also trying to explain its molecular structure, signaling cascades and interactions with other cytokines in order to develop certain therapeutic interventions. The review at hand attempts to provide a better description of the MIF, its chemical construction, and functionalities and how participated as a cytokine in disease. One of the purposes of the review is to introduce the importance of MIF in physiological and pathological processes, how translated into the synthesis of available information regarding the molecular character and the biological activity of this substance and determining the way of research in the future.

The Macrophage Migration Inhibitory Factor (MIF) is a structurally conserved cytokine that has unique biochemical and molecular features that distinguish it among classical cytokines. It is a gene that is found as chromosomes 22 and manifests in many immunological and non-immune cells. MIF is a small protein that is enzymatically active and has a distinctive trimeric structure, which allows to interact with receptors and to transmit a signal. The secretion of it is not classical and multifunctional making it so important in immune regulation, inflammation, and pathogenesis of diseases.

The gene MIF, or macrophage migration inhibitory factor, is on the chromosome 22 (22q11.2) which expressed constitutively in a broad immune and non-immune cells. It is quickly induced in reaction to inflammatory

and stress-related conditions, which emphasizes its role in immunological processes and pathogenesis of diseases. MIF is a low-sized protein made up of 114 amino acids and its molecular mass is about 12.5kDa [7]. It is interesting to note that it does not have a classic signal peptide and it is released through a non-classical pathway. MIF strongly conserved in all the species, which means that it plays an essential role in evolution in terms of immune regulation and cellular communication [11]. The crystallographic studies have been used to elucidate the three-dimensional configuration of MIF, which is a homotrimeric structure composed of three different subunits. These three-fold structures create a central channel, which is very vital in its biological action and in relations with receptors. The structural organization facilitates the work of MIF in immune signaling and inflammatory mechanisms. The monomers are bound at the interface and the catalytic sites are found with Proline-1 (Pro-1) being one of the active residues. These regions of the active site promote both the enzymatic activity and functional versatility of the protein and facilitate its role in immune regulation and disease processes [12].

MIF has unique biochemical properties, such as tautomerase enzyme activity, which unlike most classic cytokines distinguishes it. There is still no clear understanding of the nature of the physiological contribution of this activity, but it presumed that it has a role to play in its pro-inflammatory activities. Moreover, MIF has an effect on the macrophage activation and polarization, as a result on the immune responses and the inflammation. The multifunctionality of its enzymatic and cytokine-like characteristics complement each other. These biochemical characteristics also support

the dual nature of MIF as a signaling molecule and an enzyme, which is important to emphasize its importance in the immune modulation and pathophysiology [13].

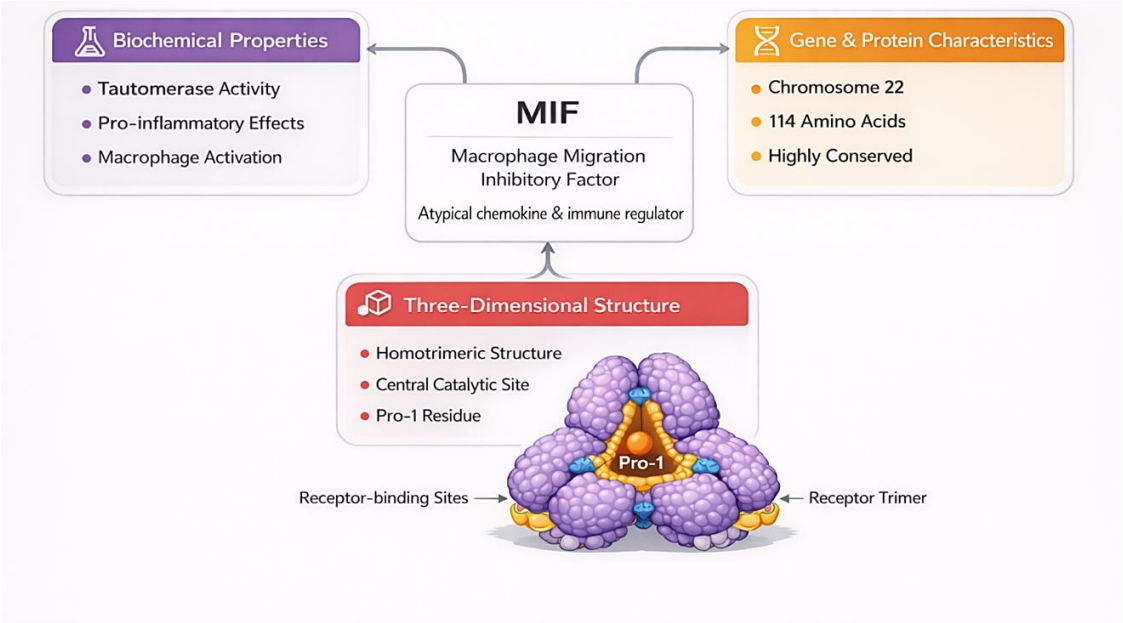


Figure 1. Schematic diagram shown the features of MIF.

Figure 1 illustrates the main structural and biochemical properties of MIF such as its gene and protein properties, three-dimensional structure that is homotrimers and its enzymatic properties. It emphasizes the preserved amino acid structure, catalytic positions like Pro-1 and its involvement in the pro-inflammatory and macrophage activation effects as a non-standard chemokine and immune regulator. Produced by a large diversity of immune cells and MIF are likewise tightly controlled at the transcriptional or post-transcriptional levels in non-immune cells. In contrast to classical cytokines, MIF is not stored in

vacuoles of the cell walls, but is released via a non-classical secretion pathway (in response to infection, hypoxia, and inflammation). It is released rapidly and is thus able to induce early immune signaling. The role of MIF production and secretion in inflammatory and immune-mediated conditions, as well as dysregulation in functions of play a key role in immune homeostasis and pathophysiology. MIF secretion and regulation is a closely regulated process that requires a number of cellular sources and stimuli as illustrated in Table 1.

Table 1: Molecular Mechanisms Governing MIF Biosynthesis and Secretion

Biological Process	Description	Reference
Cellular Sources	Immune and non-immune cells; predominantly macrophages	[14]

Regulation	Transcriptional and post-transcriptional control by inflammatory mediators	[15]
Secretion	Non-classical release from intracellular stores	[16]
Stimuli	Infection, hypoxia, oxidative stress, inflammation	[17]
Role	Cytokine production and leukocyte recruitment	[18]

The major processes involved in the MIF biosynthesis, control, and secretion summarized in Table 1, which briefly presents its cellular location, regulatory and release pathways. These processes further discussed in the following subsections and illustrated how they contributed to immune regulation and pathogenesis of diseases.

Macrophage Migration Inhibitory Factor (MIF) is a ubiquitous expression in both macrophages, T lymphocytes, dendritic cells, epithelial cells, and other immunological, as well as non-immune cells of endocrine tissues. Among them, the most important source is macrophages since the latter are at the center of immune surveillance and inflammatory processes. The wide cellular localization of MIF also indicates the significance of MIF in the coordination of innate and adaptive immunity. The rapid mobilization of immune challenges is made possible by its constitutive expression which enables it to respond quickly to stress and infection. The prevalence of this indicates the role of MIF in physiological control as well as the pathogenesis of diseases, especially those involving inflammatory and immune-mediated mechanisms [14].

MIF both transcriptional and post-transcriptional levels of expression are tightly controlled stage so maintained an equal immune response. Other cytokines and especially interleukin family members

are also important in regulating the expression of MIF gene using complicated signaling pathways. MIF is improved quickly by inflammatory pathways, such as those induced in infection or tissue damage. This control mechanism enables immune cells to react effectively to external stimuli and at the same time achieve homeostasis. Nonetheless, the disorders in the regulation of these pathways may lead to overproduction of MIF that causes persistent inflammation and the emergence of the disease. Therefore, it is crucial to regulate MIF expression to avoid excessive or insufficient immune responses and avoid pathological consequences [15].

In contrast to the classical cytokines, MIF released through a non-classical pathway, which circumvents the endoplasmic reticulum- Golgi apparatus. Rather it is accumulated in ready intracellular pools and is released immediately in response to stimulation. This special secretion system allows MIF to perform the role of an early communicator in immune responses allowing instant communication between cells. There is also the lack of a signal peptide which makes MIF unlike classical secretory proteins. Quick the activation of inflammatory pathways and immune cell responses can be achieved by rapid release of MIF. The non-conventional approach of secretion also increases its utility in the functional sense and

emphasizes its significance as a key regulator of acute and chronic inflammatory states [16].

The release of MIF provoked by numerous stimuli, among which mentioned microbial products, hypoxia, oxidative stress and inflammatory signals. These stimuli cause immune cell activation and the massive release of MIF into the extracellular space. Also, chemokine binding and receptor-mediated signaling pathways play a role in controlling MIF release, which is indicative of its incorporation into larger immune networks. The implication of cytokines and chemokine interaction on the coordination of immune responses emphasized by the role of chemokine systems. This release process, which triggered by the presence of stimuli, makes sure that MIF released during severe immune ones, which allows a proper response to pathogens and affects the process of inflammation [17].

MIF secretion is one of the essential factors in the etiology of several inflammatory and immune mediated illnesses. As an upstream regulator, MIF increases inflammatory cascades through stimulation of production of cytokines and recruitment of leukocytes. It is an important mediator of the disease progression due to its capacity to connect cellular stress signals with immune activation. High levels of MIF are linked with chronic inflammatory diseases, in which recurrent stimulation of immune systems results in the destruction of tissue. MIF has therefore attracted interest as an attractive therapeutic target. It is necessary to comprehend its dynamics of secretion and regulation in order to create specific interventions that would allow regulating excessive inflammation and achieve better clinical outcomes [18].

Receptors and Signaling Pathways. Macrophage Migration Inhibitory Factor (MIF) has its living actions by binding to

certain cell surface receptors, usually the CD74 receptor; commonly conjugated with other receptors like CD44 and chemokine receptors. These interactions promote various intracellular signaling pathways such as MAPK/ERK, PI3K/Akt and NF- κ B that control cell survival, cell proliferation and inflammatory response. MIF alters immune cell activation, cytokine production and gene expression via these signaling pathways and is a key mediator of physiological regulation, as well as of disease development [19]. MIF has biological actions that counteract through engagement with distinct cell surface receptors, predominantly CD74, which has the high affinity binding receptor. CD74 also has a tendency to bind with co-receptors such as CD44 to mediate signal transduction and the resultant cellular responses. In addition, MIF also interacts with chemokine receptors and can be an uncharacteristic chemokine, including CXCR2, CXCR4, and CXCR7, which mediate the formation of immune cells and inflammatory processes [20]. MIF and its receptors have a relationship that is crucial to the immune system regulation and pathogenesis. On the one hand, these receptor complexes activate several signaling pathways that influence the macrophage behavior including migration, activation and polarization. The MIF role in leukocyte traffic and inflammatory response enhanced by the activity of chemokine receptors; ties the interaction of receptors with immune control processes to a larger scale that observed in autoimmune and inflammatory diseases [21]. The binding of MIF triggers a number of intracellular signaling pathways in response to its receptors, including the MAPK / ERK cascade that regulates cell proliferation, differentiation and survival. The pathway plays a vital role in the mediating process of

inflammatory responses and immune cell activation.

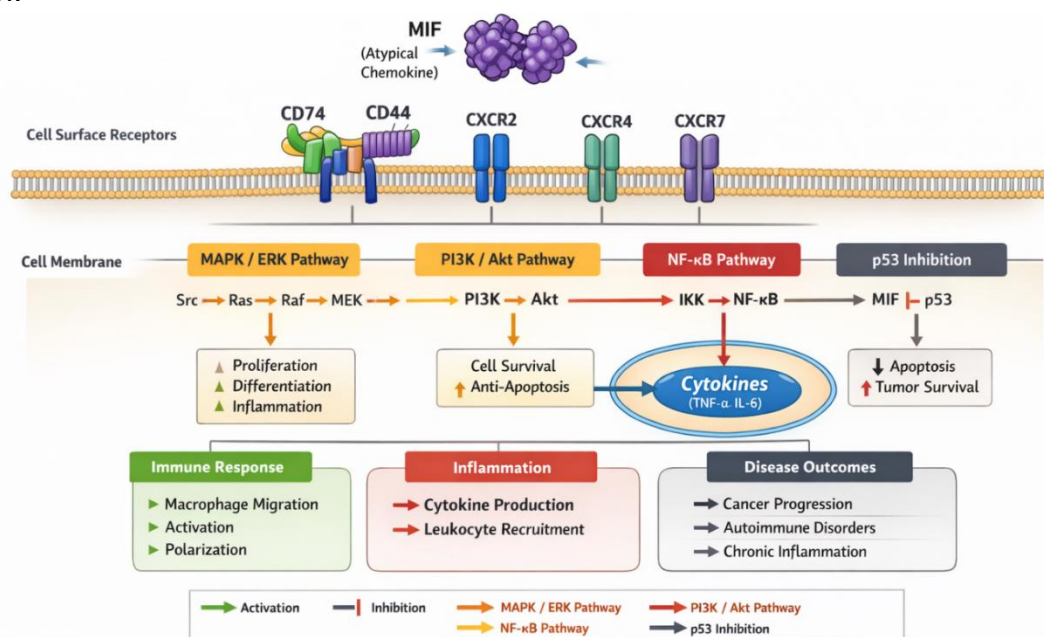


Figure 2: Schematic Diagram Shown Interaction of Receptor-Mediated Signaling Network of MIF

As shown in Figure 2, MIF can interact with cell surface receptors, such as CD74, CD44 and chemokine receptors (CXCR2, CXCR4, CXCR7), triggering the activation of MAPK/ERK, PI3K/Akt, and NF-κB pathways. All these signaling cascades control production of cytokines, cell survival and immune response and eventually leading to inflammation, macrophage activation and disease progression. The amplification of cytokine production and the overall enhancement of the immune process are signaling facilitated by MIF, which occurs in case of illnesses [22]. MIF also initiates PI3K/Akt pathway that enhances cell survival and resistance to apoptosis. It also leads to the activation of NF-κB pathway that is a significant regulator of inflammatory expression of genes. It reported that MIF interacts with the tumor suppressor protein - p53 and reduces its functions and, therefore, results in cell survival and proliferation under stressful conditions [23]. The clinical implication of the MIF involvement in these

signaling pathways is of significance particularly in cancer and inflammatory diseases. MIF is an emerging possible therapeutic target. These are MIF-neutralizing antibodies, the presence of which in clinical studies has demonstrated the possibility to control the progression of the disease and improve the therapeutic outcomes of patients with advanced malignancies [24].

MIF's function in innate immunity is it regulates the activation of macrophages the phagocytic function and the cytokine generation process. It enhances the reaction of the innate immune cells to respond rapidly to the pathogens and stress signals. Furthermore, MIF influences the macrophage polarization to a pro-inflammatory one that needed to protect the host but may eventually result in the emergence of the disease in case of a chronic disease [25]. MIF in adaptive immunity controls the T-cell activation, proliferation and differentiation thereby determining antigen specific immune

response. It aids in the preservation of the immune activation through the regulation of cytokines production and the lymphocytes recruitment. MIF also plays a role in preserving immune memory as well as augmenting of the inflammation signaling. The regulatory issues of such processes also can result in the emergence of chronic inflammatory diseases and immune-mediated pathology [26].

Macrophage Migration Inhibitory Factor (MIF) has a wide range of functional ability that spans over immunological, inflammatory, hormonal and enzymatic functions. It has a crucial part in the control of innate and adaptive immune responses, in the regulation of macrophage activity, T-cell functions and cytokine production. MIF is also a strong pro-inflammatory mediator, which increases immune signaling and leukocyte recruitment. It also overcomes immunosuppression caused by glucocorticoids, and it is enzymatic which demonstrates that it is multifunctional in

keeping the immune balance and is a part of disease pathogenesis [8].

MIF is a powerful pro-inflammatory cytokine that regulates other inflammatory mediator production and release. It enhances the manufacture of cytokine such as TNF- α , IL-1, and IL-6 that enhances the inflammatory processes. MIF is an upstream mediator of the inflammatory process, which is part of the disease process of the condition showing hyperirritable immune reactions [27]. MIF also plays a role in the process of cell recruitment of the leukocytes because it recognizes with the chemokine receptors, and helps in the movement of the cells into the inflammatory areas. It elevates the adhesion molecule expression and there is trafficking of immune cells. These functions are fundamental to effective immune responses but at excessively elevated leukocyte recruitment, tissue destruction and chronic inflammation ensues particularly in autoimmune disease and other degenerative diseases [28].

Table 2: Functional Roles of MIF in Immune and Inflammatory Processes

Category	Function	Role	Description	Reference
Immunological	Innate immunity	Macrophage activation	Enhances phagocytosis and cytokine production	[25]
Immunological	Adaptive immunity	T-cell regulation	Controls activation, proliferation, and immune memory	[26]
Pro-inflammatory	Cytokine signaling	Amplification	Upregulates TNF- α , IL-1 β , IL-6	[27]
Pro-inflammatory	Leukocyte recruitment	Migration	Promotes adhesion and immune cell trafficking	[28]
Hormonal/Enzymatic	Dual activity	Immune modulation	Counteracts glucocorticoids; enzymatic functions	[29]

Table 2 outlines the key functional activities of MIF in immunological, pro-inflammatory and enzymatic fields. This table gives a brief idea about its various biological activities, and the further subsections below discuss these functions in detail with

highlighting their importance in immune regulation and diseases progression.

MIF represents certain peculiar hormonal actions since it counter-regulated by the immunosuppressive effect of glucocorticoids. In spite of the fact that

glucocorticoids should prevent inflammation, MIF is dominant and it continues to keep the immune system active despite control. This aspect renders MIF one of the distinctive cytokines and highlights its contribution to maintaining inflammatory reactions, especially in the stressful circumstances and chronic diseases [29]. Enzymatic processes are also present in MIF including the tautomerase process and oxidoreductase process, hence, this protein can be used as a multifunctional protein in cell processes (Table 2). Although the exact physiological functions of these enzymatic properties are under current investigation, it is believed that they influence inflammatory signaling as well as the cell metabolism. These biochemical features also reiterate the duality of MIF as a cytokine and an enzyme in the body in terms of immunological regulation.

Macrophage Migration Inhibitory Factor (MIF) is a critical factor in host defense in order to orchestrate the action of innate responses as well as to increase the activity of immune cells, especially macrophages. It helps in quick immune reactions to pathogens and stress systems thus making sure that there are strong defense mechanisms. MIF is also involved in fine-tuning of inflammatory processes, ensuring the balance in effective immune response and avoiding the over damage of tissue. MIF mediates the interactions between immune surveillance and controlled inflammation by coordinating the production of cytokines and activation of

immune cells, which helps to maintain physiological stability and immune homeostasis in the normal condition [30].

The Macrophage Migration Inhibitory Factor (MIF) is also very important in many processes that occur in the body as it controls immune reactions, cell survival and tissue stability (Figure 3). It is useful in host defense as it increases innate immunity and suppresses inflammation. MIF promotes cell survival and proliferation by activating major signaling pathways as well. Also, it participates in tissue repair and regeneration by regulating the activity of immune cells and the production of cytokines. The significance of its involvement in the macrophage polarization and immune balance makes it significant in the sustenance of normal physiological functions.

MIF has a considerable impact on cell proliferation and survival, activating cell survival promoting and apoptosis inhibitory signaling pathways. These mechanisms are vital towards the maintenance of the role of immune and vascular cells, especially during physiological stress. MIF controls smooth muscle cell behavior in vascular tissues and helps to remodel the structure, which is essential in ensuring the integrity of the vessels. With the help of these ways, MIF helps adaptive cells responses and secures the functionality of the tissues. The importance of it in the control of survival pathways makes it relevant in maintaining physiological homeostasis and cellular defense against the damage caused by stress [31].

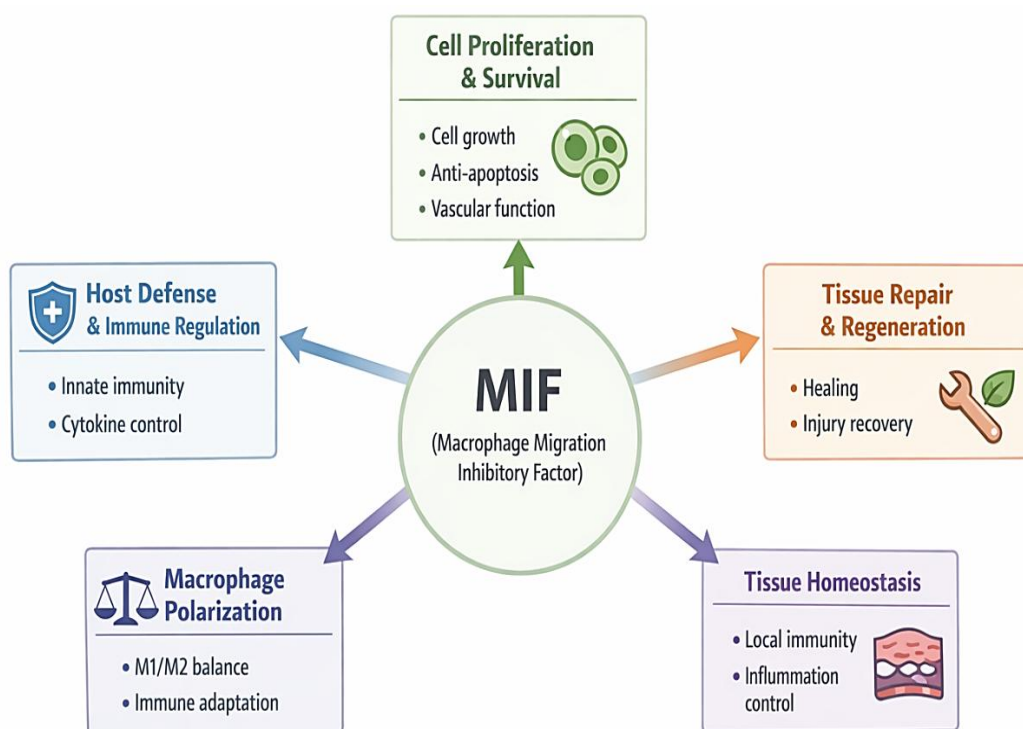


Figure 3: Schematic diagram shown the Role of MIF in Physiological Processes

Figure 3 illustrates that the MIF controls the major physiological processes such as host defense, immune control, cell growth and survival. It helps to repair tissue, sustain vascular activity as well as polarize macrophages and keep immune balance. With such coordinated efforts, MIF is at the center of tissue homeostasis and the adaptation to physiological stress. The MIF plays other roles, which explained further in the subsection.

MIF is a protective molecule that regulates the inflammatory reaction and cells regeneration after injury through repairing tissues. It improves the healing by regulating the production of cytokine and functions of immune cells, hence reduces tissue damage. Under acute injury to the kidney, MIF has shown protective properties in inhibiting cell injury and promoting regeneration. The functions show how it promotes tissue resilience and tissue recovery. MIF promotes physiological healing and repair processes by balancing the elements of inflammation

and repair, as well as normal tissue architecture restoration [32].

MIF is a major mediator of macrophage polarization, which affects the pro-inflammatory (M1) to anti-inflammatory (M2) polarization. This homeostatic equilibrium is necessary in keeping the immune homeostasis and repairing tissues. MIF controls the macrophage plasticity to make sure that there are proper responses to the immune system, whether it is inflammatory or recovery. This is because the immune system is capable of adjusting to altering physiological states through its regulatory capability to provide defense and regenerate. This control over macrophage activity highlights the need to use MIF in balancing between immune activation and inflammatory repair [33].

Besides its systemic processes, MIF is also important in ensuring homeostasis of tissues in specialized microenvironment like periodontal tissues. It controls immune signaling and cellular communications that are needed to maintain tissue integrity. MIF

provides normal tissue functionality and stability of the architecture by regulating localized inflammatory reactions and macrophage dynamics. These control mechanisms help to sustain protective immunity and avoid over-damage of inflammatory lesions. MIF, therefore, helps to ensure physiological balance, both systemic and local, and it is important to emphasize the importance of MIF in preserving normal tissue architecture and immune equilibrium [34].

Macrophage Migration inhibitory factor (MIF) is an important factor in the pathogenesis of a broad group of diseases, as it regulates immune and inflammatory reactions. It leads to the inflammatory and autoimmune diseases via the long-term effect of the production of cytokines and the activation of immunity [8].

MIF enhances the development of tumors, angiogenesis, and immune evasion in cancer. It also participates in metabolic,

cardiovascular and infectious diseases, balancing inflammation and cellular reactions and it is important to note that it is also a major founder of disease progression and a potential therapeutic goal.

The MIF is an essential factor in the pathogenesis of inflammatory and autoimmune diseases that control the production of cytokines and the activity of immune cells. It is an upstream mediator, which increases inflammatory reactions resulting in tissue destruction in rheumatoid arthritis, sepsis or inflammatory bowel disease. MIF promotes chronic inflammation and cytokine signaling sustainment in autoimmune diseases, such as systemic lupus erythematosus and multiple sclerosis. This cumulative inflammatory and autoimmune pathophysiology leads to tissue damage and severity of disease, which shows the key role of MIF in the process of these mechanisms [35].

Table 3: Pathophysiological Roles of MIF across Major Disease Categories

Category	Condition	Role	Description	Reference
Inflammatory/Autoimmune	RA, SLE, IBD, MS	Cytokine activation	Drives chronic inflammation	[35]
Cancer	Tumors	Progression	Promotes growth and angiogenesis	[36]
Metabolic	Diabetes, obesity	Inflammation	Induces insulin resistance	[37]
Cardiovascular	Atherosclerosis, MI	Vascular effects	Promotes endothelial dysfunction	[37]
Infectious	Bacterial, viral	Immune response	Enhances defense, may cause damage	[38]

Table 3 summarizes the essential pathophysiological roles of MIF in different disease categories, and it will give the reader the overview of how it is involved in immune regulation and disease progression. Its particular contributions to individual disease conditions with underlying mechanisms and clinical

importance elaborated in the following subsections.

MIF is gaining acceptance as an important controller of cancer development and progression. It stimulates tumor growth, metastasis and angiogenesis by regulating tumor microenvironment and stimulating inflammatory signaling. MIF is also implicated in immune evasion that

modulates immune cell reactions and contributes to the survival of tumors. MIF affects the macrophage polarization to tumor-supportive phenotypes, which induces the further development of cancer through the activation of the pathways including STAT3. Such mechanisms lead to a higher level of tumor aggressiveness, vascularization, and resistance to treatment, which makes MIF one of the key factors of oncogenesis and a possible cancer treatment target [36].

MIF has a significant contribution to metabolic diseases like diabetes and obesity as it enhances chronic low-grade inflammation and insulin resistance. It promotes the metabolic imbalance through control of the inflammatory pathways that suppress the glucose metabolism and energy homeostasis. Further, MIF has a role in cardiovascular diseases, such as atherosclerosis and myocardial infarction, because it affects vascular inflammation and endothelial malfunction. Its involvement in the stimulation of immune cells connects the disturbances in metabolism to the cardiovascular complication. In such ways, MIF becomes a key mediator between metabolic and cardiovascular pathologies, which is important to note and a therapeutic target [37].

MIF is also a major innate immune regulator of host responses to bacteria, viruses and parasites in infectious disease. It also augments the macrophage activation and inflammatory signaling, which helps to clear the pathogen efficiently. MIF has a dual role and assists with the protective immunity, as well as, it may also contribute to the increase in the severity of the disease in case of overexpression. Excessive inflammation or tissue damage may occur with elevated levels of MIF especially in severe infections. In respiratory diseases,

MIF play a role in the dynamics of immune responses and disease pathogenesis in relation to the changes in macrophage phenotypes. These results underscore the complicated task assigned to MIF in the process of maintaining the equilibrium between host defense and inflammatory injury [38].

The concept of therapeutic targeting of the Macrophage Migration Inhibitory Factor (MIF) has become one of the promising approaches in the treatment of inflammatory, autoimmune and malignant diseases (Figure 4). Potential strategies like small molecule inhibitors, monoclonal antibodies and immune modulatory therapy targeted to inhibit the interactions between MIF-receptor or to inhibit its enzymatic activity. These treatments can decrease inflammation, modify the polarization of macrophages and prevent the development of the disease. Moreover, MIF can be regarded as a promising disease severity and treatment response biomarker, so it is a significant object of targeted and precision-focused therapy development.

Macrophage Migration Inhibitory Factor (MIF) has become a target of interest using small molecule inhibitors, as well as monoclonal antibodies to inhibit its migration in immune-associated and inflammatory diseases. The mechanism of action of these agents is preventing the interaction between MIF and receptors or inhibiting its enzymatic activity in order to decrease the process of inflammatory signaling and disease development. It is also important to note that targeting MIF can alter macrophage plasticity and immune cell responses, which suggest its curative potential in chronic inflammatory and regenerative disease. The creation of these inhibitors demonstrates an increasing interest in MIF as an immune dysregulation

mediator and a potential target of the therapeutic intervention [39].

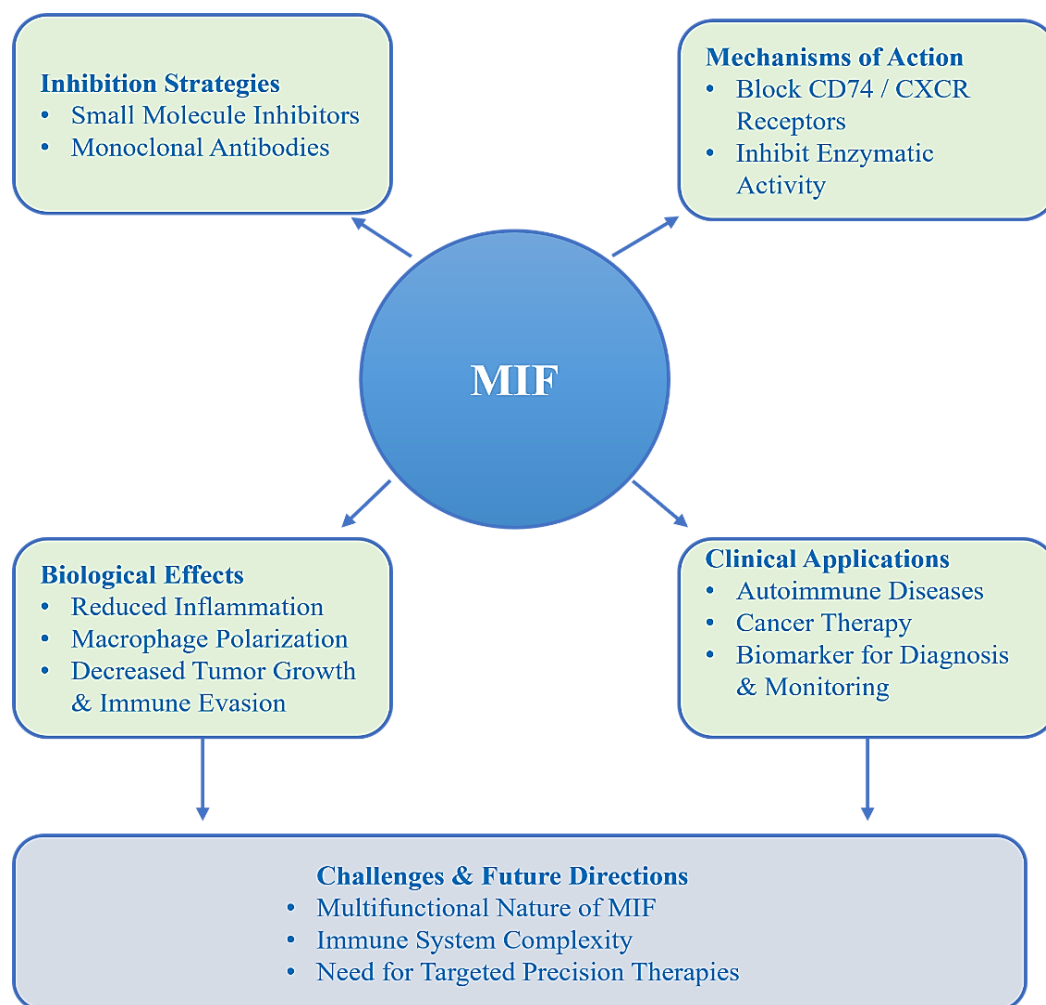


Figure 4: Therapeutic Targeting of MIF

Figure 4 shows the approaches to targeting the migration inhibitory factor (MIF) of macrophages and use of small molecule inhibitors, monoclonal antibodies. The ways inhibit CD74/CXCR receptors and enzymatic activity, which results in less inflammation, altered polarization of macrophages and lower tumor progression. It used clinically in the treatment of autoimmune diseases, cancer, and diagnostics, monitoring based on biomarkers and considered further in the following subsections.

MIF monoclonal antibodies found to have a great potential of neutralizing its biological activity especially in cancer and in autoimmune diseases. These treatments

expected to interfere with the signaling pathways mediated by MIF, which will lead to a decrease in tumor progression, immune evasion and chronic inflammation. Moreover, new cell-based therapies, including mesenchyme stem cell therapies can indirectly suppress the activity of MIF via immune modulatory actions. These modalities provide novel treatment opportunities because they act at several immune regulation levels and the opportunities of MIF-targeted therapies are further expanded [40].

MIF studied widely in clinical and experimental research as a target therapy and a biomarker. High amounts of MIF have been linked to severity of different

pathological diseases, implying that it would be useful in diagnosis and treatment follow up. Continued studies are being done to create more selective and effective MIF inhibitors which can be used in clinical practice. MIF-focused therapies used in combination with biomarker-based interventions to improve personalized treatment plans and increase patient outcomes in patients with inflammatory and immune-mediated diseases [41].

Although MIF has a therapeutic potential, a number of obstacles restrict the optimal targeting of the drug. The multifunctional character, prevalence and participation in protective, pathological mechanisms make it difficult to develop therapeutic strategies. Moreover, compensatory immune responses and cellular heterogeneity can also have an effect on treatment efficacy. The further study of the diversity of immune cells and the inflammatory signaling networks should be the key to the development of more specific and effective interventions. The future studies should aim at addressing these shortcomings to maximize on MIF-based therapies and to translate them into clinical practice [42].

Recent progress in Macrophage Migration Inhibitory Factor (MIF) studies has increased knowledge on the role of this molecule in the process of inflammation, immune compromises and disease development. Recent findings emphasize its role in cardiovascular pathology, remodeling of tumor microenvironment and polarization of macrophages, which strengthen the importance of this cytokine as a multifunctional one. According to the emerging therapeutic research, MIF can be used as biomarker or drug target in various ailments. Further studies ought to base on shedding more light on its dual protective and pathological functions, enhanced strategies of selective targeting and

translation of the experimental results to effective clinical applications.

Recent developments in the biological study of MIF have made it known to be a key mediator in inflammatory pathways and pathogenesis. Ample evidence is emerging that MIF plays a major function in the development of cardiovascular disease by regulating pro-inflammatory cytokines and immune cells, affecting vascular inflammation and remodeling. These results broaden the concept of MIF as a conventional cytokine, making it an important mediator of systemic diseases. Moreover, its role in various signaling pathways also highlights the significance of MIF in translational studies, in which the targeting MIF may expand new treatment opportunities that used to treat sophisticated inflammatory and cardiovascular diseases [43].

Recent researches have revealed that MIF is a key player in tumor microenvironment development because of its interaction with stromal and immune cells. MIF has been reported to be found in cancer-associated fibroblasts as one of the major contributors of immunosuppressive states that promote tumor growth and progression. MIF facilitates immune evasion, tumor cell growth and survival of tumor cells thus increasing cancer aggressiveness and resistance to treatment. The insights offer new directions to cancer treatment approaches, which focus on the possibility of interfering with tumor-supportive functions with the help of targeting MIF to enhance the efficacy of therapeutic approaches in oncology [44].

The future of MIF research aims at coming up with very specific therapeutic measures and its multifaceted role in the immune modulation. Innovations in the polarization of the macrophage have provided new opportunities in immunotherapy by further

connecting MIF functionality to immune cell differentiation and function. Nevertheless, several gaps in the research have not been filled, such as the necessity to provide specific targeting methods and a better comprehension of the dual functions of MIF in protective and pathological mechanisms. These issues will be required to apply the outcome of experiments to clinical practice and make the most of MIF-based treatment plans to various diseases [45].

CONCLUSION

Macrophage Migration Inhibitory Factor (MIF) has become a versatile cytokine that is essential for inflammation, immunological control and pathogenesis of diseases. Its distinctive structural characteristics, such as enzymatic activity, non-classical secretion those are what make it unlike other conventional cytokines, allow quick immune response. MIF combines several signaling pathways, including MAPK/ERK, PI3K/Akt, and NF- κ B, therefore, it controls cell survival, proliferation and inflammatory responses. Concisely, it is essential for mediating the relationship among innate and adaptive immunity, physiological and pathological effects through these mechanisms. Notably, MIF is the cause of a broad range of diseases, among which are inflammatory, autoimmune, metabolic, cardiovascular, infectious disorders and cancer. Its association with the disease progression is depicted by its position as an upstream regulator of cytokine generation and activation of immune cells. Meanwhile, MIF plays a role in both protective and pathological activities as it implicated in the physiological processes that are necessary to maintain host defense, tissue repair and immune homeostasis. MIF has therapeutic promise and there are still advances underway on small molecule inhibitors, monoclonal antibodies and immune modulatory approaches. Nevertheless,

problems associated with its multifunctionality, ubiquitous expression and intricate networks of signals continue to be major obstacles to clinical translation. Future studies are needed to enhance specificity of the target, context-dependent functions and incorporate MIF-based techniques in precision medicine. In sum, MIF is an extremely important bridge between immune control and pathology, which has much potential in diagnostic and therapeutic innovation.

REFERENCES

1. Amin MN, Siddiqui SA, Ibrahim M, et al. Inflammatory cytokines in the pathogenesis of cardiovascular disease and cancer. *SAGE Open Med.* (2020);8: 2050312120965752. doi:10.1177/2050312120965752
2. Bouras E, Karhunen V, Gill D, et al. Circulating inflammatory cytokines and risk of five cancers: a Mendelian randomization analysis. *BMC Med.* (2022); 20(1):3. doi:10.1186/s12916-021-02193-0
3. Akata K, van Eeden SF. Lung Macrophage Functional Properties in Chronic Obstructive Pulmonary Disease. *Int J Mol Sci.* (2020);21(3):853. doi:10.3390/ijms21030853
4. Cambier S, Gouwy M, Proost P. The chemokines CXCL8 and CXCL12: molecular and functional properties, role in disease and efforts towards pharmacological intervention. *Cell Mol Immunol.* (2023);20(3):217-251. doi:10.1038/s41423-023-00974-6
5. Bennett B, Bloom BR. Studies on the migration inhibitory factor associated with delayed-type hypersensitivity: cytodynamics and specificity. *Transplantation.* (1967);5(4):996-1000.

6. Bloom BR, Bennett B. Relation of the migration inhibitory factor (MIF) to delayed-type hypersensitivity reactions. *Ann N Y Acad Sci.* (1970);169(1):258-265. doi:10.1111/j.1749-6632.1970.tb55994.x
7. Aliyarbayova A, Sultanova T, Yaqubova S, Najafova T, Sadiqova G, Salimova A. Macrophage Migration Inhibitory Factor: Its Multifaceted Role in Inflammation and Immune Regulation Across Organ Systems. *Cell Physiol Biochem.* (2025);59(5):569-588. doi:10.33594/000000809
8. Aliyarbayova A., Hacıyeva Y., Pashayeva S., Qaniyeva G., Qarayeva S., Mekhtiyeva A. Macrophage Migration Inhibitory Factor and Pulmonary Immunity: a Systems Biology Perspective on Its Role in Lung Diseases *Cell Physiol Biochem* 60(2), (2026), pp.223-244. DOI: 10.33594/000000864
9. Bilsborrow JB, Doherty E, Tilstam PV, Bucala R. Macrophage migration inhibitory factor (MIF) as a therapeutic target for rheumatoid arthritis and systemic lupus erythematosus. *Expert Opin Ther Targets.* (2019);23(9):733-744. doi:10.1080/14728222.2019.1656718
10. Chen R, Zhang H, Tang B, et al. Macrophages in cardiovascular diseases: molecular mechanisms and therapeutic targets. *Signal Transduct Target Ther.* (2024);9(1):130. doi:10.1038/s41392-024-01840-1
11. Dinarello CA. The IL-1 family of cytokines and receptors in rheumatic diseases. *Nat Rev Rheumatol.* (2019);15(10):612-632. doi:10.1038/s41584-019-0277-8
12. Coperchini F, Chiovato L, Croce L, Magri F, Rotondi M. The cytokine storm in COVID-19: An overview of the involvement of the chemokine/chemokine-receptor system. *Cytokine Growth Factor Rev.* (2020);53:25-32. doi:10.1016/j.cytogfr.2020.05.003
13. Cutolo M, Campitiello R, Gotelli E, Soldano S. The Role of M1/M2 Macrophage Polarization in Rheumatoid Arthritis Synovitis. *Front Immunol.* (2022);13: 867260. doi:10.3389/fimmu.2022.867260
14. Hu C, Zhang Y, Liu J, You Y, Wu F, Zhou H. Functional Property and Regulatory Mechanism of Macrophages in Complementary and Alternative Medicine: From Bench to Clinic. *Iran J Immunol.* (2024);21(2):103-120. doi:10.22034/iji.2024.99943.2668
15. Jones SA, Jenkins BJ. Recent insights into targeting the IL-6 cytokine family in inflammatory diseases and cancer. *Nat Rev Immunol.* (2018);18(12):773-789. doi:10.1038/s41577-018-0066-7
16. Kang I, Bucala R. The immunobiology of MIF: function, genetics and prospects for precision medicine. *Nat Rev Rheumatol.* (2019);15(7):427-437. doi:10.1038/s41584-019-0238-2
17. Janssens R, Struyf S, Proost P. The unique structural and functional features of CXCL12. *Cell Mol Immunol.* (2018);15(4):299-311. doi:10.1038/cmi.2017.107
18. Kany S, Vollrath JT, Relja B. Cytokines in Inflammatory Disease. *Int J Mol Sci.* (2019);20 (23):6008. doi:10.3390/ijms20236008
19. Kapurniotu A, Gokce O, Bernhagen J. The Multitasking Potential of Alarmins and Atypical Chemokines. *Front Med (Lausanne).* (2019);6:3. doi:10.3389/fmed.2019.00003
20. Farr L, Ghosh S, Moonah S. Role of MIF Cytokine/CD74 Receptor Pathway in Protecting Against Injury and Promoting

- Repair. *Front Immunol.* 2020; 11:1273. doi:10.3389/fimmu.2020.01273
21. Ma WT, Gao F, Gu K, Chen DK. The Role of Monocytes and Macrophages in Autoimmune Diseases: A Comprehensive Review. *Front Immunol.* (2019); 10:1140. doi:10.3389/fimmu.2019.01140
 22. Meli VS, Veerasubramanian PK, Atcha H, Reitz Z, Downing TL, Liu WF. Biophysical regulation of macrophages in health and disease. *J Leukoc Biol.* (2019);106 (2):283-299. doi:10.1002/JLB.MR0318-126R
 23. Lang T, Lee JPW, Elgass K, et al. Macrophage migration inhibitory factor is required for NLRP3 inflammasome activation. *Nat Commun.* (2018);9(1):2223. doi:10.1038/s41467-018-04581-2
 24. Mahalingam D, Patel MR, Sachdev JC, et al. Phase I study of imalumab (BAX69), a fully human recombinant antioxidized macrophage migration inhibitory factor antibody in advanced solid tumours. *Br J Clin Pharmacol.* (2020); 86(9):1836-1848. doi:10.1111/bcp.14289
 25. Shapouri-Moghaddam A, Mohammadian S, Vazini H, et al. Macrophage plasticity, polarization, and function in health and disease. *J Cell Physiol.* (2018);233(9):6425-6440. doi:10.1002/jcp.26429
 26. Parisi L, Gini E, Baci D, et al. Macrophage Polarization in Chronic Inflammatory Diseases: Killers or Builders?. *J Immunol Res.* (2018):8917804. doi:10.1155/2018/8917804
 27. Nasiri E, Sankowski R, Dietrich H, et al. Key role of MIF-related neuroinflammation in neurodegeneration and cognitive impairment in Alzheimer's disease. *Mol Med.* (2020); 26(1):34. doi:10.1186/s10020-020-00163-5
 28. Park H, Kam TI, Peng H, et al. PAAN/MIF nuclease inhibition prevents neurodegeneration in Parkinson's disease. *Cell.* (2022); 185(11): pp.1943-1959. doi:10.1016/j.cell.2022.04.020
 29. Penticuff JC, Woolbright BL, Sielecki TM, Weir SJ, Taylor JA 3rd. MIF family proteins in genitourinary cancer: tumorigenic roles and therapeutic potential. *Nat Rev Urol.* (2019);16(5):318-328. doi:10.1038/s41585-019-0171-9
 30. Sinitski D, Kontos C, Krammer C, Asare Y, Kapurniotu A, Bernhagen J. Macrophage Migration Inhibitory Factor (MIF)-Based Therapeutic Concepts in Atherosclerosis and Inflammation. *Thromb Haemost.* (2019);119(4):553-566. doi:10.1055/s-0039-1677803
 31. Stenmark KR, Frid MG, Graham BB, Tudor RM. Dynamic and diverse changes in the functional properties of vascular smooth muscle cells in pulmonary hypertension. *Cardiovasc Res.* (2018);114(4):551-564. doi:10.1093/cvr/cvy004
 32. Stoppe C, Averdunk L, Goetzenich A, et al. The protective role of macrophage migration inhibitory factor in acute kidney injury after cardiac surgery. *Sci Transl Med.* (2018);10(441):eaan4886. doi:10.1126/scitranslmed.aan4886
 33. Strizova Z, Benesova I, Bartolini R, et al. M1/M2 macrophages and their overlaps - myth or reality?. *Clin Sci (Lond).* (2023);137(15):1067-1093. doi:10.1042/CS20220531
 34. Sun X, Gao J, Meng X, Lu X, Zhang L, Chen R. Polarized Macrophages in Periodontitis: Characteristics, Function, and Molecular Signaling. *Front*

- Immunol. (2021);12: 763334.
doi:10.3389/fimmu.2021.763334
35. Wautier JL, Wautier MP. Pro- and Anti-Inflammatory Prostaglandins and Cytokines in Humans: A Mini Review. *Int J Mol Sci.* (2023) ;24(11):9647. doi:10.3390/ijms24119647
36. Xia T, Zhang M, Lei W, et al. Advances in the role of STAT3 in macrophage polarization. *Front Immunol.* (2023); 14:1160719. doi:10.3389/fimmu.2023.1160719
37. Wang C, Zhou J, Wang J, et al. Progress in the mechanism and targeted drug therapy for COPD. *Signal Transduct Target Ther.* (2020);5(1):248. doi:10.1038/s41392-020-00345-x
38. Yamasaki K, Eeden SFV. Lung Macrophage Phenotypes and Functional Responses: Role in the Pathogenesis of COPD. *Int J Mol Sci.* (2018);19(2):582. doi:10.3390/ijms19020582
39. Yan L, Wang J, Cai X, et al. Macrophage plasticity: signaling pathways, tissue repair, and regeneration. *Med Comm* (2024);5(8):e658.. doi:10.1002/mco2.658
40. Yang G, Fan X, Liu Y, et al. Immunomodulatory Mechanisms and Therapeutic Potential of Mesenchymal Stem Cells. *Stem Cell Rev Rep.* (2023); 19(5): pp.1214-1231. doi:10.1007/s12015-023-10539-9
41. Yu JJ, Zhao Q, Li HN, Song JQ, Chen DC. MIFas a potential novel biomarker for cognitive function in patients with first episode schizophrenia. *Aust N Z J Psychiatry.* (2022); 56(3): 292-300. doi:10.1177/00048674211013086
42. Zhang F, Xia Y, Su J, et al. Neutrophil diversity and function in health and disease. *Signal Transduct Target Ther.* (2024);9(1):343. doi:10.1038/s41392-024-02049-y
43. Zhang H, Dhalla NS. The Role of Pro-Inflammatory Cytokines in the Pathogenesis of Cardiovascular Disease. *Int J Mol Sci.* (2024);25(2):1082. doi:10.3390/ijms25021082
44. Zhu GQ, Tang Z, Huang R, et al. CD36+ cancer-associated fibroblasts provide immunosuppressive microenvironment for hepatocellular carcinoma via secretion of macrophage migration inhibitory factor. *Cell Discov.* (2023);9(1):25. doi:10.1038/s41421-023-00529-z
45. Zhu W, Qiong D, Changzhi X, Meiyu J, Hui L. Macrophage polarization regulation shed lights on immunotherapy for CaOx kidney stone disease. *Biomed Pharmacother.* (2024);179:117336. doi:10.1016/j.biopha.2024.117336

MAKROFAQ MİQRASIYASINI İNHİBİSİYA EDƏN FAKTOR: KİMYƏVİ QURULUŞU, FUNKSIONAL XÜSUSİYYƏTLƏRİ VƏ XƏSTƏLİKLƏRDƏ SİTOKİN KİMİ ROLU

Əliyərbəyova A. Ə.^{1*}, Həsənov İ.Ə.², Ünal G.³, Çinar- Asa S.⁴

¹ Azərbaycan Tibb Universiteti, Sitologiya, embriologiya və histologiya kafedrası, Bakı, Azərbaycan

² "Ozon" Tibb Mərkəzi, Gəncə, Azərbaycan

³ Erciyes Universiteti, Farmakologiya kafedrası, Kayseri, Türkiyə

⁴ Bursa Uludağ Universiteti, Biologiya kafedrası, Bursa, Türkiyə

* Yazışma üçün əlaqə E-mail: aeliyarbeyova@amu.edu.az

Xülasə

Makrofaq Miqrasiyasını İnhibisiya edən Faktor (MIF) immun tənzimlənməsində, iltihabda və xəstəlik patogenezinə əsas rol oynayan çoxfunksiyalı sitokindir. Klassik sitokinlərdən fərqli olaraq, MIF, sürətli immun reaksiyalarına imkan verən fermentativ aktivlik və qeyri-klassik sekresiya daxil olmaqla unikal struktur və biokimyəvi xüsusiyyətlərə malikdir. Bu icmal, MIF-in molekulyar quruluşu, biosintezi, siqnal yolları, funksional xüsusiyyətləri və patofizioloji rolları haqqında ətraflı məlumat vermək məqsədi daşıyır və onun terapeutik potensialına diqqət yetirir. MIF kimyəvi quruluşu, reseptor vasitəçiliyi ilə siqnal, immun funksiyaları və müxtəlif xəstəliklərdə iştirakı ilə bağlı məlumatları birləşdirən mövcud ədəbiyyat araşdırılmış, həmçinin terapeutik hədəfləmə və biomarker tətbiqlərindəki son nailiyyətlər təhlil edilmişdir. MIF, MAPK/ERK, PI3K/Akt və NF-κB daxil olmaqla əsas siqnal yollarını tənzimləyir, iltihaba, hüceyrə fəaliyyətinə və immun reaksiyalara təsir göstərir. O, orqanizmin müdafiəsi, toxuma bərpası və immun homeostazı kimi fizioloji proseslərdə mühüm rol oynayır. MIF-in pozulmuş tənzimi iltihaba, autoimmun, metabolik, ürək-damar, yoluxucu xəstəliklər və xərçəngə səbəb olur. Kiçik molekulalı inhibitorlar və monoklonal antikorlar da daxil olmaqla, MIF-i hədəf alan yeni müalicə üsulları ümidverici klinik potensial nümayiş etdirir. MIF immun tənzimlənməni xəstəliyin irəliləməsi ilə əlaqələndirən mərkəzi vasitəçi rolunu oynayır. Terapeutik vədinə baxmayaraq, onun çoxfunksiyallığı və mürəkkəb siqnal şəbəkələri ilə bağlı çətinlikləri qalmaqdadır. Gələcək tədqiqatlar MIF əsaslı terapiyaları optimallaşdırmaq üçün hədəflənmiş müdaxilələrə və dəqiq tibb yanaşmalarına yönəlməlidir.

Açar sözlər: Makrofaq miqrasiyasını inhibisiya edən faktor, sitokin siqnalı, iltihab, immun tənzimlənmə, terapeutik hədəfləmə

ФАКТОР ИНГИБИРОВАНИЯ МИГРАЦИЮ МАКРОФАГОВ: ХИМИЧЕСКАЯ СТРУКТУРА, ФУНКЦИОНАЛЬНЫЕ СВОЙСТВА И ЕГО РОЛЬ В КАЧЕСТВЕ ЦИТОКИНА ПРИ ЗАБОЛЕВАНИЯХ.

Алиярбекова А. А.^{1*}, Гасанов И. А.², Унал Г.³, Чинар Аса С.⁴

¹ Азербайджанский медицинский университет, кафедра цитологии, эмбриологии и гистологии, Баку, Азербайджан

² Медицинский центр «Озон», Гянджа, Азербайджан

³ Университет Эрчиес, Кайсери, кафедра фармакологии, Кайсери, Турция

⁴ Университета Улудаг, Бурса, Турция, факультет биологии

* Для корреспонденции E-mail: aaliyarbeyova@amu.edu.az

Резюме

Фактор ингибирования миграции макрофагов (MIF) — это многофункциональный цитокин, играющий ключевую роль в регуляции иммунитета, воспалении и патогенезе заболеваний. В отличие от классических цитокинов, MIF обладает уникальными структурными и биохимическими свойствами, включая ферментативную активность и неклассическую секрецию, что позволяет быстро реагировать на иммунные реакции. Цель данного обзора — предоставить подробный обзор молекулярной структуры, биосинтеза, сигнальных путей, функциональных свойств и патофизиологической роли MIF, с акцентом на его терапевтический потенциал. Проведен нарративный синтез современной литературы, интегрирующий данные о структуре MIF, рецептор-опосредованной передаче сигналов, иммунных функциях и участии в различных заболеваниях, а также проанализированы последние достижения в области терапевтического воздействия и применения биомаркеров. MIF регулирует ключевые сигнальные пути, включая MAPK/ERK, PI3K/Akt и NF-κB, влияя на воспаление, выживаемость

клеток и иммунные реакции. Он играет критическую роль в физиологических процессах, таких как защита организма, восстановление тканей и иммунный гомеостаз. Нарушение регуляции MIF способствует развитию воспалительных, аутоиммунных, метаболических, сердечно-сосудистых, инфекционных заболеваний и рака. Новые методы лечения, направленные на MIF, включая ингибиторы малых молекул и моноклональные антитела, демонстрируют многообещающий клинический потенциал. MIF выступает в качестве центрального медиатора, связывающего иммунную регуляцию с прогрессированием заболевания. Несмотря на его терапевтический потенциал, остаются проблемы, связанные с его многофункциональностью и сложными сигнальными сетями. Будущие исследования должны быть сосредоточены на целенаправленных вмешательствах и подходах прецизионной медицины для оптимизации терапии на основе MIF.

Ключевые слова: *Ингибирующий фактор миграции макрофагов, цитокиновая сигнализация, воспаление, иммунная регуляция, терапевтическое воздействие*

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AÇIQ GİRİŞ (OPEN ACCESS)

DOI: <https://doi.org/10.28942/atuj.v6i3y2026.160>INBORN ERRORS OF IMMUNITY IN THE ERA OF GLOBALIZATION:
EVOLVING CONCEPTS AND CLINICAL IMPLICATIONSNasrullayeva G.M.^{1,2}, Mammadova V.R.^{1*}, Nazirzade M.M.¹, Mollayeva N.O.²**Abstract**

Globalization, migration, urbanization, and environmental changes exert a significant impact on human health, particularly on immune system function. These factors have contributed to evolving perspectives on congenital immunodeficiencies, now more accurately referred to as inborn errors of immunity (IEI). Previously considered rare and underdiagnosed, these disorders are currently recognized as a major global health concern.

Advances in molecular genetic technologies have enabled early and precise diagnosis of IEI. International collaboration, the development of global registries, and the standardization of clinical protocols have increased detection rates worldwide. Environmental influences also affect immune function through modulation of the microbiota and microbiome.

Neonatal screening using TREC/KREC assays is increasingly becoming standard practice in many countries for the early detection of severe forms of IEI, such as severe combined immunodeficiency (SCID). The concept of IEI reflects a paradigm shift, recognizing immune disorders as complex, dynamic conditions. The immune system is now viewed not only as a collection of cells and molecules but as an integrated ecosystem interacting with genetic and environmental factors.

Keywords: *Inborn Errors of Immunity, Primary Immunodeficiency, Globalization, Neonatal Screening, Microbiome*

INTRODUCTION

In recent decades, globalization has profoundly influenced medicine alongside

economic and communication systems. It has facilitated the discovery of previously unrecognized diseases and enabled the rapid global exchange of scientific knowledge regarding diagnosis and treatment. Enhancing the translation of scientific findings into clinical practice has become a key priority in modern healthcare [1,4].

Globalization, migration, urbanization, and environmental changes significantly affect immune system function. Increasing

Yazışma üçün əlaqə:

Nasrullayeva G.M.^{1,2}, Mammadova V.R.^{1*}, Nazirzade M.M.¹, Mollayeva N.O.²

1Azerbaijan Medical University, Scientific Research Laboratory of Immunology

2Azerbaijan Medical University, I children department, Baku, Azerbaijan

*For correspondence E-mail: vafaimmun26@gmail.com



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interactions across geographical, ethnic, and social boundaries have further shaped immune responses. These developments have led to a transformation in the understanding of congenital immunodeficiencies. Once considered rare and poorly characterized, these conditions are now recognized as a significant global health issue [2,3,8]. Historically, primary immunodeficiencies were frequently misdiagnosed or overlooked, with patients receiving only symptomatic treatment for recurrent infections. Advances in immunology and genetics have demonstrated that these disorders comprise a heterogeneous group of diseases with diverse clinical manifestations. Consequently, the term

inborn errors of immunity (IEI) has been adopted to reflect this complexity [2,7,8].

Changing Perspectives in a Globalizing World

Globalization has accelerated progress in medical technologies and genetic research. Modern molecular diagnostic methods allow early and accurate identification of IEI. International cooperation, global databases, and standardized clinical protocols have significantly increased detection rates. Although these conditions were previously under recognized, substantial progress has been achieved in recent years. Nevertheless, globalization presents both advantages and challenges (Figure 1) [1,3].

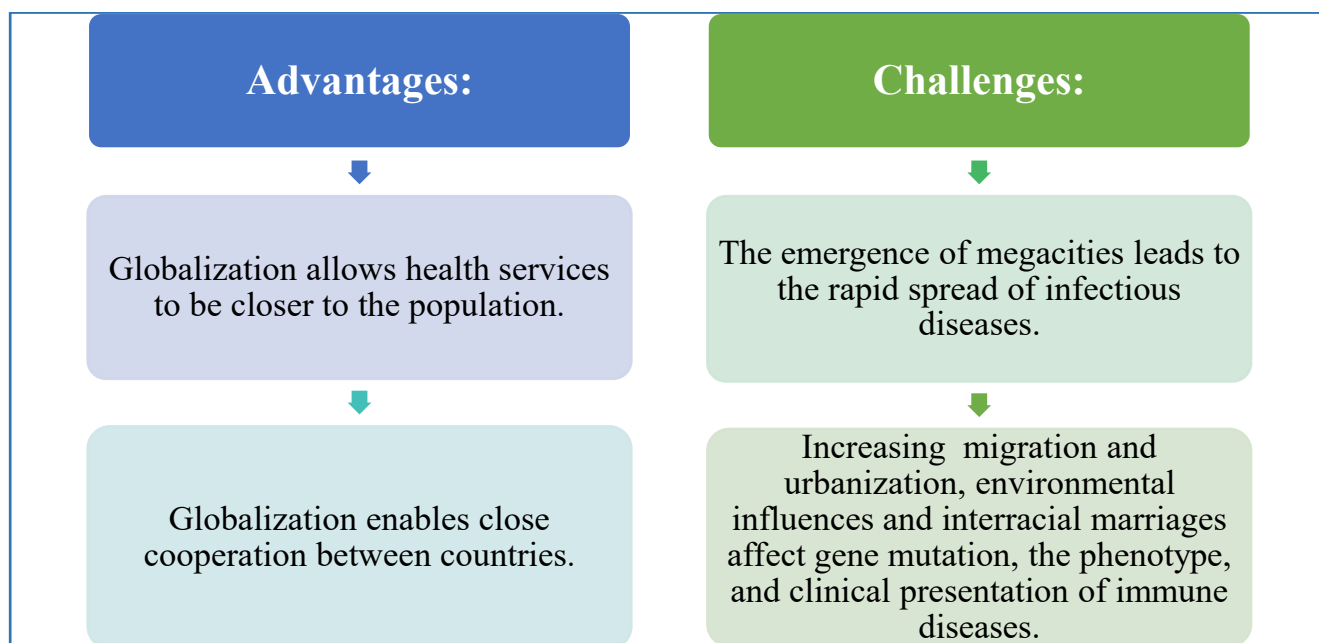


Figure 1. Advantages and challenges of globalization.

The foundation of immune function lies within the human genome. Epigenetic modifications regulate gene expression and are highly sensitive to environmental factors such as infections, microbiome composition, toxins, and stress. As a result, individuals with the same genetic mutation may exhibit markedly different phenotypes,

ranging from severe combined immunodeficiency (SCID) to mild antibody deficiencies. Conversely, similar clinical phenotypes may arise from different genetic mutations [5,12].

Key mechanisms underlying genetic variability include:

- Gene frequency

- Diversity of gene expression
- Gene–gene interactions
- Environmental influences [6,7]

Globalization, urbanization, environmental pollution, and changes in the microbial environment can lead to the following consequences: increased incidence of certain genetic diseases in the population, changes in the clinical symptoms of genetic diseases, increased risk of genetic predisposition, and the spread of rare mutations in consanguineous marriages. From the perspective of modern genetics and immunology, immunodeficiency diseases are of particular interest.

Previously, many children with severe primary immunodeficiencies died at an early age. Today, thanks to modern and effective treatments such as immunoglobulin therapy, bone marrow transplantation, and gene therapy, patients survive into adulthood and have children.

As a result, some rare mutations (CTLA4 deficiency, LRBA deficiency, APDS (PIK3CD), STAT3, and ADA deficiency) can persist and become more common in the population.

Environmental factors also influence immune function through their effects on the microbiota and microbiome. The microbiome comprises all microorganisms and their genetic material residing in and on the human body, forming a complex ecosystem that affects metabolism, immunity, and overall health. Its composition is highly individualized and influenced by diet, lifestyle, and environmental exposures.

The microbiota plays a critical role in immune system development, immune memory formation, and immune regulation. Under normal conditions, the immune system and microbiota maintain a balanced relationship that ensures protection against pathogens while preserving tolerance to

harmless antigens. However, factors such as excessive antibiotic use and dietary additives may disrupt this balance, leading to immune dysregulation.

The development of the immune system and the maintenance of homeostasis are just two of the many areas in which the microbiota is important. It is known that a healthy microbiota balance is important in the management of inflammatory responses by supporting immune tolerance. In this regard, first of all, the interactions between the microbiota and the immune system are investigated, and the influence of the microbiota on the immune system is studied in detail. Then, the molecular and cellular mechanisms of the microbiota balance in specific autoimmune diseases can be evaluated. In this regard, the interactions and clinical manifestations of these two mechanisms should be studied, and therapeutic approaches targeting the microbiota, such as prebiotics, probiotics, and fecal microbiota transplantation, may be prescribed [12].

Microbiome alterations in patients with IEI may result in dysbiosis, microbial translocation, mucosal immune dysfunction, chronic inflammation, and impaired immune regulation. These processes contribute to complications such as autoimmunity, lymph proliferation, and malignancy, significantly affecting patient outcomes and quality of life [9,10,12].

IEI in the Context of the COVID-19 Pandemic

The COVID-19 pandemic highlighted variability in immune responses across populations. Environmental factors such as urban density, pollution, and microbiome diversity may influence susceptibility and disease severity.

The pandemic also exposed vulnerabilities in healthcare systems, particularly affecting patients with IEI. Disruptions in plasma

donation negatively impacted the availability of immunoglobulin replacement therapy, a cornerstone of treatment. Patients with IEI are at increased risk of infections, and ongoing research explores genetic predisposition to severe COVID-19. Additionally, vaccine responses in these patients may be suboptimal due to impaired B- and T-cell immunity. Consequently, efforts have intensified to develop more effective and durable immunization strategies [5].

New Concepts in Inborn Errors of Immunity

Advances in molecular genetics have redefined the classification of immune disorders, leading to the adoption of the term *inborn errors of immunity (IEI)*. The immune system is now conceptualized as a dynamic and integrated ecosystem. The concept of the *immunome* has emerged, reflecting the interaction between genetic factors and environmental influences [3,4,6].

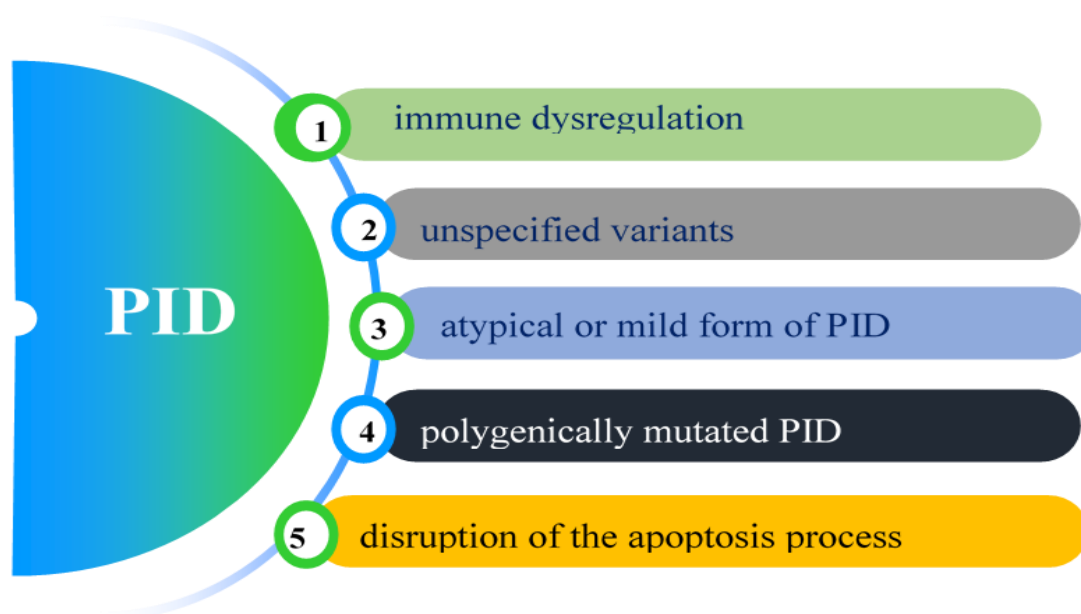


Figure 2. New forms of primary immunodeficiency

Next-generation sequencing (NGS) has identified more than 690 gene defects associated with IEI. Thus, there has been a shift the paradigm from isolated genetic defects to complex networks of immune dysregulation, from the importance of a single gene to the multiple interactions of genetic changes [1, 5, 12]. As a result, new forms of PID have emerged (Figure 2).

Newborn Screening Programs

Newborn screening programs are implemented in many countries and now it is part of routine healthcare, although the

range of screened conditions varies widely. Initially introduced for the detection of phenylketonuria, these programs now have expanded significantly over time [12, 13]. This program allows us to detect several serious hereditary diseases before clinical symptoms even appear. Also, it plays a critical role in preventing severe complications. This is particularly important for primary immunodeficiencies, where early diagnosis can be life-saving.

In 2022, this program in Azerbaijan is dedicated to the following diseases: phenylketonuria, cystic fibrosis, adrenogenital syndrome, hypothyroidism,

and galactosemia. In 2026-2027, the program plans to include testing for severe combined immunodeficiency (SCID) using a heel-prick blood test.

TREC/KREC-based screening is increasingly used for early detection of severe IEI, particularly SCID. TREC reflects newly formed T-lymphocytes. It is a small DNA fragment formed during T-cell development in the thymus and indicates the number of naive T cells. KREC reflects newly formed B cell.

SCID is characterized by: impaired T-cell development, reduced or absent B cells, dysfunction of natural killer (NK) cells

Early diagnosis enables timely hematopoietic stem cell transplantation (HSCT), significantly improving outcomes. This underscores the importance of early identification in reducing morbidity and mortality [1, 6].

Future Directions

- Integration of artificial intelligence in immunological diagnostics
- Development of global registries combining genomic and clinical data
- Strengthening multidisciplinary collaboration
- Expansion of telemedicine to improve access in low-resource settings [4,5]

CONCLUSION

A multidisciplinary approach is essential in the management of patients with IEI, involving immunologists, pediatricians, geneticists, infectious disease specialists, and other healthcare professionals.

Globalization has transformed the understanding and management of primary immunodeficiencies. Advances in diagnostics, treatment, and international collaboration have improved patient outcomes. Continued progress in this field will further enhance our understanding of immune system disorders and support the

development of innovative therapeutic strategies.

REFERENCES

1. Rivière JG, Cantenys-Saba R, Carot-Sans G, et al. Current perspectives and challenges of using artificial intelligence in immunodeficiencies. *J Allergy Clin Immunol.* 2025;156(4):878–888. doi:10.1016/j.jaci.2025.06.015
2. Reyes S, Condino-Neto A, Stepensky P. Global perspectives on primary immune deficiency diseases. In: *Stiehm's Immune Deficiencies.* 2020. p. 1129–1142. doi:10.1016/B978-0-12-816768-7.00054-5
3. Condino-Neto A, Sullivan K. The relevance of primary immunodeficiency registries on a global perspective. *J Allergy Clin Immunol.* 2021;148(5):1170–1171. doi:10.1016/j.jaci.2021.08.023
4. Abolhassani H, Azizi G, Sharifi L, et al. Global systematic review of primary immunodeficiency registries. *Expert Rev Clin Immunol.* 2020;16(7):717–732. doi:10.1080/1744666X.2020.1801422
5. Meyts I, Bousfiha A, Duff C, et al. Primary immunodeficiencies: a decade of progress and a promising future. *Front Immunol.* 2021;11:625753. doi:10.3389/fimmu.2020.625753
6. Poli C, Aksentijevich I, Bousfiha A, et al. Human inborn errors of immunity: 2024 update on the classification from the International Union of Immunological Societies Expert Committee. *J Hum Immun.* 2025;1(1):e20250003. doi:10.70962/jhi.20250003
7. Bousfiha A, Jeddane L, Moundir M, et al. The 2024 update of IUIS phenotypic classification of human inborn errors of immunity. *J Hum Immun.* 2025;1(1):e20250002. doi:10.70962/jhi.20250002

8. Vichare V, Kilich G, Cunningham-Rundles C, et al. Automated data extraction model for the USIDNET registry: bigger, faster, and better data collection. *J Hum Immun.* 2026;2(1):e20250118. doi:10.70962/jhi.20250118
9. Yurtseven B, Aydemir E, Ayaz F. The role of intestinal microbiota and immune system interactions in autoimmune diseases. *Immunotargets Ther.* 2025;14:1347–1372. doi:10.2147/ITT.S569016
10. Al-Nesf MA, Morgan D, Mohamed-Ali V. Primary immunodeficiency and the microbiome. *Curr Opin Pediatr.*

2021;33(6):633–638.

doi:10.1097/MOP.0000000000001067

11. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell.* 2014;157(1):121–141. doi:10.1016/j.cell.2014.03.011

12. Guliyeva LM, Hajiyeva NN. The role of genetic testing in optimizing screening in newborns. *Azerbaijan Journal of Perinatology and Pediatrics.* 2025;11(1). doi:10.28942/jpp.v11i1y2025.106

13. Ismayilova Sh. Neonatal screening: international experience. *Eurasian Journal of Clinical Sciences.* 2020;2(2):63–70. doi:10.28942/ejcs.v2i2.90

QLOBALLAŞMA DÖVRÜNDƏ İMMUNITETİN ANADANGƏLMƏ SƏHVLƏRİ: ELMI KONSEPSİYALARIN İNKİŞAFI VƏ KLİNİK ƏHƏMİYYƏTİ

Nasrullayeva G.M.^{1,2}, Məmmədova V.R.^{1*}, Nazirzadə M.M.¹, Mollayeva N.O.²

¹ Azərbaycan Tibb Universiteti, İmmunologiya Elmi-Tədqiqat Laboratoriyası

² Azərbaycan Tibb Universiteti, I Uşaq xəstəlikləri kafedrası, Bakı, Azərbaycan

* Yazışma üçün E-mail: vafaimmun26@gmail.com

Xülasə

Qloballaşma, miqrasiya, urbanizasiya və ətraf mühitdə baş verən dəyişikliklər insan sağlamlığına, xüsusilə immun sisteminin funksional vəziyyətinə əhəmiyyətli təsir göstərir. Bu amillərin təsiri nəticəsində anadangəlmə immun çatışmazlıqları haqqında elmi təsəvvürlər əhəmiyyətli dərəcədə dəyişmiş və hazırda bu xəstəliklər daha geniş anlayış olan *immunitetin anadangəlmə səhvləri* (Inborn Errors of Immunity – IEI) termini ilə ifadə olunur. Əvvəllər nadir və çox zaman gec diaqnostika olunan xəstəliklər kimi qiymətləndirilən bu patologiyalar hazırda qlobal səhiyyənin mühüm problemlərindən biri hesab edilir.

Molekulyar-genetik texnologiyaların sürətli inkişafı immunitetin anadangəlmə səhvlərinin erkən və dəqiq diaqnostikasına imkan yaratmışdır. Beynəlxalq əməkdaşlığın genişlənməsi, qlobal xəstə registrlərinin yaradılması və klinik protokolların standartlaşdırılması bütün dünyada bu xəstəliklərin aşkar edilmə səviyyəsinin yüksəlməsinə şərait yaratmışdır. Eyni zamanda, ətraf mühit amillərinin mikrobiota və mikrobiomun tərkib və funksiyasına təsiri immun sisteminin fəaliyyətinin formalaşmasında mühüm rol oynayır.

TREC/KREC analizlərindən istifadə etməklə neonatal skrining bir sıra ölkələrdə ağır immun çatışmazlığı formalarının, xüsusilə ağır kombine olunmuş immun çatışmazlığının (SCID) erkən aşkarlanması məqsədilə getdikcə daha geniş tətbiq olunur və standart diaqnostik yanaşmaya çevrilməkdədir.

İmmunitetin anadangəlmə səhvləri konsepsiyasının formalaşması immun pozğunluqlarına yanaşmada yeni paradigmanı əks etdirir. Bu konsepsiyaya əsasən, immun sisteminin xəstəlikləri sadəcə ayrı-ayrı immun hüceyrələrinin və ya molekulalarının funksional pozğunluğu kimi deyil, genetik və ətraf mühit

amilləri ilə qarşılıqlı əlaqədə olan mürəkkəb və dinamik proseslər kimi qiymətləndirilir. Müasir yanaşmada immun sistemi yalnız hüceyrə və molekulların məcmusu deyil, genetik xüsusiyyətlər və ətraf mühit amilləri ilə qarşılıqlı əlaqədə fəaliyyət göstərən inteqrasiya olunmuş bioloji ekosistem kimi nəzərdən keçirilir.

Açar sözlər: *immunitetin anadangəlmə səhvləri, birincili immun çatışmazlığı, qloballaşma, neonatal skrining, mikrobiom.*

ВРОЖДЕННЫЕ ОШИБКИ ИММУНИТЕТА В ЭПОХУ ГЛОБАЛИЗАЦИИ: ЭВОЛЮЦИЯ КОНЦЕПЦИЙ И КЛИНИЧЕСКИЕ АСПЕКТЫ

Насруллаева Г.М.^{1,2}, Мамедова В.Р.^{1}, Назирзаде М.М.¹, Моллаева Н.О.²*

¹ *Азербайджанский медицинский университет, Научно-исследовательская лаборатория иммунологии, Баку*

² *Азербайджанский медицинский университет, кафедра детских болезней I, Баку, Азербайджан*

** Для корреспонденции E-mail: vafaimmun26@gmail.com*

Резюме

Глобализация, миграция, урбанизация и изменения окружающей среды оказывают существенное влияние на здоровье человека, в частности на функционирование иммунной системы. Воздействие этих факторов обусловило изменение современных представлений о врожденных иммунодефицитах, которые в настоящее время более корректно обозначаются термином *врожденные ошибки иммунитета* (Inborn Errors of Immunity, IEI). Ранее рассматривавшиеся как редкие и часто недодиагностируемые заболевания, данные состояния в настоящее время признаны значимой глобальной проблемой здравоохранения.

Достижения в области молекулярно-генетических технологий обеспечили возможности для ранней и точной диагностики врожденных ошибок иммунитета. Международное сотрудничество, создание глобальных регистров и стандартизация клинических протоколов способствовали повышению выявляемости данных заболеваний во всем мире. Существенное влияние на функционирование иммунной системы оказывают также факторы окружающей среды, в том числе посредством модуляции микробиоты и микробиома.

Неонатальный скрининг с использованием методов определения TREC/KREC все чаще становится стандартной практикой в ряде стран для раннего выявления тяжелых форм врожденных ошибок иммунитета, в частности тяжелого комбинированного иммунодефицита (SCID).

Современная концепция врожденных ошибок иммунитета отражает смену парадигмы в понимании иммунных нарушений как сложных и динамических состояний. Иммунная система в настоящее время рассматривается не только как совокупность клеток и молекул, но и как интегрированная биологическая система, функционирование которой определяется взаимодействием генетических факторов и факторов окружающей среды.

Ключевые слова: *врожденные ошибки иммунитета, первичные иммунодефициты, глобализация, неонатальный скрининг, микробиома.*

ORIJINAL MƏQALƏ

AÇIQ GİRİŞ (OPEN ACCESS)

DOI: <https://doi.org/10.28942/atu.j.v6i3y2026.161>**DEVELOPMENT, STANDARDIZATION, AND STUDY OF THE BIOLOGICAL EFFICACY OF A SYNERGISTIC PHYTOEXTRACT OF *SYZYGIUM AROMATICUM* L., *ACHILLEA MILLEFOLIUM* L., AND *HIBISCUS SABDARIFFA* L. BY HIGH-SPEED EXTRACTION**Mehraliyeva S.J.^{1*}, Mammadov B.S.^{1*}, Gadirova H.A.², Süleymanova T.H.², Nasirli I.U.³, Ece Bayır⁴, A. Alper Öztürk⁵**Abstract**

The present study aimed to develop and optimize a multifunctional phytoextract composed of clove (*Syzygium aromaticum*), yarrow (*Achillea millefolium*), and hibiscus (*Hibiscus sabdariffa*) for potential topical pharmaceutical and cosmetic applications. Three different phytocompositions were prepared and comparatively evaluated in terms of antimicrobial activity, antioxidant potential, physicochemical properties, safety, and long-term stability. Among the investigated systems, the composition containing clove, yarrow, and hibiscus in a ratio of 6:1:3 exhibited the most favorable profile and was therefore selected as the optimized formulation. The extraction process was systematically optimized by investigating the influence of extraction method, extraction time, plant-to-solvent ratio, temperature, solvent composition, and rotation speed. High-speed extraction performed for 7 min at 26 °C using a 95:5 ethanol: propylene glycol mixture, a raw material-to-solvent ratio of 1:10, and a rotation speed of 3200 rpm yielded the most suitable extract in terms of quality, antimicrobial activity, and long-term stability. The optimized phytocomposition demonstrated pronounced antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, with minimum inhibitory concentration values of 0.25 mL, while complete inhibition of *Candida albicans* was achieved at 0.50 mL. The extract also exhibited strong antioxidant activity in the DPPH assay, with an IC₅₀ value of 1.58 mg/mL. Long-term stability studies demonstrated that the optimized extract remained stable for up to 24 months when stored in dark glass packaging. Only minor changes in rutin and eugenol content, dry residue, and pH were observed, whereas storage in plastic containers resulted in marked physicochemical deterioration. The optimized extract was found to be non-irritant in the HET-CAM assay, with an irritation index of 2.45 ± 0.35. Overall, the developed phytocomposition combined strong antimicrobial and antioxidant activity with good stability and excellent tolerability. These findings indicate that the optimized phytoextract may serve as a promising active ingredient for the development of topical pharmaceutical and cosmetic formulations.

Keywords: *Syzygium aromaticum*, *Achillea millefolium*, *Hibiscus sabdariffa*, high-speed extraction, antimicrobial activity, antioxidant activity, HET-CAM, topical phytocomposition



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INTRODUCTION

Medicinal plant extracts have attracted considerable attention owing to their antioxidant, antimicrobial, anti-inflammatory and regenerative properties. The management of superficial skin damage, minor wounds, inflammatory dermatoses, and microbial skin infections often requires multifunctional preparations capable of reducing oxidative stress, preventing microbial colonization, and supporting tissue repair. In this context, medicinal plants rich in polyphenols, flavonoids, and volatile constituents represent promising sources of bioactive compounds for the development of effective topical formulations [1–5].

Among the selected medicinal plants, clove buds (*Syzygium aromaticum* L.) are particularly rich in eugenol, eugenol acetate, and β -caryophyllene, which are responsible for pronounced antibacterial, antifungal, antioxidant, and anti-inflammatory activities. Clove extracts have demonstrated inhibitory effects against both Gram-positive and Gram-negative microorganisms and are widely used in traditional medicine for the treatment of infected wounds, inflammatory skin disorders, and superficial lesions [6,7]. Yarrow (*Achillea millefolium* L.) contains flavonoids, tannins,

sesquiterpene lactones, and phenolic acids, which contribute to its well-documented wound-healing, anti-inflammatory, antimicrobial, and regenerative properties. In addition, yarrow has been reported to promote tissue repair and reduce local irritation, making it a valuable component of topical herbal preparations [8,9]. Hibiscus flowers (*Hibiscus sabdariffa* L.) are characterized by high levels of anthocyanins, organic acids, and polyphenolic compounds, particularly gallic acid, quinic acid, quercetin, rutin, and related flavonoids. These constituents exhibit strong free radical scavenging capacity and may contribute to protection against oxidative stress and inflammation [10,11]. Therefore, the rational combination of clove, yarrow, and hibiscus extracts may provide broader and potentially synergistic biological activity than each extract used individually, owing to the complementary actions of volatile oils, flavonoids, tannins, and phenolic compounds. The use of multicomponent phytocompositions is especially attractive because plant-derived constituents may interact synergistically. A rational combination of plant extracts may improve antimicrobial activity through the simultaneous action of volatile oils, phenolic acids, and flavonoids on different microbial targets, while also enhancing antioxidant capacity and reducing irritation. However, the biological efficacy of combined herbal systems strongly depends on the ratio of the individual components [12–16]. For this reason, the optimization of the proportion between clove, yarrow, and hibiscus is essential to obtain a phytocomposition with maximum biological activity and acceptable physicochemical properties. Preliminary microbiological observations indicated that the 6:1:3 ratio provided superior antimicrobial activity and comparable antifungal activity compared with other tested combinations, suggesting that this composition may represent the most

Yazışma üçün əlaqə:

Mehraliyeva S.J.1*, Mammadov B.S.1*, Gadirova H.A.2, Süleymanova T.H.2, Nasirli I.U.3, Ece Bayır4, A. Alper Öztürk5

1*Azerbaijan Medical University, Department of Pharmaceutical Technology and Management, Baku, Azerbaijan

2Azerbaijan Medical University, Department of Medical Microbiology and Immunology, Baku, Azerbaijan

3Azerbaijan Medical University, Department of Pharmaceutical Toxicology and Chemistry, Baku, Azerbaijan

4Ege Üniversitesi, Central Research Test and Analysis Laboratory Application and Research Center, İzmir, Türkiye

5Anadolu University, Department of Pharmaceutical Technology, Eskişehir, Türkiye

*For correspondence E-mail:

prof.nanopharmacy@gmail.com

promising balance between the active plant constituents [17–19].

In addition to phytochemical composition, the extraction method plays a crucial role in determining the quality and activity of the obtained extract. Conventional methods such as maceration and percolation are widely used for the extraction of biologically active substances from plant materials; however, these methods are generally time-consuming and may provide limited extraction efficiency. In contrast, high-speed extraction based on cavitation and intense shear forces significantly accelerates mass transfer processes, increases the contact surface between plant material and solvent, and facilitates the release of intracellular compounds. High-speed dispersion technologies are also advantageous for the extraction of thermolabile and volatile constituents because they allow efficient extraction within a short period of time and under relatively mild conditions [18–24].

The use of rotor–stator homogenizers and high-speed dispersers has recently emerged as a promising strategy for the intensification of pharmaceutical and phytochemical processes. These systems generate turbulence, cavitation, and strong hydrodynamic forces, leading to rapid disruption of plant cell walls and enhanced release of active compounds. Compared with conventional extraction methods, high-speed extraction can reduce processing time several-fold while simultaneously improving extract yield and preserving biologically active constituents [25–29]. Nevertheless, despite the growing interest in these technologies, there is still limited information regarding their application to multicomponent phytocompositions intended for topical pharmaceutical use.

Furthermore, although the antioxidant, antimicrobial, and anti-inflammatory

properties of individual extracts obtained from clove buds (*S.aromaticum* L.), yarrow (*A.millefolium* L.), and hibiscus flowers (*H.sabdariffa* L.) have been reported previously, their combined use in a rationally optimized ratio has not been sufficiently investigated. There is limited information regarding the simultaneous optimization of plant ratio and extraction conditions, together with comprehensive evaluation of antioxidant activity, antimicrobial efficacy, irritation potential, and cytotoxicity. Moreover, no previous study has been identified in which a high-speed extracted phytocomposition consisting of clove, yarrow, and hibiscus was optimized according to extraction parameters and component ratio, chemically characterized by GC/MS and LC/MS, and biologically evaluated using antimicrobial, antioxidant, HET-CAM, and MTT assays.

Therefore, the aim of the present study was to develop and optimize a multicomponent phytocomposition based on clove buds, yarrow, and hibiscus flowers, and to determine the most suitable extraction conditions for obtaining a biologically active extract. Different extraction methods, extraction times, solvent systems, and plant ratios were systematically compared to identify the optimal phytocomposition. The resulting extracts were subsequently characterized by modern chromatographic techniques and evaluated in terms of their antimicrobial, antioxidant, irritation, and cytotoxic properties. To the best of our knowledge, this is the first study to report the comprehensive development and biological evaluation of a high-speed extracted phytocomposition composed of *S.aromaticum*, *A.millefolium*, and *H.sabdariffa* in an optimized 6:1:3 ratio for potential application in topical pharmaceutical preparations [18, 32].

High-Speed Extraction of Phytoextract from Clove, Hibiscus, and Achillea millefolium and Development of Phytoextract



MATERIALS and METHODS

Materials

Dried flower buds of *S. aromaticum* L. (Myrtaceae), aerial parts of *A. millefolium* L. (Asteraceae), and flowers of *H. sabdariffa* L. (Malvaceae) were used as plant materials. The botanical materials were cleaned, dried under ambient conditions, and pulverized prior to extraction. Ethanol (95%, v/v),

propylene glycol, and distilled water were used as extraction solvents. All other chemicals and reagents were of analytical grade. Preliminary studies were performed using different extraction methods, solvent systems, extraction times, and plant ratios to determine the optimum phytocomposition. Based on microbiological and physicochemical screening, the most active

phytochemical composition consisted of *S.aromaticum*, *A.millefolium*, and *H.sabdariffa* in a ratio of 6:1:3[18].

Preparation of Phytoextracts

The volume of extractant required for the extraction procedure was calculated according to the following equation (Eq. 1.):

$$V_{extractant} = V + mK$$

Eq.1.

where (V) is the required volume of extract (mL), (m) is the amount of plant material (g), and (K) is the water absorption coefficient of the plant material.

The amount of dry residue in the obtained extracts was determined using the following equation (Eq. 2.):

$$Dry\ residue = \frac{m_2 - m_1}{V} \times 100$$

Eq.2.

where (m_1) is the weight of the empty container (g), (m_2) is the weight of the container after evaporation of the sample (g), and (V) is the volume of extract used for the analysis (mL).

Three different extraction methods were compared: maceration (12–48 h), percolation (8–24 h), and high-speed extraction (1–10 min). The powdered plant materials were mixed with an ethanol: propylene glycol solvent system (95:5, v/v) and subjected to high-speed extraction using a Stegler DG-360 high-speed rotor–stator disperser operated at 3200 rpm, 50 Hz, and 360 W. The extraction process was carried out at 26 ± 1 °C to minimize thermal degradation of volatile and thermolabile constituents [18].

Different extraction times (1, 3, 5, 7, and 10 min) and plant ratios were evaluated. The optimum conditions were determined as a 6:1:3 ratio of *S.aromaticum*: *A.millefolium*: *H.sabdariffa*, extracted for 7 min using the ethanol: propylene glycol (95:5, v/v) solvent system[18].

Determination of Alcohol Content

The alcohol content of the obtained phytoextract was determined by pycnometric analysis after distillation. Briefly, 25 mL of

liquid extract was transferred into a 250 mL distillation flask and diluted with distilled water to a total volume of 75 mL Porcelain boiling chips were added and the sample was distilled. Approximately 48 mL of distillate was collected in a 50 mL receiving flask cooled in an ice-water bath. The distillate was adjusted to 20 °C, and its density was measured using a pycnometer. The alcohol concentration of the extract was found to be 95%.

Stability Evaluation of the Phytoextract

The stability of the optimized phytoextract was evaluated by determining its organoleptic and physicochemical properties, including appearance, color, odor, pH, density, dry residue, and extractive matter content. Samples were filled into dark glass and plastic containers and stored at 12–15 °C under long-term conditions. The influence of packaging material on the stability of the extract was assessed periodically during the storage period.

Determination of Antioxidant Activity

The antioxidant activity of the optimized phytoextract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay according to the method of Brand-Williams et al. with slight modifications. Briefly, different concentrations of the phytoextract were prepared in methanol and mixed with freshly prepared DPPH solution. The reaction mixtures were incubated in the dark at room temperature for 30 min, and the absorbance was measured at 517 nm using a UV–Vis spectrophotometer. Ascorbic acid was used as the reference antioxidant. The radical scavenging activity was calculated as the percentage inhibition of DPPH radicals, and the IC_{50} value (concentration required to inhibit 50% of DPPH radicals) was obtained from the concentration–response curve. All experiments were performed in triplicate, and the results are presented as mean $\pm$ SD.[30–33, 43–45].

The radical scavenging activity was calculated according to the following equation (Eq. 3.):

$$DPPH \text{ inhibition (\%)} = \frac{A_0 - A_s}{A_0} \times 100$$

Eq. 3.

where (A_0) is the absorbance of the control and (A_s) is the absorbance of the sample.

Microbiological Evaluation

The antimicrobial activity of the phytoextracts was investigated in the Department of Medical Immunology and Microbiology using both agar disk diffusion and serial dilution methods. The following test microorganisms were used: *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*.

For the disk diffusion assay, sterile paper disks were impregnated with the extracts and placed on inoculated agar plates. Following incubation, the diameters of the inhibition zones were measured and expressed in millimeters [32].

The minimum inhibitory concentration was further evaluated by serial dilution. Briefly, four serial dilutions of each extract (1:100, 1:200, 1:400, and 1:800) were prepared in sterile distilled water. A standardized microbial suspension containing approximately 5×10^8 CFU/mL was added to each tube. After exposure for 10, 20, 40, and 60 min, aliquots were inoculated onto agar media. Fungal cultures were incubated at 28 °C for 48 h, while bacterial cultures were incubated at 37 °C for 24 h. Among the investigated formulations, the composition containing *S.aromaticum*, *A.millefolium*, and *H.sabdariffa* showed the highest antimicrobial activity against both Gram-positive and Gram-negative microorganisms as well as *C.albicans* [17,32,42]:

HET-CAM Assay

The anti-inflammatory and irritation potential of the optimized extract was evaluated using the Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM). Fertilized chicken eggs were incubated for 9 days at 37.8 °C and

60% relative humidity. On day 9, the eggshell was carefully opened at the blunt end, and the inner membrane was removed to expose the chorioallantoic membrane [33, 34].

Aliquots of the test samples were applied directly onto the membrane surface. The occurrence of vascular hemorrhage, coagulation, and lysis was visually monitored for 5 min. Sodium lauryl sulfate was used as the positive control and physiological saline as the negative control.

The irritation score (IS) was calculated according to the Luepke method (Eq. 4.) [34]:

$$IS = \frac{(301 - H) \times (301 - L) \times 7 + (301 - C) \times 9}{300}$$

Eq. 4.

where (H), (L), and (C) represent the times (s) required for the appearance of hemorrhage, vascular lysis, and coagulation, respectively.

Cytotoxicity Analysis

The cytotoxicity of the optimized phytoextract was evaluated by the MTT assay. Cells were seeded into 96-well plates and incubated under standard culture conditions. After reaching appropriate confluence, the cells were treated with different concentrations of the extract samples for 24 h. Following incubation, MTT solution was added to each well and the plates were incubated for an additional 3–4 h. The resulting formazan crystals were dissolved in dimethyl sulfoxide, and the absorbance was measured at 570 nm using a microplate reader [32,35].

Cell viability was calculated as follows (Eq. 5.):

$$Cell \text{ viability (\%)} = \frac{A_s}{A_c} \times 100$$

Eq. 5.

where (A_s) is the absorbance of the treated cells and (A_c) is the absorbance of the untreated control cells. The cytotoxicity results were interpreted together with the antimicrobial and HET-CAM findings to determine the suitability of the Composition II

for potential topical pharmaceutical applications.

Results and Discussion

Selection of the Optimal Phytocomposition and Synergistic Effect

Three phytocompositions composed of clove buds (*S.aromaticum*), yarrow herb (*A.millefolium*), and hibiscus flowers (*H.sabdariffa*) were prepared in different ratios to identify the most suitable composition for the development of a stable and biologically active topical formulation. The compositions differed markedly in both antimicrobial performance and physicochemical behavior. Composition I contained the plant materials at a ratio of 3:6:1, Composition II at 6:1:3, and Composition III at 1:3:6 [17, 36-40, 42].

The obtained results demonstrated that the biological activity of the compositions was strongly dependent on the relative amount of clove extract. Composition II, which contained the highest proportion of clove together with a moderate amount of hibiscus, exhibited the most pronounced antimicrobial and antioxidant activity. This finding may be attributed to the high content of eugenol and other phenolic derivatives originating from clove. Eugenol is known to disrupt bacterial cell membranes, increase membrane permeability, denature intracellular proteins, and induce leakage of cytoplasmic contents. In addition, the presence of hibiscus extract likely enhanced the overall activity due to its anthocyanin and organic acid content, while

yarrow contributed flavonoids and sesquiterpenes with additional anti-inflammatory and regenerative properties. Composition I demonstrated intermediate activity. Although the high proportion of yarrow improved the anti-inflammatory potential of the mixture, the lower amount of clove reduced the overall antibacterial effect. Furthermore, the lower concentration of hibiscus resulted in a less intense antioxidant activity. The composition remained physically acceptable during short-term storage; however, slight precipitation and gradual loss of color intensity were observed after prolonged storage. Composition III exhibited the least favorable profile. The low concentration of clove markedly reduced the antibacterial activity, whereas the high hibiscus content caused progressive precipitation, increased acidity, and intense color instability. The predominance of hydrophilic hibiscus constituents probably decreased the compatibility of the extract with the solvent system and reduced the stability of the final product [32, 36-40,42].

As summarized in **Table 1**, the tested phytocompositions exhibited distinct physicochemical and biological profiles depending on the relative proportions of *S.aromaticum*, *A.millefolium*, and *H.sabdariffa*. Among them, Composition II demonstrated the most favorable balance between antimicrobial activity, antioxidant potential, physicochemical stability, and overall suitability for further formulation development.

Table 1. Comparative physicochemical and biological evaluation of the tested phytocompositions

Parameter	Composition I (3:6:1)	Composition II (6:1:3)	Composition III (1:3:6)
Main dominant constituents	Flavonoids, sesquiterpenes	Eugenol, anthocyanins, flavonoids	Anthocyanins, organic acids
Antimicrobial activity	Moderate	Highest	Weak
Antioxidant activity	Moderate	High	Moderate–high

Physicochemical stability	Acceptable	Excellent	Poor
Precipitation tendency	Slight	None	Marked
Color stability	Moderate	Stable	Unstable
Overall suitability	Acceptable	Optimal	Unsuitable

The superior behavior of Composition II suggests that the simultaneous presence of eugenol-rich clove extract, anthocyanin-containing hibiscus, and anti-inflammatory yarrow creates a synergistic phytochemical system. The combination appears to provide

a balance between antimicrobial potency, antioxidant protection, and physicochemical stability. Therefore, Composition II was selected for all subsequent experiments (**Table 2**).

Table 2. Comparative evaluation of different plant ratios used for preparation of the phytoextract.

Ratio (Syzygium : Achillea : Hibiscus)	Appearance	Stability	Antimicrobial activity	Final assessment
3:6:1	Slight turbidity	Moderate	Lower	Not selected
6:1:3	Homogeneous, no sediment	Stable	Highest	Selected
1:3:6	Darkening / sedimentation	Poor	Lower	Not selected

The superior behavior of Composition II provides empirical validation of a true synergistic effect achieved by modulating the multi-component plant ratios. The baseline methodology and raw microbiological dynamics of this system have been fully validated and published in our previous study [18, 32, 42]. Our standardized serial dilution screening, referenced strictly to 0.5 McFarland (1.5×10^8 CFU/mL) for bacterial strains and 2.0 McFarland (6×10^8 CFU/mL) for *C. albicans*, revealed that the biological response is strictly dependent on the architectural alignment of the matrices. While Compositions I (3:6:1) and III (1:3:6) exhibited a standard baseline Minimum Inhibitory Concentration (MIC) of 0.5 mL against *St. aureus* and *E. coli*, Composition II (6:1:3) demonstrated a notable 2-fold increase in antibacterial potency, dropping the MIC threshold to just 0.25 mL.

This strict formulation synergy is further manifested in the significant enlargement of the zones of inhibition; for instance, the inhibition zone against encapsulated *Klebsiella pneumoniae* increased by over 50%, jumping from 14 ± 0.9 mm (standalone clove extract) to 22 ± 1.28 mm within the composite system. Crucially, this synergistic performance has been formally certified via an Official Act of Laboratory Evaluation/Implementation.

Mechanistically, this synergistic interaction is hypothetically attributed to multi-target biochemical pathways described in ethnopharmacological literature. It is proposed that the phenolics within *S. aromaticum*, particularly eugenol, disrupt bacterial outer cell wall integrity, induce intracellular lipid leakage, and alter cell membrane permeability [38, 39, 40].

This structural destabilization operates cooperatively, thereby facilitating the

accelerated intracellular influx and localized efficacy of the co-delivered *H.sabdariffa* organic acids (such as hibiscus and citric acids) and anthocyanins, which effectively lower internal pH [11, 36, 37], alongside *A.millefolium* flavonoids that inhibit essential bacterial enzyme synthesis [8, 9]. Consequently, this literature-supported molecular cooperation firmly positions Composition II as the optimal certified matrix for subsequent formulation development.

Antimicrobial Activity and MIC Determination

The antimicrobial activity of the prepared phytocompositions was evaluated against *St. aureus*, *E.coli*, and *C.albicans*, representing Gram-positive bacteria, Gram-negative bacteria, and fungi, respectively. The tested microorganisms were selected because they are commonly associated with infected skin lesions and impaired wound healing. The obtained results clearly demonstrated that

Composition II possessed the strongest antibacterial activity among all investigated samples. Against *S. aureus*, the MIC value of Composition II was 0.25 mL, whereas Compositions I and III required 0.50 mL to inhibit microbial growth. A similar trend was observed for *E. coli*. The lower MIC value of Composition II indicates a more potent antibacterial effect and suggests that the higher content of clove-derived phenolic compounds was sufficient to overcome both the thick peptidoglycan layer of Gram-positive bacteria and the more resistant outer membrane of Gram-negative bacteria.

The MIC values presented in **Table 3** clearly indicate that Composition II exhibited the strongest antimicrobial activity against both *St.aureus* and *E.coli*, whereas all tested phytocompositions showed comparable antifungal activity against *C.albicans* [17-18, 32-33, 42].

Table 3. Minimum inhibitory concentration values of the tested phytocompositions

Sample	<i>S. aureus</i> (mL)	<i>E. coli</i> (mL)	<i>C. albicans</i> (mL)
Composition I	0.50	0.50	0.50
Composition II	0.25	0.25	0.50
Composition III	0.50	0.50	0.50

The difference between the antibacterial activities against *S. aureus* and *E. coli* was relatively small, which indicates that the Composition II exerted a broad-spectrum antimicrobial effect. This broad activity may arise from the coexistence of several phytochemicals acting through different mechanisms. Eugenol is known to penetrate and disrupt lipid membranes, while hibiscus acids lower the local pH and thereby create an unfavorable environment for bacterial survival. Yarrow-derived flavonoids may further contribute by interfering with bacterial enzyme systems.

The antifungal activity against *C. albicans* was lower and more comparable among all compositions. Complete inhibition was

achieved at 0.50 mL for all samples. This finding suggests that the phytochemical composition mainly influenced the antibacterial rather than antifungal effect. Nevertheless, the observed antifungal activity remains important because *Candida* colonization is frequently associated with chronic skin lesions.

Overall, these findings indicate that Composition II was the most promising phytocomposition owing to its superior broad-spectrum antimicrobial activity. The optimized ratio of *S.aromaticum*, *A.millefolium*, and *H.sabdariffa* appeared to provide the most favorable balance between membrane-disrupting volatile compounds and biologically active phenolic constituents.

Therefore, Composition II was selected as the optimized extract for subsequent extraction optimization and stability studies [18, 32].

Optimization of the Extraction Process

The extraction process was systematically optimized to maximize the recovery of biologically active compounds while maintaining good physicochemical stability. The influence of extraction method, extraction time, plant-to-solvent ratio, temperature, extractant composition, and rotation speed was investigated [18, 43].

Effect of Extraction Method

Among the investigated techniques, high-speed extraction provided the highest dry residue value ($2.56 \pm 0.01\%$), followed by

maceration and percolation (**Table 4**). The superiority of high-speed extraction may be explained by the rapid mechanical destruction of plant tissues, which increases the contact area between the plant material and solvent. Intensive mixing likely enhanced the diffusion of eugenol, flavonoids, anthocyanins, and other phenolic constituents into the extractant. In addition, the relatively short duration of this method minimized the degradation of volatile and thermolabile compounds. By contrast, percolation and maceration required a longer extraction period and therefore probably resulted in greater losses of volatile compounds [18].

Table 4. *Effect of extraction method on dry residue content*

Method	Dry residue (%)
Percolation	2.01 ± 0.03
Maceration	2.13 ± 0.02
High-speed extraction	2.56 ± 0.01

Effect of Extraction Time

The amount of dry residue increased gradually with increasing extraction time and reached a maximum value after 7 min (**Table 5**). The sharp increase between 1 and 7 min indicates that most extractable constituents were released during the early stages of extraction. The subsequent decrease observed after 10 min most likely reflects the evaporation of essential oil components and oxidative degradation of phenolic compounds. Since eugenol and several anthocyanins are sensitive to prolonged exposure to oxygen and mechanical stress, excessive extraction time may reduce the quality of the extract despite a similar total dry residue. The extraction efficiency increased progressively with increasing processing time and reached its maximum at 7 min. This finding indicates that high-speed extraction

provided sufficient disruption of plant tissues and promoted rapid release of bioactive constituents within a relatively short period. However, extending the extraction time beyond 7 min resulted in a slight decrease in dry residue content. This reduction may be associated with the degradation or volatilization of thermolabile and volatile compounds, particularly eugenol and other aromatic constituents present in *S.aromaticum*. Prolonged processing may also promote oxidation of polyphenolic compounds and partial destruction of sensitive phytochemicals due to increased shear stress and cavitation intensity. Therefore, 7 min was considered the optimal extraction time, providing the best balance between extraction efficiency and preservation of biologically active compounds [18, 42-44].

Table 5. *Effect of extraction time on dry residue content*

Time (min)	Dry residue (%)
1	1.06 ± 0.00
3	2.21 ± 0.00
5	2.43 ± 0.00
7	2.58 ± 0.00
10	2.47 ± 0.00

Effect of Raw Material-to-Extractant Ratio

Although the 1:5 ratio produced the highest dry residue, the resulting extract was excessively viscous, difficult to filter, and contained a substantial amount of ballast material. In contrast, the 1:10 ratio provided a more homogeneous extract with lower

viscosity and superior physical stability. The lower number of insoluble substances obtained at 1:10 also facilitated subsequent incorporation into the final semisolid dosage form. Therefore, the ratio of 1:10 was considered optimal (**Table 6**).

Table 6. *Effect of raw material-to-extractant ratio on dry residue content*

Ratio	Dry residue (%)
1:5	3.16 ± 0.02
1:10	2.57 ± 0.03
1:15	2.25 ± 0.02
1:20	1.52 ± 0.01

Effect of Extraction Temperature

The highest extraction efficiency was obtained at 26 °C (**Table 7**). Temperatures above this value led to a gradual decrease in dry residue. This reduction probably resulted from the instability of thermolabile compounds such as anthocyanins and volatile terpenes.

Elevated temperatures may also accelerate oxidation reactions and evaporation of clove essential oil constituents. Consequently, the use of mild temperatures is advantageous for preserving the biological activity of the extract [18].

Table 7. *Effect of temperature on dry residue content*

Temperature (°C)	Dry residue (%)
22	2.41 ± 0.04
24	2.52 ± 0.03
26	2.79 ± 0.02
30	2.56 ± 0.01
38	2.42 ± 0.01

Effect of Ethanol: Propylene Glycol Ratio

The influence of the solvent composition was one of the most critical parameters affecting the quality of the obtained phytoextract (**Table 8**). Although the highest dry residue was obtained with the 80:20 ethanol:propylene glycol system, this solvent mixture also extracted a larger amount of undesired ballast

substances, including sugars, mucilage, and other high-molecular-weight constituents. As a consequence, the resulting extract exhibited higher viscosity, lower transparency, more difficult filtration, and reduced long-term stability. Increasing the proportion of propylene glycol above 5% also appeared to impair the efficiency of high-speed extraction

because the higher viscosity of the medium limited solvent penetration and mass transfer within the plant particles. Similar observations have been reported for plant extraction systems in which excessive amounts of propylene glycol decreased extraction selectivity and complicated subsequent processing. By contrast, the 95:5 ethanol:propylene glycol mixture yielded a lower total dry residue but produced a cleaner and more selective extract enriched in the desired phenolic and volatile compounds. This solvent system provided improved extract clarity, lower viscosity, easier filtration, better microbiological stability, and a more

reproducible composition. The lower proportion of propylene glycol was still sufficient to enhance the solubility of semi-polar constituents, while the high ethanol content favored the extraction of eugenol-rich and flavonoid-rich fractions [18-19, 32, 42]. Therefore, the final selection of the 95:5 ethanol:propylene glycol ratio was based not only on the total extraction yield but also on the superior qualitative composition and stability of the resulting extract. This finding indicates that the highest dry residue does not necessarily correspond to the most suitable phytochemical composition for further pharmaceutical development.

Table 8. *Effect of ethanol: propylene glycol ratio on dry residue content*

Ratio	Dry residue (%)
80:20	3.73 ± 0.03
90:10	3.46 ± 0.02
95:5	2.56 ± 0.04
60:40	2.48 ± 0.05
50:50	2.13 ± 0.02
40:60	1.68 ± 0.04
30:70	1.04 ± 0.04

Effect of Rotation Speed

Among the investigated rotation speeds, 3200 rpm provided the highest extraction efficiency and yielded the most suitable physicochemical characteristics of the phytoextract (**Table 9**). At lower rotation speeds, the mechanical force was insufficient to achieve complete disruption of the plant material, resulting in lower extraction efficiency. In contrast, higher rotation speeds negatively affected the extract quality, most

likely because of excessive aeration, foaming, and possible degradation of thermolabile constituents. Therefore, 3200 rpm was selected as the optimum rotation speed for further studies. Consequently, the final optimized extraction conditions consisted of high-speed extraction for 7 min at 26 °C using a 95:5 ethanol: propylene glycol mixture, a raw material-to-solvent ratio of 1:10, and a rotation speed of 3200 rpm [18].

Table 9. *Effect of rotation speed on dry residue content*

Rotation speed (rpm)	Dry residue (%)
2800	2.37 ± 0.04
3000	2.43 ± 0.02
3200	2.56 ± 0.04
3400	2.18 ± 0.01
3600	1.65 ± 0.05

Elemental Composition of the Optimized Extract

The macro- and microelement composition of the optimized extract (Composition II) was evaluated to assess both its potential biological benefits and its safety (**Table 10**). The relatively high calcium content may contribute to keratinocyte proliferation and epidermal regeneration, whereas magnesium and potassium are involved in cellular metabolism and osmotic balance. Copper is

of particular interest because it is closely associated with collagen synthesis and wound healing. Therefore, the elemental profile of the extract may additionally support its biological activity. Importantly, the heavy metal contents remained well below accepted pharmacopoeia and toxicological limits. This result confirms that the developed extract is suitable for topical use and that the selected raw plant materials were of adequate quality [18, 31-33, 42].

Table 10. *Elemental composition of the optimized phytoextract*

Element	Content
Ca (%)	0.23
P (%)	0.05
Mg (%)	0.0201
K (%)	0.02
Fe (%)	0.0012
Cu (mg/kg)	5.34
Ni (mg/kg)	1.62
Co (mg/kg)	0.22
Cr (mg/kg)	0.41
Sr (mg/kg)	11.4
Pb (mg/kg)	0.11
As (mg/kg)	0.07
Cd (mg/kg)	0.011
Hg (mg/kg)	0.003

Stability Study

The long-term stability study demonstrated that the packaging material had a pronounced effect on the quality of the optimized phytoextract. When stored in dark glass containers, the extract retained its original appearance, characteristic odor, pH, and phytochemical composition throughout the storage period. Only minor reductions in dry residue, extractive substances, rutin, and eugenol content were observed after 24 months, indicating gradual but limited degradation of the bioactive constituents (**Table 11**). In contrast, samples stored in

plastic containers underwent substantially greater changes during storage. Progressive darkening, marked reduction in odor intensity, lower pH values, and significant decreases in dry residue, extractives, rutin, and eugenol content were observed. These findings suggest that the permeability of plastic packaging to oxygen and volatile compounds accelerated the oxidation and loss of phenolic and aromatic constituents (**Table 12**).

Therefore, storage in dark glass containers at 12–15 °C should be recommended for the final phytoextract formulation.

Table 11. *Stability of the optimized phytoextract during storage in dark glass containers*

Storage period	Color	Odor	Dry residue (%)	Extractives (%)	Rutin (mg/mL)	Eugenol (%)	pH
Initial	Cherry red	Characteristic aromatic odor	2.56	32.5	26.90	95	4.5
6 months	Cherry red	No significant change	2.50	31.8	25.9	93	4.4
12 months	Slightly lighter red	Slight decrease	2.44	31.0	24.8	91	4.2
24 months	Slight fading	Slight decrease	2.30	29.8	20.82	89	3.9–5.1

Table 12. *Stability of the optimized phytoextract during storage in plastic containers*

Storage period	Color	Odor	Dry residue (%)	Extractives (%)	Rutin (mg/mL)	Eugenol (%)	pH
Initial	Cherry red	Characteristic aromatic odor	2.56	32.5	26.90	95	4.5
6 months	Dark red	Noticeable decrease	2.28	26.4	18.7	84	4.0
12 months	Brownish red	Markedly reduced	2.12	22.1	14.5	78	3.7
24 months	Brown	Marked decrease	2.00	19.3	10.0	73	3.5

Antioxidant Activity

The antioxidant activity of the optimized extract (Composition II) was assessed by the DPPH method (**Table 13**). The extract exhibited strong concentration-dependent free radical scavenging activity. The low IC₅₀ value indicates that only a relatively small amount of extract was required to neutralize 50% of the DPPH radicals. This pronounced activity is likely related to the high content of

eugenol, flavonoids, and anthocyanins present in the optimized composition. The strong antioxidant capacity is particularly important for the intended topical application because oxidative stress is one of the major factors responsible for delayed wound healing and chronic inflammation. Therefore, the developed extract may contribute not only to microbial control but also to tissue regeneration[30,32].

Table 13. *Antioxidant activity of the optimized extract determined by DPPH assay*

Parameter	Value
IC ₅₀ (mg/mL)	1.58
R ² (Coefficient of Determination)	0.998

3.7. HET-CAM Assay and Irritation Potential

The irritation potential of the optimized phytoextract (Composition II) was evaluated using the HET-CAM assay, and the obtained

irritation scores are presented in **Table 14**. As expected, 0.9% NaCl produced no irritation, whereas sodium lauryl sulfate (SLS) caused a severe irritant response with an irritation index of 18.0 ± 1.15 . In contrast, Composition

II exhibited a markedly lower irritation index (2.45 ± 0.35) and was therefore classified as non-irritant [59]. The low irritation potential may be attributed to the balanced phytochemical composition of the extract. Although the formulation contains phenolic compounds with strong antimicrobial activity, the presence of yarrow- and hibiscus-derived constituents may contribute to reducing local

irritation. The observed result is particularly important because the intended application involves damaged or sensitive skin. Taken together with its pronounced antimicrobial and antioxidant properties, the very low irritation potential indicates that the optimized phytoextract is suitable for further development as a topical pharmaceutical or cosmetic ingredient.

Table 14. HET-CAM irritation scores of the optimized extract.

Group	Irritation index	Classification
0.9% NaCl	0.00 ± 0.00	Non-irritant
SLS	18.0 ± 1.15	Severe irritant
Composition II	2.45 ± 0.35	Non-irritant

Overall, the study demonstrated that the biological and physicochemical properties of the investigated phytocompositions depended strongly on the ratio of the three plant extracts and on the selected extraction conditions. The optimized 6:1:3 composition provided the most favorable combination of antimicrobial efficacy, antioxidant activity, physicochemical stability, and safety. The results further showed that obtaining the highest extraction yield is not necessarily equivalent to obtaining the highest quality extract. Several conditions, such as the 80:20 solvent system and the 1:5 plant-to-solvent ratio, produced higher dry residue values but also extracted undesirable ballast substances and reduced the stability of the final product. Therefore, the selected extraction parameters represent a compromise between quantitative yield and qualitative composition. The final optimized extract (Composition II) remained stable for 24 months in dark glass packaging, demonstrated significant antioxidant activity, exhibited broad-spectrum antimicrobial activity, and showed no irritation in the HET-CAM assay. These findings support the potential use of the developed phytocomposition as a safe and effective topical ingredient for pharmaceutical or cosmetic applications.

CONCLUSION

In the present study, a multifunctional phytoextract based on *Syzygium aromaticum*, *Achillea millefolium*, and *Hibiscus sabdariffa* was successfully developed and optimized for potential topical pharmaceutical and cosmetic applications. Among the investigated phytocompositions, the 6:1:3 ratio exhibited the most favorable combination of physicochemical properties, antimicrobial activity, antioxidant potential, and tolerability. The extraction procedure was successfully optimized using high-speed extraction, and the most suitable conditions were identified as 7 min extraction time, 26 °C, a 95:5 ethanol: propylene glycol solvent system, a raw material-to-solvent ratio of 1:10, and a rotation speed of 3200 rpm. Under these conditions, the optimized extract (Composition II) demonstrated strong inhibitory activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, together with pronounced antioxidant activity.

Long-term stability studies further demonstrated that the Composition II retained its quality for up to 24 months when stored in dark glass containers, whereas marked deterioration occurred in plastic packaging. In addition, the extract was found to be non-irritant in the HET-CAM assay. Overall, the

results obtained indicate that the optimized phytoextract is a promising natural active ingredient for the preparation of stable, effective, and well-tolerated topical formulations (ointment, cream, nanogel with Ag/Au nanoparticles) possessing high prospective potential for the management of small skin lesions, inflammatory dermatoses, and microbially contaminated skin conditions.

REFERENCES

1. Wojciechowska K, Dos Santos Szewczyk K. The Skin Microbiome and Bioactive Compounds: Mechanisms of Modulation, Dysbiosis, and Dermatological Implications. *Molecules*. 2025;30:4363. <https://doi.org/10.3390/molecules30224363>
2. Criollo-Mendoza MS, Contreras-Angulo LA, Leyva-López N, Gutiérrez-Grijalva EP, Jiménez-Ortega LA, Heredia JB. Wound Healing Properties of Natural Products: Mechanisms of Action. *Molecules*. 2023;28:598. <https://doi.org/10.3390/molecules28020598>
3. Pereira RF, Bártolo PJ. Traditional Therapies for Skin Wound Healing. *Adv Wound Care (New Rochelle)*. 2016;5:208–29. <https://doi.org/10.1089/wound.2013.0506>
4. Albahri G, Badran A, Hijazi A, Daou A, Baydoun E, Nasser M, et al. The Therapeutic Wound Healing Bioactivities of Various Medicinal Plants. 2023;13:317. <https://doi.org/10.3390/life13020317>
5. Brar G, Nandy SK, Sharma A, Siddiqui AJ, Sharma L. Antimicrobial and Anti-Infective Potential of Herbal Creams in Dermatology: Efficacy, Safety, and Challenges in Skin Infection Management. *Infect Drug Resist*. 2025;Volume 18:6289–311. <https://doi.org/10.2147/IDR.S565852>
6. Maggini V, Semenzato G, Gallo E, Nunziata A, Fani R, Firenzuoli F. Antimicrobial Activity of *Syzygium aromaticum* Essential Oil in Human Health Treatment. *Molecules*. 2024;29:999. <https://doi.org/10.3390/molecules29050999>
7. Pandey VK, Srivastava S, Ashish, Dash KK, Singh R, Dar AH, et al. Bioactive properties of clove (*Syzygium aromaticum*) essential oil nanoemulsion: A comprehensive review. *Heliyon*. 2024;10:e22437. <https://doi.org/10.1016/j.heliyon.2023.e22437>
8. Strzępek-Gomółka M, Gawel-Bęben K, Kukula-Koch W. Achillea Species as Sources of Active Phytochemicals for Dermatological and Cosmetic Applications. *Oxid Med Cell Longev*. 2021;2021. <https://doi.org/10.1155/2021/6643827>
9. Rezaei S, Ashkar F, Koohpeyma F, Mahmoodi M, Gholamalizadeh M, Mazloom Z, et al. Hydroalcoholic extract of *Achillea millefolium* improved blood glucose, liver enzymes and lipid profile compared to metformin in streptozotocin-induced diabetic rats. *Lipids Health Dis*. 2020;19:81. <https://doi.org/10.1186/s12944-020-01228-4>
10. Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M. *Hibiscus sabdariffa* L. – A phytochemical and pharmacological review. *Food Chem*. 2014;165:424–43. <https://doi.org/10.1016/j.foodchem.2014.05.002>
11. Riaz G, Chopra R. A review on phytochemistry and therapeutic uses of *Hibiscus sabdariffa* L. *Biomedicine & Pharmacotherapy*. 2018;102:575–86. <https://doi.org/10.1016/j.biopha.2018.03.023>
12. Rahman MdM, Rahaman MdS, Islam MdR, Hossain MdE, Mannan Mithi F, Ahmed M, et al. Multifunctional Therapeutic Potential of Phytocomplexes and Natural Extracts for Antimicrobial Properties. *Antibiotics*. 2021;10:1076. <https://doi.org/10.3390/antibiotics10091076>
13. Mehraliyeva S, İsmaylova N, Öztürk AA, Jahangirova İ, Jafarova Sh, Phyto-functionalized niosomal gel for acne therapy: Dual delivery of clindamycin and botanical

- actives via a sustained topical platform - *in vitro*, *ex vivo*, *in silico* and *in vivo* evaluation. J Drug Deliv Sci Technol. 2026 Apr; <https://doi.org/10.1016/j.jddst.2026.108313>
14. Neacșu SM, Mititelu M, Ozon EA, Musuc AM, Iuga IDM, Manolescu BN, et al. Comprehensive Analysis of Novel Synergistic Antioxidant Formulations: Insights into Pharmacotechnical, Physical, Chemical, and Antioxidant Properties. Pharmaceuticals. 2024;17:690. <https://doi.org/10.3390/ph17060690>
15. Sharma K, Guleria S, Razdan VK, Babu V. Synergistic antioxidant and antimicrobial activities of essential oils of some selected medicinal plants in combination and with synthetic compounds. Ind Crops Prod. 2020;154:112569. <https://doi.org/10.1016/j.indcrop.2020.112569>
16. Vaou N, Stavropoulou E, Voidarou C (Chrysa), Tsakris Z, Rozos G, Tsigalou C, et al. Interactions between Medical Plant-Derived Bioactive Compounds: Focus on Antimicrobial Combination Effects. Antibiotics. 2022;11:1014. <https://doi.org/10.3390/antibiotics11081014>
17. Suleymanova T. Yeni sintez olunmuş aromatik və heterosiklik etil törəmələrinin antimikrob və antifunqal təsirinin öyrənilməsi//Azərbaycan Əczaçılıq və farmakoterapiya jurnalı, 2022, 22(1), s.61-65 176008848976370891
PDF (azpharmjournal.org)
18. Mehraliyeva, S. D., & Mammadov, B. S. (2026). *Method for obtaining a phytoextract by high-speed extraction*. Eurasian Patent No. 052812. Eurasian Patent Organization. [online] Available at: [https://www.eapo.org/pubservices/info/registr](https://www.eapo.org/pubservices/info/registry/inventions/patents/052812)
19. Capaldi G, Binello A, Aimone C, Mantegna S, Grillo G, Cravotto G. New trends in extraction-process intensification: Hybrid and sequential green technologies. Ind Crops Prod. 2024;209:117906. <https://doi.org/10.1016/j.indcrop.2023.117906>
20. Sindhu PM, Sethi S, Budnimath SH, Joshi A, Raghavendra HR. Extraction Technologies for Horticultural Crops. Advances in Postharvest and Analytical Technology of Horticulture Crops. Singapore: Springer Nature Singapore; 2024. p. 125–50. https://doi.org/10.1007/978-981-97-7247-6_7
21. Cui Q, Shi J-Y, Wen L-L, Kong W-H, Jiang L-J, Liu J-Z. Synergistic coupling of ultrasonic cavitation with tailored deep eutectic solvent systems for intensified extraction of Scutellaria Radix flavonoids: Modelling and optimization by genetic algorithm. Ultrason Sonochem. 2025;119:107386. <https://doi.org/10.1016/j.ultsonch.2025.107386>
22. Kamal RM, Abo-Elghiet F, Hal DM, Hammam WE, El-Dessouki AM, Mahrous MH, et al. From conventional to green extraction of saponins: A review on plant matrices and byproducts. J Agric Food Res. 2026;25:102607. <https://doi.org/10.1016/j.jafr.2025.102607>
23. Sun S, Yu Y, Jo Y, Han JH, Xue Y, Cho M, et al. Impact of extraction techniques on phytochemical composition and bioactivity of natural product mixtures. Front Pharmacol. 2025;16. <https://doi.org/10.3389/fphar.2025.1615338>
24. Kumar A, P N, Kumar M, Jose A, Tomer V, Oz E, et al. Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. Molecules. 2023;28:887. <https://doi.org/10.3390/molecules28020887>
25. Avdieieva LYu, Makarenko AA, Turchyna TYa, Dekusha HV, Kozak MM. Cavitation Technology for Intensification of Plant Raw Materials Extraction. Food Science and Technology. 2023;17. <https://doi.org/10.15673/fst.v17i1.2559>
26. Wu Z, Ferreira DF, Crudo D, Bosco V, Stevanato L, Costale A, et al. Plant and Biomass Extraction and Valorisation under

Hydrodynamic Cavitation. Processes. 2019;7:965.

<https://doi.org/10.3390/pr7120965>

27. Ghenabzia I, Hemmami H, Ben Amor I, Zeghaoun S, Ben Seghir B, Hammoudi R. Different methods of extraction of bioactive compounds and their effect on biological activity: A review. International Journal of Secondary Metabolite. 2023;10:469–94. <https://doi.org/10.21448/ijsm.1225936>

28. Abubakar AR, Haque M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. J Pharm Bioallied Sci. 2020;12:1–10.

https://doi.org/10.4103/jpbs.JPBS_175_19

29. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. Afr J Tradit Complement Altern Med. 2011;8:1–10.

30. Qarayev E.A, Paşayeva S.A, Hüseynquliyeva K.F, Nəsirli İ.Ü. Toksikologiyada antioksidant fəallıq və təyini sınaqları. Azərbaycan Tibb Universitetinin Jurnalı: Daxili xəstəliklər. 2025;5(1). Available from:

<https://www.atuj.az/uploads/issues/29/174599159742681548.pdf>

31. Nəsirli İ.Ü./Azərbaycan Florasında yayılmış *Thalictrum minus* L. Bitkisinde Makro-və mikroelementlərin orqanlar üzrə paylanması//Azərbaycan Tibb Universitetinin Jurnalı, cild 5 №2,səh.112-121 DOI: <https://doi.org/10.28942/atuj.v5i2y2025.121>

32. Mamedov BS, Mehraliyeva SJ, Suleymanova TH. Comparative Analysis of Antimicrobial and in Vitro Cytotoxic Studies in Regenerative and Antioxidant Containing Products. Am J Biomed Sci Res. 2024;22(6):693-699. doi: 10.34297/AJBSR.2024.22.003004

33. Мамедов М.К., Кадырова А.А., Гудратов Н.О., Мамедов Г.М. Клеточная цитотоксичность

иммуноцитов и методические подходы к ее оценке in vitro. Биомедицина, 2022, N.1, с.14-23.

34. Schlage WK, Bülles H, Kurkowsky B. Use of the HET–CAM Test for the Determination of the Irritant Potential of Cigarette Sidestream Smoke. Toxicology in Vitro. 1999;13:829–35. [https://doi.org/10.1016/S0887-2333\(99\)00059-4](https://doi.org/10.1016/S0887-2333(99)00059-4)

35. Kurban B, Tuncel T, Görgülü Ş, Kar F, Öztürk A, Özek T. Elemi essential oil nanocapsulated drug ameliorates lung cancer via oxidative stress, apoptosis and inflammation pathway. J Cell Mol Med. Wiley Online Library; 2023;27:1887–99.

36. Ziyenok Demirtaş S. Investigating the Impact of Hibiscus Extracts on Paraoxonase and Antioxidant Activities in Diabetic Rats. Online Türk Sağlık Bilimleri Dergisi. 2024;9:246–53.

<https://doi.org/10.26453/otjhs.1475794>

37. Nogueira da Cruz ACS, Madruga Mesquita MJTA, Dantas G kauan R, Silva ER, Falcone De Oliveira VC, Rezende HM, et al. Applicability of Hibiscus sabdariffa L. Extract With Anti-Inflammatory Potential in Human Health: A Review. Food Sci Nutr. 2025;13. <https://doi.org/10.1002/fsn3.70938>

38. Devi KP, Nisha SA, Sakthivel R, Pandian SK. Eugenol (an essential oil of clove) acts as an antibacterial agent against Salmonella typhi by disrupting the cellular membrane. J Ethnopharmacol. 2010;130:107–15. <https://doi.org/10.1016/j.jep.2010.04.025>

39. Pandey VK, Srivastava S, Ashish, Dash KK, Singh R, Dar AH, et al. Bioactive properties of clove (*Syzygium aromaticum*) essential oil nanoemulsion: A comprehensive review. Heliyon. 2024;10:e22437. <https://doi.org/10.1016/j.heliyon.2023.e22437>

40. Wadi MA. Evaluation of antibacterial activity and chemical analysis of clove aqueous extract (*Syzygium aromaticum*). BMC Complement Med Ther. 2025;25:146. <https://doi.org/10.1186/s12906-023-04243-x>

41. Verap D, Ateş S, Karaoğlu E. A Review of Extraction Methods for Obtaining Bioactive Compounds in Plant-Based Raw Materials. Bartın Orman Fakültesi Dergisi. 2023;25:492–513. <https://doi.org/10.24011/barofd.1303285>
42. Mammadov BS. GC-MS analysis of essential oils in antimicrobial and antifungal phytocomposition. Medicine and Science (Tibb və Elm). 2022;2(28):61-66. Available from: [https://jurnal.adhti.edu.az/pdf/No2\(28\).pdf](https://jurnal.adhti.edu.az/pdf/No2(28).pdf). Azerbaijani.
43. Huang H-W, Cheng M-C, Chen B-Y, Wang C-Y. Effects of high pressure extraction on the extraction yield, phenolic compounds, antioxidant and anti-tyrosinase activity of Djulis hull. J Food Sci Technol. 2019;56:4016–24. <https://doi.org/10.1007/s13197-019-03870-y>
44. Tzanova M, Atanasov V, Yaneva Z, Ivanova D, Dinev T. Selectivity of Current Extraction Techniques for Flavonoids from Plant Materials. Processes. 2020;8:1222. <https://doi.org/10.3390/pr8101222>
45. Shi L, Zhao W, Yang Z, Subbiah V, Suleria HAR. Extraction and characterization of phenolic compounds and their potential antioxidant activities. Environmental Science and Pollution Research. 2022;29:81112–29. <https://doi.org/10.1007/s11356-022-23337-6>

YÜKSƏKSÜRƏTLİ EKSTRAKSİYA ÜSULU İLƏ SYZYGIUM AROMATICUM L., ACHILLEA MILLEFOLIUM L. VƏ HIBISCUS SABDARİFFA L. BİTKİLƏRİNDƏN SİNƏRGİSTİK FİTOEKSTRAKTIN İŞLƏNİB HAZIRLANMASI, STANDARTLAŞDIRILMASI VƏ BİOLOJİ EFFEKTİVLİYİNİN TƏDQIQI

Mehraliyeva S.C.^{1*}, Məmmədov B.S.^{1*}, Qədirova H.A.², Süleymanova T.H.², Nəsirli I.Ü.³, Ece Bayır⁴, A. Alper Öztürk⁵

^{1*} Azərbaycan Tibb Universiteti, Əczaçılıq texnologiyası və idarəçiliyi kafedrası, Bakı, Azərbaycan

² Azərbaycan Tibb Universiteti, Tibbi mikrobiologiya və immunologiya kafedrası, Bakı, Azərbaycan.

³ Azərbaycan Tibb Universiteti, Əczaçılıq toksikologiyası və kimya kafedrası, Bakı, Azərbaycan.

⁴Ege Universiteti, Mərkəzi Araşdırma Test və Analiz Laboratoriyası (EGE-MATAL), İzmir, Türkiyə

⁵Anadolu Universiteti, Əczaçılıq Texnologiyası Bölümü, Eskişehir, Türkiyə

* Yazışma üçün əlaqə: prof.nanopharmacy@gmail.com

Xülasə

Bu tədqiqatın məqsədi potensial topikal əczaçılıq və kosmetik tətbiqlər üçün mixək (*Syzygium aromaticum*), boymadərən (*Achillea millefolium*) və hibiskusdan (*Hibiscus sabdariffa*) ibarət çoxfunksiyalı fitoekstrakt hazırlamaq və optimallaşdırmaqdır. Üç fərqli fitokompozisiya hazırlanmış və antimikrob aktivliyi, antioksidant potensialı, fiziki-kimyəvi xüsusiyyətləri, təhlükəsizliyi və uzunmüddətli stabilliyi baxımından müqayisəli şəkildə qiymətləndirilmişdir. Tədqiqat sistemləri arasında mixək, boymadərən və hibiskusdan ibarət tərkib 6:1:3 nisbətində ən əlverişli profil nümayiş etdirmiş və buna görə də optimallaşdırılmış formulasıya kimi seçilmişdir. Ekstraksiya prosesi ekstraksiya metodunun, ekstraksiya müddətinin, bitki-həlləddici nisbətinin, temperaturun, həlləddici tərkibinin və fırlanma sürətinin təsirini araşdırmaqla sisteməlik şəkildə optimallaşdırılmışdır. 95:5 etanol: propilen qlikol qarışığı, xammal-həlləddici nisbəti 1:10 və fırlanma sürəti 3200 dövr/dəq istifadə edilərək 26 °C-də 7 dəqiqə ərzində yüksək sürətli ekstraksiya keyfiyyət, antimikrob aktivliyi və uzunmüddətli stabillik baxımından ən uyğun ekstraktı verdi. Optimallaşdırılmış fitokompozisiya, minimum inhibitor konsentrasiyası 0,25 ml olmaqla *Staphylococcus aureus* və *Escherichia coli*-yə qarşı açıq antimikrob aktivliyi nümayiş

etdirdi, *Candida albicans*-ın tam inhibisiyonu isə 0,50 ml-də əldə edildi. Ekstrakt həmçinin DPPH analizində 1,58 mq/ml IC50 dəyəri ilə güclü antioksidan aktivlik nümayiş etdirdi. Uzunmüddətli stabillik tədqiqatları göstərdi ki, optimallaşdırılmış ekstrakt tünd şüşə qablaşdırmada saxlanıldıqda 24 aya qədər sabit qalıb. Rutin və evgenol tərkibində, quru qalıqda və pH-da yalnız kiçik dəyişikliklər müşahidə edildi, plastik qablarda saxlama isə nəzərəcarpacaq dərəcədə fiziki-kimyəvi pisləşməyə səbəb oldu. Optimallaşdırılmış ekstraktın HET-CAM analizində qıcıqlanmayan olduğu və qıcıqlanma indeksinin $2,45 \pm 0,35$ olduğu aşkar edilmişdir. Ümumilikdə, hazırlanmış fitokompozisiya güclü antimikrob və antioksidant aktivliyi yaxşı stabillik və əla tolerantlıqla birləşdirmişdir. Bu tapıntılar optimallaşdırılmış fitoekstraksın topikal əczaçılıq və kosmetik formulaların hazırlanması üçün perspektivli aktiv tərkib hissəsi kimi xidmət edə biləcəyini göstərir.

Açar sözlər: *Syzygium aromaticum*, *Achillea millefolium*, *Hibiscus sabdariffa*, yüksək sürətli ekstraksiya, antimikrob aktivlik, antioksidan aktivlik, HET-CAM, topikal fitokompozisiya

РАЗРАБОТКА, СТАНДАРТИЗАЦИЯ И ИССЛЕДОВАНИЕ БИОЛОГИЧЕСКОЙ ЭФФЕКТИВНОСТИ СИНЕРГЕТИЧЕСКОГО ФИТОЭКСТРАКТА *SYZYGIUM AROMATICUM* L., *ACHILLEA MILLEFOLIUM* L. И *HIBISCUS SABDARIFFA* L. МЕТОДОМ ВЫСОКОСКОРОСТНОЙ ЭКСТРАКЦИИ

Мехралиева С.Дж.^{1*}, Мамедов Б.С.^{1*}, Гадирова Х.А.², Сулейманова Т.Х.², Насирли И.У.³, Эдже Байыр⁴, А. Алпер Озтюрк⁵

^{1*}Азербайджанский медицинский университет, кафедра фармацевтической технологии и менеджмента, Баку, Азербайджан

²Азербайджанский медицинский университет, кафедра медицинской микробиологии и иммунологии, Баку, Азербайджан

³Азербайджанский медицинский университет, кафедра фармацевтической токсикологии и химии, Баку, Азербайджан

⁴Эгейский университет, Центр прикладных исследований и разработок, Центр испытаний и анализа, Измир, Турция

⁵Анадолуский университет, фармацевтический факультет, кафедра фармацевтической технологии, Эскишехир, Турция

*Для корреспонденции E-mail: prof.nanopharmacy@gmail.com

Резюме

Целью настоящего исследования было разработать и оптимизировать многофункциональный фитоэкстракт, состоящий из гвоздики (*Syzygium aromaticum*), тысячелистника (*Achillea millefolium*) и гибискуса (*Hibiscus sabdariffa*), для потенциального применения в фармацевтике и косметике. Были приготовлены три различных фитокомпозиции, которые были сравнительно оценены с точки зрения антимикробной активности, антиоксидантного потенциала, физико-химических свойств, безопасности и долговременной стабильности. Среди исследованных систем композиция, содержащая гвоздику, тысячелистник и гибискус в соотношении 6:1:3, показала наиболее благоприятный профиль и поэтому была выбрана в качестве оптимизированной рецептуры. Процесс экстракции был систематически оптимизирован путем исследования влияния метода экстракции, времени экстракции, соотношения растения и растворителя, температуры, состава растворителя и скорости вращения. Высокоскоростная экстракция, проведенная в течение 7 минут при 26 °C с использованием смеси этанола и пропиленгликоля в соотношении 95:5, соотношения сырья к растворителю 1:10 и скорости вращения 3200 об/мин, позволила получить наиболее подходящий экстракт с точки зрения качества, антимикробной активности и долговременной стабильности. Оптимизированный

фитокомпонит продемонстрировал выраженную антимикробную активность против *Staphylococcus aureus* и *Escherichia coli* с минимальной ингибирующей концентрацией 0,25 мл, в то время как полное ингибирование *Candida albicans* было достигнуто при 0,50 мл. Экстракт также проявил сильную антиоксидантную активность в тесте DPPH со значением IC₅₀ 1,58 мг/мл. Исследования долговременной стабильности показали, что оптимизированный экстракт оставался стабильным до 24 месяцев при хранении в темной стеклянной упаковке. Наблюдались лишь незначительные изменения содержания рутина и эвгенола, сухого остатка и pH, тогда как хранение в пластиковых контейнерах привело к заметному ухудшению физико-химических свойств. Оптимизированный экстракт оказался не вызывающим раздражения в тесте HET-CAM, с индексом раздражения $2,45 \pm 0,35$. В целом, разработанная фитокомпозиция сочетает в себе сильную антимикробную и антиоксидантную активность с хорошей стабильностью и отличной переносимостью. Эти результаты показывают, что оптимизированный фитоэкстракт может служить перспективным активным ингредиентом для разработки лекарственных препаратов для местного применения и косметических средств.

Ключевые слова: *Syzygium aromaticum*, *Achillea millefolium*, *Hibiscus sabdariffa*, высокоскоростная экстракция, антимикробная активность, антиоксидантная активность, HET-CAM, фитокомпозиция для местного применения

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ANTIMICROBIAL ACTIVITY OF *SALVIA ROSMARINUS* SPENN. INTRODUCED UNDER ABSHERON CONDITIONS AGAINST THE PRINCIPAL ETIOLOGICAL AGENTS OF SCALP DERMATOSES

Gadirova H.A.¹, Aliyeva H.M.^{1*}, Mammadova N.H.^{2*}, Bayramova R.S.¹, Mammadova V.V.², Mehtiyev B.E.²

Abstract

This study investigates the antimicrobial activity of *Salvia rosmarinus* Spenn. cultivated under the conditions of the Absheron peninsula against pathogens associated with scalp dermatoses. The relevance of the research is driven by the high prevalence of these conditions and the increasing antimicrobial resistance of pathogens.

The aim of the study was to comparatively evaluate the antimicrobial activity of essential oil and hydroalcoholic extracts obtained from aerial and underground parts of the plant. Clinical isolates from 78 patients, along with reference strains *Staphylococcus aureus* ATCC 29213 and *Candida albicans* ATCC 10231, were used. Antimicrobial activity was assessed using the disk diffusion method and determination of minimum inhibitory concentration (MIC).

The essential oil, rich in 1,8-cineole and α -pinene, demonstrated the highest antimicrobial activity (MIC 0.5–1.25%). Aerial part extracts showed moderate activity, while root extracts exhibited weak effects. Similar patterns were observed against dermatophytes and yeast-like fungi.

The obtained data confirm the potential of using the essential oil of *Salvia rosmarinus* Spenn. in the development of phytotherapeutic agents for the treatment of infectious dermatoses of the scalp.

Keywords: *Salvia rosmarinus* Spenn, rosemary, antimicrobial activity, essential oil, scalp dermatoses, dermatophytes, phytotherapy

INTRODUCTION

Investigations into the phytochemical composition of *Salvia rosmarinus* Spenn. have demonstrated that different plant organs, including the roots, contribute to the formation of a unified phytocomplex with a broad spectrum of biological activity. Moreover, comprehensive processing of the entire rosemary biomass is regarded as a promising strategy for obtaining biologically active compounds.

Salvia rosmarinus Spenn. (synonym: *Rosmarinus officinalis* L.) is an evergreen aromatic shrub of the family *Lamiaceae*, widely recognized as an essential oil-bearing plant. Under the conditions of the Absheron peninsula, it reaches a height of 1.5–2.0 m. Its leaves are linear and leathery, with prominent essential oil glands. Flowering occurs in May–June and lasts approximately 4–6 weeks, corresponding to the period of maximal



accumulation of secondary metabolites; the flowers are light blue and arranged in false whorls [21].

The biological activity of the essential oil of *S. rosmarinus* is largely attributable to its high content of terpene compounds, the predominant constituents being 1,8-cineole, α -pinene, β -pinene, α -terpinene, linalool, sabinene, and other components [19]. These compounds account for the antioxidant, anti-inflammatory, and antimicrobial properties of the plant [1, 2]. Beyond the essential oil, extracts from both the aerial and underground parts of rosemary are of considerable interest. Extracts derived from the aerial organs (leaves and stems) are characterized by a high content of phenolic compounds, such as rosmarinic acid, carnosic acid, and carnosol. These compounds exhibit pronounced antioxidant activity, inhibit lipid peroxidation, and possess anti-inflammatory properties [1,4]. Rosemary leaf extracts have also been shown to display antimicrobial activity against a broad range of bacterial and fungal pathogens. However, the available data remain fragmentary and require further systematization, particularly with regard to the sensitivity of clinically significant microorganisms to these phytocomponents [20].

Extracts from the underground part (roots) have been investigated to a lesser extent; nevertheless, there is evidence indicating the presence of phenolic acids and other secondary metabolites with antioxidant activity [5]. These compounds are believed to participate in plant adaptation mechanisms under stressful conditions, including abiotic environmental factors [6]. A number of studies have pointed to the potential pharmacological significance of root extracts; however, this field still requires further investigation.

Thus, the biological activity of *S. rosmarinus* is determined not only by its essential oil, but also by a complex of biologically active substances present in extracts of both the aerial and underground parts of the plant, thereby broadening its potential applications in medicine and pharmaceuticals.

Despite the substantial body of research devoted to the antimicrobial properties of the essential oil of *S. rosmarinus* comparative analyses of the activity of different plant parts (aerial and underground) remain insufficiently explored. Data concerning differences in the phytochemical composition of these parts are limited, and their influence on antimicrobial activity against clinical strains isolated from patients with scalp disorders has likewise been inadequately studied. The lack of such investigations is particularly evident in regions with specific climatic and environmental conditions [10]. The city of Baku, a major industrial center of the Caspian region, is characterized by a combination of unfavorable factors, including a dry subtropical climate, high wind activity, and air pollution. Together with the stress-related effects of the urban environment, these conditions may contribute to impairment of the skin barrier

Yazışma üçün əlaqə:

Gadirova H.A.¹, Aliyeva H.M.^{1*}, Mammadova N.H.^{2*}, Bayramova R.S.¹, Mammadova V.V.², Mehtiyev B.E.²

1 Azerbaijan Medical University, Department of Medical Microbiology and Immunology, Baku, Azerbaijan

2 Azerbaijan Medical University, Department of Pharmacognosy, Baku, Azerbaijan

1*For correspondence E-mail: heliyeva@amu.edu.az

2* nmemmedova1@amu.edu.az

and to an increased risk of scalp dermatoses.

Accordingly, it appears warranted to investigate the antimicrobial potential of the essential oil, as well as extracts from the aerial and underground parts of *S. rosmarinus* cultivated in the Absheron region of Azerbaijan, against reference and clinical strains of microorganisms associated with scalp diseases. The results obtained may contribute to the development of novel phytotherapeutic agents for the prevention and treatment of these pathologies.

The aim of the study was to evaluate the antimicrobial activity of the essential oil and extracts from the aerial and underground parts of *S. rosmarinus* against the principal microorganisms responsible for scalp diseases.

To achieve this aim, the following objectives were formulated:

- to perform a comparative analysis of the phytochemical composition of the essential oil and the aerial and underground parts of *S. rosmarinus*;
- to isolate and identify the principal microbial strains from clinical specimens obtained from patients with scalp diseases;
- to assess and compare the antimicrobial activity of the essential oil and extracts of the aerial and underground parts against reference strains and clinical isolates;
- to determine the minimum inhibitory concentrations (MICs) and to conduct a comparative evaluation of the efficacy of the different plant fractions.

MATERIALS AND METHODS

The study used aerial (leaves and shoots) and underground (roots) organs of plants introduced and cultivated on the Absheron peninsula (Azerbaijan). Plant material was collected during the flowering stage in the Baku Dendrological Garden. The harvested

raw material was shade-dried at 25–30°C for 10 days under natural ventilation until an air-dry state was achieved. The dried material was then ground in a laboratory mill to a particle size passing through a 2 mm sieve.

The essential oil was isolated from the aerial parts of the plant by hydrodistillation (steam distillation) according to the Ginzburg method.

Aqueous-alcoholic extracts were prepared separately from the aerial and underground parts of the plant by maceration in 70% ethanol. The use of an aqueous-alcoholic solvent ensured efficient extraction of a broad range of biologically active compounds, including phenolic compounds, flavonoids, and organic acids. The study included clinical specimens obtained from 78 patients with scalp diseases. Samples were collected by sterile scraping of the affected skin areas and by hair epilation using sterile instruments.

Microorganisms were identified using microscopic, cultural, and molecular methods. Microscopic examination was performed with a 10–20% potassium hydroxide (KOH) solution. Fungal isolates, including yeasts of the genus *Malassezia* and dermatophytes of the genera *Trichophyton* and *Microsporum*, were identified on the basis of morphological characteristics and further confirmed by polymerase chain reaction (PCR). *Candida albicans* was identified using the germ tube test and cultivation on chromogenic media. *Malassezia* yeasts were cultured on lipid-dependent media (Dixon agar, Leeming-Notman agar) at 32–34°C. Standard reference strains of *Staphylococcus aureus* ATCC 29213 and *C. albicans* ATCC 10231 were used as control test cultures, thereby ensuring reproducibility of the results and comparability with international data in

accordance with the recommendations of the Clinical and Laboratory Standards Institute and the European Committee on Antimicrobial Susceptibility Testing.

Determination of antimicrobial activity.

The antimicrobial activity of the essential oil and extracts was evaluated by the disk diffusion method and by determination of the minimum inhibitory concentration (MIC) using both clinical isolates and reference strains. Prior to testing, the samples were dissolved in 1–5% dimethyl sulfoxide (DMSO). For the disk diffusion assay, microbial suspensions were standardized to 0.5 McFarland ($\approx 1.5 \times 10^8$ CFU/mL for bacteria and $\approx 1.0 \times 10^6$ CFU/mL for fungi) and inoculated onto solid nutrient media (Mueller–Hinton agar and Sabouraud Dextrose Agar). Sterile paper disks (6 mm) were impregnated with the test samples and placed on the agar surface. Incubation was carried out at 37°C for 24 h for bacteria and at 28–30°C for 48–72 h for fungi. The results were assessed by measuring the diameters of growth inhibition zones (mm). The minimum inhibitory concentration was determined by two-fold serial dilutions in liquid nutrient media (Mueller–Hinton broth and Sabouraud broth). The essential oil was pre-dissolved in DMSO to obtain a stock solution with a concentration of 10% (v/v). The final concentration range was 5.0–0.312% (v/v). MIC was defined as the lowest concentration at which visible microbial growth was completely inhibited. Statistical analysis was performed using GraphPad Prism software. Differences between groups were assessed using Student's t-test and were considered statistically significant at $p < 0.005$.

RESULTS AND DISCUSSION:

The content of essential oil obtained from the aerial parts of *S. rosmarinus* was 2.06% of the absolute dry weight of the raw

material, which is consistent with published data for plants cultivated under warm climatic conditions [22–25].

The high content of oxygenated monoterpenes, primarily 1,8-cineole, underlies the pronounced antimicrobial activity of the essential oil through disruption of microbial cell membrane integrity and inhibition of enzymatic systems [19, 26, 29]. Water-alcohol extracts of the aerial organs were characterized by a higher content of phenolic compounds (including rosmarinic acid and flavonoids), whereas the underground organs contained these compounds in smaller quantities, which determined differences in biological activity [30–33].

The observed differences in chemical composition may be associated with the ecological and geographical conditions of the Absheron peninsula, including high insolation and an arid climate, which influence the biosynthesis of secondary metabolites [34–37].

The study included 78 patients with scalp diseases (mean age 34.6 ± 12.3 years), among whom fungal infections predominated, in agreement with current epidemiological data [38–42]. The most frequently identified taxa were representatives of the genera *Malassezia* (41.0%), *Trichophyton* (28.2%), and *Microsporum* (12.8%), as well as *C. albicans* (17.9%).

Polymicrobial associations were identified in 50% of patients, most commonly combinations of fungal and bacterial flora, including *S. aureus*, indicating the complex nature of scalp infections and the need for an integrated therapeutic approach [38, 42–45].

The antimicrobial activity of the tested samples was assessed against the reference strains *S. aureus* ATCC 29213

and *C. albicans* ATCC 10231 [46]. The essential oil demonstrated pronounced antimicrobial activity: the diameters of growth inhibition zones were 25 mm for *S. aureus* and 20 mm for *C. albicans*, indicating high susceptibility of these microorganisms, as reported in the literature from 2020 to 2026. These data

are comparable to the findings of other studies, in which inhibition zones ranged from 18 to 30 mm [45,47–49] (Table 1). Extracts of the aerial organs exhibited moderate activity, whereas extracts of the underground organs were characterized by weak antimicrobial effects.

Table 1. Antimicrobial activity of *Salvia rosmarinus* Spenn. against reference strains (disk diffusion method)

Microorganism	Essential oil (mm)	Extracts - Aboveground part (mm)	Extracts - Underground part (mm)	DMSO
<i>S. aureus</i> ATCC 29213	22-25	15–18	10–13	5-6
<i>C. albicans</i> ATCC 10231	18-20	14–17	10–12	5-6

The results obtained indicate higher antimicrobial activity in the aerial organs of the plant, which is associated with increased levels of phenolic compounds and flavonoids possessing pronounced antimicrobial properties [50–53].

Investigation of the clinical strains showed that the essential oil exhibited the highest activity among all samples studied. The diameters of inhibition zones ranged from 16 to 25 mm (Table 2).

Table 2. Antimicrobial activity of *Salvia rosmarinus* Spenn. against clinical strains.

Microorganism	Essential oil (mm)	Extracts - aboveground part (mm)	Extracts - belowground part (mm)
<i>S. aureus</i>	22–25	15–18	10–13
<i>C. albicans</i>	18–20	14–17	10–12
<i>Malassezia</i> spp.	18	15	11
<i>Trichophyton</i> spp.	16–17	13–15	10–12
<i>Microsporum</i> spp.	17–18	14–16	11–13

The obtained data demonstrate that antimicrobial activity depends on the type of plant material in the following order: essential oil > aerial parts > underground parts. This pattern is attributable to differences in the accumulation of secondary metabolites in various plant organs and is consistent with contemporary research [54–57].

To quantify antimicrobial activity, the minimum inhibitory concentration (MIC) method was employed.

The essential oil demonstrated the most pronounced activity: the MIC was 0.5% for *S. aureus* and 1.0% for *C. albicans*. For dermatophytes, MIC values ranged from 0.5 to 1.25%, whereas for yeasts they were approximately 1.25%. Extracts obtained from the aerial parts showed moderate

antimicrobial activity (MIC 1.0–2.5%), whereas extracts from the underground parts exhibited weak activity (MIC ≥ 2.0 –2.5%), which may be related to the lower

content of biologically active compounds and the organ-specific features of their distribution [58–61] (Figure 1).

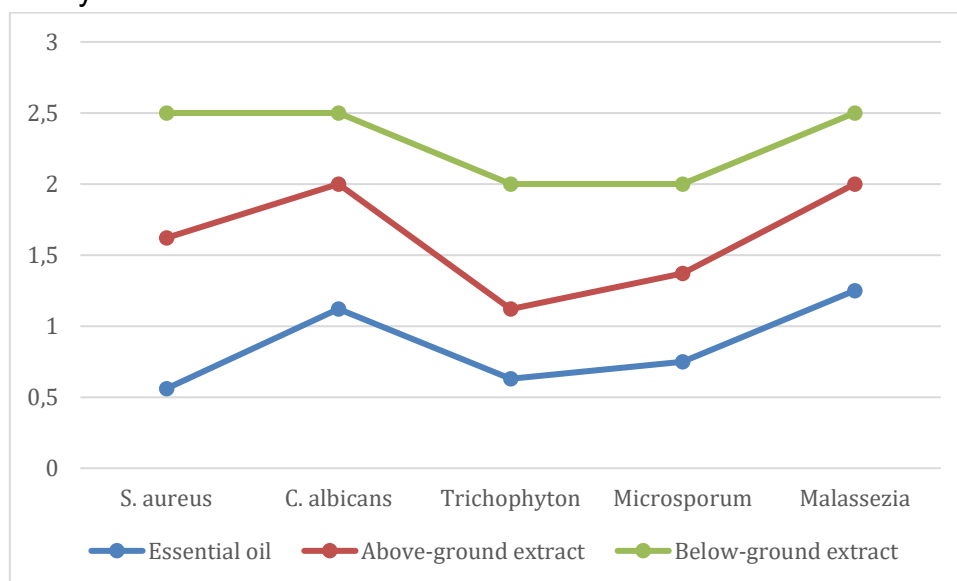


Figure 1. Minimum inhibitory concentrations of *Salvia rosmarinus* Spenn. (MIC)

The high antimicrobial activity of the essential oil is attributable to the presence of monoterpenes (1,8-cineole, α -pinene), which are capable of disrupting cell membrane permeability and inhibiting microbial metabolic processes [62–64].

CONCLUSION.

Thus, the findings of the present study demonstrate that the essential oil of *S. rosmarinus* cultivated in the Absheron region exhibits pronounced antimicrobial activity against both reference and clinical microbial strains, whereas extracts of the aerial parts exert a moderate effect and those of the underground parts only a weak effect. These data confirm the potential applicability of the essential oil in phytotherapeutic and dermatocosmetic formulations intended for the treatment of infectious and inflammatory diseases of the scalp.

REFERENCES

1. Trüeb RM. The scalp microbiome and its role in dermatological disorders. *Dermatology*. 2021;237(5):647–656.
2. Borda LJ, Wikramanayake TC. Seborrheic dermatitis and dandruff: a comprehensive review. *J Clin Investig Dermatol*. 2022;10(1):1–8.
3. Gupta AK, Versteeg SG. Dermatophyte infections in the modern era. *Mycopathologia*. 2020;185(5):747–758.
4. Nenoff P, Verma SB, Ebert A, Süß A, Fischer E, Auerswald E. The current situation of dermatophytoses worldwide. *J Fungi*. 2021;7(2):117.
5. Dréno B, Pécastaings S, Corvec S, Veraldi S, Khammari A, Roques C. *Cutibacterium acnes* (Propionibacterium acnes) and skin diseases. *J Eur Acad Dermatol Venereol*. 2020;34(4):645–654.
6. World Health Organization. Global antimicrobial resistance and use surveillance system (GLASS) report 2023. Geneva: WHO; 2023.

7. Nieto G, Ros G, Castillo J. Antioxidant and antimicrobial properties of rosemary (*Salvia rosmarinus*): a review. *Plants* (Basel). 2022;11(3):345.
8. Man A., Santacroce L., Jacob R., Mare A., Man L. Antimicrobial activity of six essential oils against a group of human pathogens: A comparative study. *Pathogens*. 2019; 8(1): 15. <https://doi.org/10.3390/pathogens8010015>
9. Carneiro V.A., Melo R.S., Pereira A.M.G., Azevedo A.M.A., Matos M.N.C. et al. Essential oils as an innovative approach against biofilm of multidrug-resistant *Staphylococcus aureus*. 2020.
10. Sakkas H., Papadopoulou C. Antimicrobial activity of basil, oregano, and thyme essential oils // *J.Microbiol. Biotechnol.* 2017. Vol. 27, no. 3. P. 429–438. <https://doi.org/10.4014/jmb.1608.08024>.
11. Moumni S., Elaissi A., Trabelsi A., Merghni A., Chraief I., Jelassi B., Chemli R., Ferchichi S. Correlation between chemical composition and antibacterial activity of some Lamiaceae species essential oils from Tunisia // *BMC Complement. Med. Ther.* 2020. Vol. 20, art. 103. <https://doi.org/10.1186/s12906-020-02888-6>.
12. Panahi Y., Taghizadeh M., Marzony E.T., Sahebkar A. Rosemary (*Rosmarinus officinalis*) oil in the treatment of androgenetic alopecia: a randomized comparative trial. *Skinmed.* 2015;13(1):15–21.
13. Koba K., et al. Chemical composition and biological activity of rosemary essential oil. *Food Chem.* 2017;214:462–471.
14. Mousavi S., et al. Topical rosemary oil versus 2% minoxidil for hair growth in androgenetic alopecia. *J Dermatolog Treat.* 2016;27(3):1–7.
15. Al-Sereiti M.R., Abu-Amer K.M., Sen P. Pharmacology of rosemary (*Rosmarinus officinalis* Linn.) and its therapeutic potentials. *Indian J Exp Biol.* 1999;37(2):124–130.
16. Shimizu K., et al. Antioxidant and hair growth-promoting activities of rosemary extract. *Phytother Res.* 2010;24(4):485–490.
17. Bozin B., et al. Antimicrobial properties of rosemary essential oil. *Lett Appl Microbiol.* 2007;44(6):408–412.
18. Farias M.A., et al. Rosemary as a potential agent for hair growth: current evidence and future perspectives. *Dermatol Ther.* 2020;33:e13548.
19. Morphobiological characteristics and essential oil of *Rosmarinus Officinalis* L. introduced in Absheron. *BIO Web of Conferences* 100, 04049 (2024) https://www.bioconferences.org/articles/bioconf/abs/2024/19/bioconf_ifbioscfu2024_04049/bioconf_ifbioscfu2024_04049.html
20. Nieto G., Ros G., Castillo J. (2018). Antioxidant and antimicrobial properties of rosemary (*Rosmarinus officinalis*, L.): A review. DOI: 10.3390/medicines5030098
21. Əsgərov A. Azərbaycanın bitki aləmi. – Bakı : aa TEAS Press, 2016. – 444 s.
22. Elansary H.O., Szopa A., Kubica P., Ekiert H. (2020). *Rosmarinus officinalis* L.: Chemical composition, antioxidant and antimicrobial activity. *Plants*, 9(8), 1040. <https://doi.org/10.3390/plants9081040>
23. Nieto G., Ros G., Castillo J. (2020). Antioxidant and antimicrobial

- properties of rosemary (*Salvia rosmarinus*). *Molecules*, 25(6), 1293. <https://doi.org/10.3390/molecules25061293>
24. Hussain A.I., Anwar F., Sherazi S.T.H., Przybylski R. (2021). Chemical composition and antioxidant activity of rosemary essential oil. *Journal of Essential Oil Research*, 33(2), 120–128. <https://doi.org/10.1080/10412905.2020.1863797>
 25. Yeshiwas Y., Tadesse A. (2022). Effect of environmental factors on essential oil yield of rosemary. *Industrial Crops and Products*, 176, 114318. <https://doi.org/10.1016/j.indcrop.2021.114318>
 26. Borges A., et al. (2020) Antibacterial activity and mode of action of essential oils and their components. *Frontiers in Microbiology* <https://doi.org/10.3389/fmicb.2020.00388> ; Nieto G., et al. (2020)
 27. Antioxidant and antimicrobial properties of rosemary essential oil (*Salvia rosmarinus*). *Molecules* <https://doi.org/10.3390/molecules25061293> ; Hussain A.I., et al. (2021)
 28. Chemical composition of rosemary essential oil and variability with environment. *Journal of Essential Oil Research* <https://doi.org/10.1080/10412905.2020.1863797>
 29. Nazzaro F., et al. (2021) Essential oils and antimicrobial activity. *Molecules* <https://doi.org/10.3390/molecules26020345>
 30. Andrade J.M., et al. (2022) Bioactivity of phenolic compounds in medicinal plants. *Phytomedicine*. <https://doi.org/10.1016/j.phymed.2021.153910>;
 31. Sharma S., et al. (2023) Rosmarinic acid: biological properties and antimicrobial potential. *Food Chemistry*. <https://doi.org/10.1016/j.foodchem.2022.134217>.
 32. Liu X., et al. (2022) Distribution of secondary metabolites in plant organs and ecological regulation. *Industrial Crops and Products* <https://doi.org/10.1016/j.indcrop.2022.114560>;
 33. Alimpić A., et al. (2021) Phytochemical variation in medicinal plants depending on plant organs. *Plants* <https://doi.org/10.3390/plants10081564>
 34. Selmar D., Kleinwächter M. (2020) Stress enhances the synthesis of secondary metabolites in plants. *Plants* <https://doi.org/10.3390/plants9050591>;
 35. Hussain A.I. et al. (2021) Environmental factors affecting essential oil composition of medicinal plants. *Journal of Essential Oil Research*. <https://doi.org/10.1080/10412905.2020.1863797>;
 36. Dong C.J., et al. (2021) Drought stress induces accumulation of phenolic compounds in plants. *Frontiers in Plant Science* <https://doi.org/10.3389/fpls.2021.680998>;
 37. Sánchez-Gómez P., et al. (2022) Geographical variation in essential oil composition of aromatic plants. *Industrial Crops and Products* <https://doi.org/10.1016/j.indcrop.2022.115123>.
 38. Seebacher C. et al. (2021) The dermatophytes: epidemiology and pathogenesis of tinea infections.

- Mycoses
<https://doi.org/10.1111/myc.13250>;
39. Gaitanis G. et al. (2020) The *Malassezia* genus in skin disease. *Frontiers in Cellular and Infection Microbiology*
<https://doi.org/10.3389/fcimb.2020.00140>;
 40. Krcmery V., Barnes A.J. (2021) Non-albicans *Candida* spp. causing infections: epidemiology and clinical relevance. *Clinical Microbiology and Infection*.
<https://doi.org/10.1016/j.cmi.2020.10.029>;
 41. Hay R.J. et al. (2020) Dermatophytosis: epidemiology and treatment update. *The Lancet Infectious Diseases*
[https://doi.org/10.1016/S1473-3099\(20\)30164-8](https://doi.org/10.1016/S1473-3099(20)30164-8);
 42. Gupta A.K. et al. (2022). Fungal infections of the scalp: current epidemiology and management. *Journal of Fungi*.
<https://doi.org/10.3390/jof8040351>.
 43. Totte J.E. et al. (2020) *Staphylococcus aureus* in skin disease: atopic dermatitis and beyond. *Frontiers in Microbiology*.
<https://doi.org/10.3389/fmicb.2020.591853>;
 44. Crespo-Erchiga V., Florencio V.D. (2021) *Malassezia* and bacterial interactions in skin disorders. *Journal of Fungi*
<https://doi.org/10.3390/jof7010006>;
 45. Fox E.P. et al. (2020) Biofilms in mixed bacterial-fungal infections. *Nature Reviews Microbiology*.
<https://doi.org/10.1038/s41579-020-0402-7>;
 46. Mihaylova Y. et al. In vitro study of antimicrobial activity of essential oils of *Lamiaceae* family against *Staphylococcus aureus* ATCC 29213 and *Candida albicans* ATCC 10231. *Proceedings of CBU in Natural Sciences and ICT*.
<https://doi.org/10.12955/pns.v3.318>.
 47. Tkachenko H. et al. (2023) In vitro antibacterial activity of rosemary essential oil against clinical and reference strains. *Scripta Scientifica Pharmaceutica*.
<https://doi.org/10.32999/ksu2524-0838/2023-35-5>;
 48. Kullberg B.J., Arendrup M.C. (2021) Invasive candidiasis. *Clinical Microbiology and Infection*.
<https://doi.org/10.1016/j.cmi.2020.10.029>;
 49. Nieto G. et al. (2020) *Rosmarinus officinalis* essential oil: chemical composition and antimicrobial activity. *Molecules*.
<https://doi.org/10.3390/molecules25061293>.
 50. Daglia M. (2021) *Molecules*. Polyphenols as antimicrobial agents in plant-based systems
<https://doi.org/10.3390/molecules26092300>;
 51. Cushnie T.P.T., Lamb A.J. (2020) *International Journal of Antimicrobial Agents*
Recent advances in understanding antibacterial properties of flavonoids
<https://doi.org/10.1016/j.ijantimicag.2020.106010> ;
 52. Alimpić A. et al. (2021). *Plants (MDPI)* Phytochemical variability among plant organs in medicinal species.
<https://doi.org/10.3390/plants10081564>;
 53. Xu L., Wang X. (2025). *International Journal of Molecular Sciences* Distribution of secondary metabolites in plant organs under environmental regulation
<https://doi.org/10.3390/ijms26125767>.
 54. Jin D. et al. (2020) Secondary metabolites profiled in

- different plant organs (leaves, stems, roots).
Scientific Reports.
<https://doi.org/10.1038/s41598-020-60172-6>;
55. Pang Z. et al. (2021). Linking plant secondary metabolites and plant microbiomes. *Frontiers in Plant Science*.
<https://doi.org/10.3389/fpls.2021.621276>;
56. Zhou P. et al. (2024). Spatial metabolome of aerial and underground plant parts. *Phytomedicine*.
<https://doi.org/10.1016/j.phymed.2023.155259>
57. Palazon J., Alcalde M.A. (2025). Secondary metabolites in plants. *Plants*.
<https://doi.org/10.3390/plants14142146>
58. Elshafie H.S., Camele I. (2023). A comprehensive review on plant secondary metabolites and their biological activity. *International Journal of Molecular Sciences*.
<https://doi.org/10.3390/ijms24043266>;
59. Yang W. et al. (2024). Plant secondary metabolites-mediated plant defense against bacteria and fungi pathogens. *Plant Physiology and Biochemistry*.
<https://doi.org/10.1016/j.plaphy.2024.109224>;
60. Li X.C. et al. (2024). Antimicrobial secondary metabolites: sources and opportunities. *Frontiers in Plant Science*.
<https://doi.org/10.3389/fpls.2024.1382604>;
61. Zhang Y. et al. (2026). The rhizosphere as a source of bioactive compounds. *Rhizosphere*.
<https://doi.org/10.1016/j.rhisph.2026.101298>.
62. Merghni A. et al. (2023). 1,8-Cineol (Eucalyptol) disrupts membrane integrity and induces oxidative stress in MRSA. *Antioxidants*.
<https://doi.org/10.3390/antiox12071388>;
63. Shahina Z. et al. (2022). Rosemary essential oil and its components induce cell death in *Candida albicans*. *PLOS ONE*.
<https://doi.org/10.1371/journal.pone.0277097>;
64. Sharifi-Rad J. et al. (2021). Natural products and antimicrobial mechanisms of essential oils. *Frontiers in Microbiology*.
<https://doi.org/10.3389/fmicb.2021.621276>;
65. Silva N.C.C., Fernandes Júnior A. (2020). Biological properties of medicinal plant terpenes. *Molecules*.
<https://doi.org/10.3390/molecules25163663>;

SAÇ DƏRİSİNİN DERMATOZLARININ ƏSAS ETIOLOJİ TÖRƏDİCİLƏRİNƏ QARŞI, ABŞERON ŞƏRAİTİNDƏ İNTRODUKSIYA OLUNMUŞ *SALVIA ROSMARINUS* SPENN. BITKİSİNİN ANTİMİKROB AKTİVLİYİ

Qədirova H.A.¹, Əliyeva H.M.^{1*}, Bayramova R.S.¹, Məmmədova N.H.^{2*}, Məmmədova V.V.², Mehtiyev B.E.²

¹Azərbaycan Tibb Universiteti, tibbi Mikrobiologiya və İmmunologiya kafedrası, Bakı, Azərbaycan,

² Azərbaycan Tibb Universiteti, Farmakoqnoziya kafedrası, Bakı, Azərbaycan,

^{1*} Yazışma üçün əlaqə: heliyeva@amu.edu.az

^{2*} nmemmedova1@amu.edu.az

Xülasə

Tədqiqat Abşeron yarımadası şəraitində introduksiya olunmuş *Salvia rosmarinus* Spenn. bitkisinin saç dərisinin dermatozlarının törədicilərinə qarşı antimikrob aktivliyinin öyrənilməsinə həsr edilmişdir. İşin aktuallığı bu xəstəliklərin geniş yayılması və mikroorqanizmlərin ənənəvi antimikrob preparatlara qarşı artan davamlılığı ilə bağlıdır.

Tədqiqatın məqsədi *Salvia rosmarinus* Spenn. bitkisinin efir yağı, həmçinin yerüstü və yeraltı hissələrindən alınmış su-spirit ekstraktlarının antimikrob aktivliyinin müqayisəli qiymətləndirilməsi olmuşdur. Tədqiqat obyektlərini 78 pasiyentdən ayrılmış klinik mikroorqanizm ştammları, eləcə də *Staphylococcus aureus* və *Candida albicans* referent kulturaları təşkil etmişdir, maddələrin aktivliyi isə disk-diffuziya üsulu və minimal inhibəedici konsentrasiyanın (MIC) təyini metodu ilə qiymətləndirilmişdir.

Müəyyən edilmişdir ki, tədqiq olunan efir yağı ən yüksək antimikrob aktivlik göstərmişdir (MIC 0,5–1,25%). Yerüstü hissələrin ekstraktları orta dərəcədə təsir göstərdiyi halda, yeraltı hissələr zəif aktivlik nümayiş etdirmişdir. Oxşar nəticələr dermatofitlər və mayayabənzər göbələklər üçün də müşahidə edilmişdir.

Əldə edilmiş nəticələr *Salvia rosmarinus* Spenn. efir yağının saç dərisinin infeksiya dermatozlarının müalicəsi üçün fitoterapevtik vasitələrin hazırlanmasında perspektivli olduğunu təsdiqləyir.

Açar sözlər: *Salvia rosmarinus* Spenn., rozmarin, antimikrob aktivlik, efir yağı, saç dərisinin dermatozları, dermatofitlər, fitoterapiya

АНТИМИКРОБНАЯ АКТИВНОСТЬ SALVIA ROSMARINUS SPENN. ИНТРОДУЦИРОВАННОГО В УСЛОВИЯХ АПШЕРОНА, ПРОТИВ ОСНОВНЫХ ЭТИОЛОГИЧЕСКИХ ВОЗБУДИТЕЛЕЙ ДЕРМАТОЗОВ КОЖИ ГОЛОВЫ

Гадирова Х.А.¹, Алиева Х.М.^{1*}, Байрамова Р.С.¹, Мамедова Н.Х.^{2*}, Мамедова В.В.², Мехмиев Б.Е.²

¹Азербайджанский Медицинский Университет, Кафедра медицинской микробиологии и иммунологии, Баку

² Азербайджанский Медицинский Университет, Кафедра фармакогнозии, Баку

^{1*}Для корреспонденции E-mail: heliyeva@amu.edu.az

^{2*} nmemmedova1@amu.edu.az

Резюме

Исследование посвящено изучению антимикробной активности *Salvia rosmarinus* Spenn., культивируемого в условиях Апшеронского полуострова, в отношении возбудителей дерматозов волосистой части головы. Актуальность работы обусловлена высокой распространённостью данных заболеваний и ростом устойчивости микроорганизмов к традиционным противомикробным препаратам.

Целью исследования являлась сравнительная оценка антимикробной активности эфирного масла и водно-спиртовых экстрактов надземной и подземной частей растения. Объектами исследования служили клинические штаммы микроорганизмов, выделенные у 78 пациентов, а также референтные культуры *Staphylococcus aureus* и *Candida albicans*. Активность оценивали диско-диффузионным методом и методом определения минимальной ингибирующей концентрации (MIC).

Установлено, что эфирное масло проявляет наиболее выраженную антимикробную активность (MIC 0,5–1,25%). Экстракты надземной части характеризуются умеренным эффектом, тогда как подземные органы демонстрируют слабую активность. Аналогичные результаты получены для дерматофитов и дрожжеподобных грибов.

Полученные данные подтверждают перспективность использования эфирного масла *Salvia rosmarinus* Spenn., в разработке фитотерапевтических средств для лечения инфекционных дерматозов волосистой части головы.

Ключевые слова: *Salvia rosmarinus* Spenn., розмарин лекарственный, антимикробная активность, эфирное масло, дерматозы волосистой части головы, дерматофиты, фитотерапия

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SAFETY AND CLINICAL OUTCOMES OF SIMULTANEOUS ABDOMINAL PROCEDURES DURING LAPAROSCOPIC CHOLECYSTECTOMY

Zeynalov S.M., Zeynalov B.M., İbrahimova A.A., Namazov A.E., İmanova S.S., Qapaqov F.M., Rəfiyev S.F., Bayramov N.Y.

Abstract

Gallstone disease remains one of the most common surgical conditions worldwide, and laparoscopic cholecystectomy is widely accepted as the gold standard for its treatment. A considerable proportion of patients undergoing laparoscopic cholecystectomy present with concomitant abdominal or pelvic pathologies, raising the clinical question of whether simultaneous surgical procedures can be safely performed during the same operative session. This retrospective observational study analyzed clinical outcomes in patients who underwent laparoscopic cholecystectomy combined with simultaneous abdominal surgical procedures between 2015 and 2025. A total of 410 patients were included. Demographic characteristics, types of simultaneous procedures, surgical approaches, postoperative complications, and length of hospital stay were evaluated using descriptive statistical methods. Results. Female patients predominated (83.4%), and the majority belonged to middle-aged and elderly groups. The most frequently performed simultaneous procedures were gynecological operations and abdominal wall hernia repairs. A completely laparoscopic approach was used in 70.48% of cases, whereas a combined laparoscopic–open strategy was required in the remaining patients. Postoperative complications occurred in 2.7% of cases and were limited to minor events corresponding to Clavien–Dindo grades I–II. No mortality was observed. Patients treated with a fully laparoscopic approach had shorter hospital stay and faster postoperative recovery.

Simultaneous abdominal surgical procedures performed during laparoscopic cholecystectomy appear to be safe and effective in appropriately selected patients. The findings suggest that this strategy may reduce the need for repeated anesthesia and hospitalization, thereby offering potential clinical and socioeconomic benefits.

Keywords: *Laparoscopic cholecystectomy, simultaneous surgery, gallstone disease, abdominal surgical procedures, minimally invasive surgery, surgical outcomes*

INTRODUCTION

Gallstone disease is one of the most

prevalent disorders of the hepatobiliary system and represents a major public health and socioeconomic burden worldwide. Epidemiological studies indicate that its prevalence ranges from 10% to 20% in the adult population and increases with age, female sex, obesity, metabolic syndrome, and dietary factors [1].

Yazışma üçün əlaqə:

Zeynalov S.M., Zeynalov B.M., İbrahimova A.A.,
Namazov A.E., İmanova S.S., Qapaqov F.M., Rəfiyev
S.F., Bayramov N.Y.

Azerbaijan Medical University, Department of Surgical
Diseases I, Baku, Azerbaijan

*For correspondence E-mail: dr.a.ibrahimova@gmail.com



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Laparoscopic cholecystectomy is currently recognized as the gold standard for the treatment of symptomatic gallstone disease, because of its low morbidity, short hospital stays and rapid postoperative recovery [2]. With increasing life expectancy and the growing prevalence of comorbid conditions, many patients scheduled for laparoscopic cholecystectomy also present with concomitant abdominal or pelvic diseases requiring surgical treatment [3].

Traditionally, these disorders have been managed in separate operative sessions. However, staged surgery requires repeated anesthesia, additional hospitalization, delayed recovery, and increased healthcare costs. Simultaneous (one-stage) surgery has therefore emerged as an attractive alternative, enabling treatment of multiple pathologies during a single operative session while potentially reducing overall treatment time and improving patient convenience [2–6].

Although recent studies have demonstrated that simultaneous laparoscopic procedures are generally safe and feasible in appropriately selected patients, the available evidence remains heterogeneous because of differences in patient selection, operative techniques, and outcome assessment [5,13,15]

A major limitation is the lack of unified clinical guidelines and standardized recommendations regarding indications, contraindications, optimal sequencing of surgical steps, and risk factors influencing outcomes. Consequently, decision-making often relies on individual surgeon experience [13,18].

Therefore, analyzing clinical outcomes of simultaneous abdominal procedures performed during laparoscopic cholecystectomy in a large patient cohort is of significant scientific and practical importance.

MATERIALS AND METHODS

This retrospective observational clinical study included 410 consecutive patients who underwent simultaneous abdominal procedures during laparoscopic cholecystectomy between 2015 and 2025. The patients' age ranged from 28 to 86 years, with females predominating (83.4%). Age and sex distribution are presented in Table 1.

Preoperative evaluation included medical history, physical examination, routine laboratory investigations, abdominal ultrasonography, and additional imaging studies when clinically indicated. The indication for simultaneous surgery was established individually according to the patient's clinical condition, concomitant pathology, and the surgeon's judgment.

Table 1. Age and Sex Distribution of Patients

Age group	Male n (%)	Female n (%)	Total n (%)
≤30	4 (0.98)	31 (7.56)	35 (8.54)
31–40	8 (1.95)	65 (15.85)	73 (17.80)
41–50	14 (3.42)	76 (18.53)	90 (21.95)
51–60	21 (5.12)	56 (13.66)	77 (18.78)
61–70	15 (3.66)	71 (17.32)	86 (20.98)
>70	6 (1.46)	43 (10.49)	49 (11.95)

Comorbidities were considered when determining surgical tactics. The most common conditions included arterial

hypertension, diabetes mellitus, ischemic heart disease, and chronic respiratory diseases.

Clinical data were extracted retrospectively from medical records and analyzed using descriptive statistical methods. Continuous variables are presented as ranges,

whereas categorical variables are expressed as absolute numbers (n) and percentages (%).

Table 2. Structure of Comorbidities

Comorbidity	n (%)
Hypertension	93 (22.7)
Ischemic heart disease	43 (10.5)
Diabetes mellitus	65 (15.9)
Chronic bronchitis/COPD	43 (10.5)

Comorbidities were considered when determining surgical tactics. The most common conditions included arterial hypertension, diabetes mellitus, ischemic heart disease, and chronic respiratory diseases.

All patients underwent laparoscopic cholecystectomy as the primary procedure. Additional simultaneous interventions were performed during the same operative session according to the type of concomitant abdominal or pelvic pathology

Table 3. Types of Simultaneous Procedures

Procedure	N
LC + gynecological surgery	214
LC + abdominal wall hernia repair	72
LC + adhesiolysis	49
LC + appendectomy	7
LC + liver resection/cyst	5
Others	63

According to the surgical approach, patients were classified into two groups:
Group I – Fully laparoscopic approach

Group II – Combined laparoscopic and open approach

Table 4. Surgical Approach Distribution

Group	Approach	n (%)
I	Full laparoscopic	289 (70.48)
II	Laparoscopic + open	121 (29.52)

Preoperative evaluation included detailed medical history, physical examination, routine laboratory tests, abdominal ultrasonography, and additional imaging studies when indicated. Surgical strategy (fully laparoscopic or combined laparoscopic–open approach) was determined according to the type of

concomitant pathology, technical feasibility, and intraoperative findings.

Data were analyzed using descriptive statistical methods and are presented as absolute numbers (n) and percentages (%). No inferential statistical analysis was performed because of the descriptive retrospective design of the study.

RESULTS

Among 410 patients, 342 (83.4%) were female and 68 (16.6%) were male. The majority of patients belonged to the middle-aged and elderly groups (Table 1).

Gynecological procedures constituted the largest proportion of simultaneous interventions (214 cases), followed by abdominal wall hernia repair (72 cases), adhesiolysis (49 cases), appendectomy (7 cases), and liver procedures (5 cases) (Table 3).

A fully laparoscopic approach was used in 289 patients (70.48%), whereas 121 patients (29.52%) required a combined laparoscopic–open approach (Table 4)

The most frequently observed comorbidities were hypertension (22.7%), diabetes mellitus (15.9%), and ischemic heart disease (10.5%) (Table 2).

Postoperative complications occurred in 11 patients (2.7%). These were mild and managed conservatively, including superficial wound inflammation and residual pain syndrome. All complications were classified as Clavien–Dindo Grade I–II, and no mortality was recorded.

The mean length of hospital stay was 2.8 ± 0.9 days in the fully laparoscopic group and 4.1 ± 1.2 days in the combined laparoscopic–open group ($p = 0.003$).

DISCUSSION

Simultaneous abdominal surgery during laparoscopic cholecystectomy remains a topic of growing interest in minimally invasive surgery. The present study demonstrates that, in carefully selected patients, performing multiple procedures in a single session does not significantly increase perioperative risk. These findings are consistent with previous studies reporting that simultaneous laparoscopic procedures can be performed safely without increasing postoperative morbidity [1–6,13].

The complication rate of 2.7% confirms the acceptable safety profile of this approach and is consistent with literature data reporting complication rates between 2–5%, mostly mild and manageable conservatively [2,5,13].

The type of surgical approach appeared to influence postoperative outcomes. The fully laparoscopic technique was associated with shorter hospitalization and faster recovery, confirming the advantages of minimally invasive surgery. However, because only descriptive statistical analysis was performed, these findings should be interpreted with caution.

The predominance of gynecological procedures and hernia repairs reflects the high prevalence of these conditions, particularly among women with gallstone disease. These combined procedures are technically feasible due to anatomical proximity and have also been reported by other authors as the most frequently performed simultaneous interventions [4,5,15–18].

Simultaneous surgery also offers economic advantages by reducing hospitalization, anesthesia exposure, and recovery time, although economic parameters were not directly analyzed in this study. A major concern is prolonged operative time; however, advances in laparoscopic techniques and anesthesia have minimized its clinical impact [13,18].

Patient selection remains crucial. High-risk patients with severe cardiopulmonary conditions require careful evaluation. The optimal sequence of surgical steps remains controversial and should be individualized based on patient condition and technical considerations.

The present study has several limitations. Its retrospective single-center design and the absence of inferential statistical analysis limit the strength and generalizability of the conclusions.

Therefore, prospective multicenter studies with comparative statistical analyses are warranted to further validate these findings.

CONCLUSION

Simultaneous abdominal procedures during laparoscopic cholecystectomy appear to be a safe and effective treatment strategy in appropriately selected patients. In the present study, postoperative complications were infrequent and limited to Clavien–Dindo Grade I–II events, and no mortality was observed. Fully laparoscopic techniques were associated with shorter hospitalization and better recovery outcomes.

The findings suggest that simultaneous surgery may reduce the need for repeated anesthesia and hospitalization, thereby offering potential clinical and socioeconomic benefits.

Further prospective multicenter studies with comparative statistical analyses are needed to confirm these findings and establish standardized recommendations for patient selection and surgical strategy

REFERENCES

1. Savita KS, Khedkar I, Bhartia VK. Combined procedures with laparoscopic cholecystectomy. *Indian J Surg.* 2010;72(5):377–380.
2. Orr NT, Davenport DL, Roth JS. Outcomes of simultaneous laparoscopic cholecystectomy and ventral hernia repair compared to laparoscopic cholecystectomy alone. *Surg Endosc.* 2013;27(1):67–73.
3. Sarli L, Villa F, Marchesi F. Hernioplasty and simultaneous laparoscopic cholecystectomy: a prospective randomized study of open tension-free versus laparoscopic inguinal hernia repair. *Surgery.* 2001;129(5):530–536.
4. Brekhov EI, Savinova EB, Lebedeva EA. Experience of simultaneous laparoscopic cholecystectomy and gynecologic operations. *Khirurgiia (Mosk).* 2010;(12):23–26.
5. Claus CMP, Ruggeri JRB, Ramos EJB, et al. Simultaneous laparoscopic inguinal hernia repair and cholecystectomy: does it cause mesh infection? *ABCD Arq Bras Cir Dig.* 2021;34(2):e1600.
6. Lehmann A, Piatkowski J, Nowak M, et al. Simultaneous TAPP and cholecystectomy. *Pol Przegl Chir.* 2014;86(2):73–76.
7. Savoliuk SI, et al. The influence of simultaneous laparoscopic interventions in abdominal surgery on postoperative outcomes. *Emergency Medicine (Ukraine).* 2025;11(2):23–29.
8. Parkhomenko KY, et al. Laparoscopic simultaneous operations: indications and results. *J Educ Health Sport.* 2020;10(4):13–17.
9. Boyko VV, et al. Simultaneous surgical operations and hernia repair: analysis of results. *J Educ Health Sport.* 2021;11(7):26–34.
10. Savita KS, Resutra R, et al. Laparoscopic cholecystectomy in combination with other abdominal procedures: safety and feasibility. *Int Surg J.* 2019;6(11):3991–3995.
11. Panchal JH, et al. A study of laparoscopic (simple or complex) cholecystectomy with or without ERCP and CBD stenting associated with other general, bariatric, gynecological and renal surgical procedures in a single session. *Medtigo J.* 2025; Volume (Issue):25–29.
12. Aljohani E, et al. Safety of simultaneous laparoscopic cholecystectomy and hernia repair: a meta-analysis. *Heliyon.* 2025;S2405-8440(25)01965-6.

13. Nakamoto H, et al. Simultaneous laparoscopic cholecystectomy and endoscopic full-thickness resection for colonic lesions (CELS). *Case Rep Gastroenterol.* 2019;13(3):76–87.
14. Hart S, et al. Laparoendoscopic single-site combined cholecystectomy and hysterectomy. *J Minim Invasive Gynecol.* 2011;18(4): 430
15. Gümüş İl, et al. Minimally invasive approach for combined gynecologic procedures and laparoscopic cholecystectomy. *Gynecol Obstet Reprod Med.* 2010;16(3):18-21
16. Gençtürk M, et al. Simultaneous scarless laparoscopic cholecystectomy with abdominoplasty. *Genel Tıp Dergisi.* 2018;28(3):9–14.
17. Earle D, et al. Guidelines for laparoscopic ventral hernia repair. *Surg Endosc.* 2016;30(8):3163–3183.
18. Liang B, Dai M, Zou Z. Safety and efficacy of antibiotic prophylaxis in patients undergoing elective laparoscopic cholecystectomy: a systematic review and meta-analysis. *J Gastroenterol Hepatol.* 2016;31(5):921–928.

LAPAROSKOPIK XOLESISTEKTOMIYA ZAMANI SIMULTAN ABDOMINAL ƏMƏLIYYATLARIN TƏHLÜKƏSİZLİYİ VƏ KLİNİK NƏTİCƏLƏRİ

Zeynalov S.M., Zeynalov B.M., İbrahimova A.A., Namazov A.E., İmanova S.S., Qapaqov F.M., Rəfiyev S.F., Bayramov N.Y.

Azərbaycan Tibb universiteti, I Cərrahi xəstəliklər kafedrası, Bakı, Azərbaycan

** Yazışma üçün əlaqə E-mail: dr.a.ibrahimova@gmail.com*

Xülasə

Öd daşı xəstəliyi dünyada ən geniş yayılmış cərrahi patologiyalardan biri olaraq qalmaqdadır və laparoskopik xolesistektomiya onun müalicəsində “qızıl standart” hesab olunur. Laparoskopik xolesistektomiya icra olunan pasiyentlərin əhəmiyyətli hissəsində yanaşı abdominal və ya kiçik çanaq patologiyalarının mövcudluğu eyni əməliyyat seansında simultan cərrahi müdaxilələrin təhlükəsizliyinin və məqsədəuyğunluğunun qiymətləndirilməsini aktuallaşdırır.

Bu retrospektiv müşahidə xarakterli tədqiqatda 2015–2025-ci illər ərzində laparoskopik xolesistektomiya zamanı simultan abdominal cərrahi əməliyyatlar icra edilmiş 410 pasiyentin klinik nəticələri təhlil edilmişdir. Demografik göstəricilər, simultan əməliyyatların növləri, cərrahi taktika, əməliyyatsonrası ağrılaşmalar və hospitalizasiya müddəti deskriptiv statistik üsullarla qiymətləndirilmişdir.

Pasiyentlərin 83,4%-ni qadınlar təşkil etmiş, əksəriyyəti isə orta və yaşlı yaş qruplarına aid olmuşdur. Ən çox icra edilən simultan əməliyyatlar ginekoloji cərrahi müdaxilələr və qarın ön divarı yırtıqlarının plastikası olmuşdur. Xəstələrin 70,48%-də əməliyyatın bütün mərhələləri tam laparoskopik üsulla həyata keçirilmişdir. Əməliyyatsonrası ağrılaşmalar 2,7% hallarda müşahidə edilmiş və yalnız Clavien–Dindo təsnifatına görə I–II dərəcəli yüngül ağrılaşmalar qeydə alınmışdır. Letallıq halı müşahidə edilməmişdir. Tam laparoskopik yanaşma daha qısa hospitalizasiya müddəti və daha sürətli əməliyyatsonrası bərpa ilə əlaqələndirilmişdir.

Laparoskopik xolesistektomiya zamanı simultan abdominal əməliyyatlar düzgün seçilmiş pasiyentlərdə təhlükəsiz və effektiv cərrahi yanaşma hesab oluna bilər. Əldə olunan nəticələr bu yanaşmanın təkrar narkoz və hospitalizasiya ehtiyacını azalda biləcəyini, bununla da klinik və potensial sosial-iqtisadi üstünlüklər təmin edə biləcəyini göstərir. Bu nəticələrin təsdiqlənməsi və pasiyent seçimi ilə cərrahi

taktikanın standartlaşdırılması üçün müqayisəli statistik təhlilləri əhatə edən gələcək prospektiv, çoxmərkəzli tədqiqatlara ehtiyac vardır.

Açar sözlər: *laparoskopik xolesistektomiya; simultan cərrahiyyə; öd daşı xəstəliyi; abdominal cərrahi əməliyyatlar; minimal invaziv cərrahiyyə; cərrahi nəticələr*

БЕЗОПАСНОСТЬ И КЛИНИЧЕСКИЕ ИСХОДЫ ОДНОВРЕМЕННЫХ АБДОМИНАЛЬНЫХ ОПЕРАЦИЙ ПРИ ЛАПАРОСКОПИЧЕСКОЙ ХОЛЕЦИСТЭКТОМИИ

Зейналов С.М., Зейналов Б.М., Ибраимова А.А., Намазов А.Е., Иманова С.С., Гапагов Ф.М., Рафиев С.Ф., Байрамов Н.Ю.

Азербайджанский медицинский университет, Кафедра хирургических болезней I, Баку, Азербайджан

** Для корреспонденции E-mail: dr.a.ibrahimova@gmail.com*

Резюме

Желчнокаменная болезнь остается одним из наиболее распространенных хирургических заболеваний в мире, а лапароскопическая холецистэктомия широко признана «золотым стандартом» ее лечения. Значительная часть пациентов, которым выполняется лапароскопическая холецистэктомия, имеет сопутствующие патологии органов брюшной полости или малого таза, что ставит вопрос о возможности безопасного выполнения симультанных хирургических вмешательств в рамках одной операции. В данном ретроспективном обсервационном исследовании были проанализированы клинические исходы лечения пациентов, перенесших лапароскопическую холецистэктомию с симультанными хирургическими вмешательствами на органах брюшной полости в период с 2015 по 2025 год. В исследование было включено 410 пациентов. Оценивались демографические характеристики, виды симультанных вмешательств, хирургические подходы, послеоперационные осложнения и длительность госпитализации с использованием методов описательной статистики. Среди пациентов преобладали женщины (83,4%), при этом большинство относилось к группе среднего и пожилого возраста. Наиболее часто выполняемыми симультанными вмешательствами были гинекологические операции и операции по поводу грыж брюшной стенки. Полностью лапароскопический подход был применен в 70,48% случаев, тогда как в остальных случаях использовался комбинированный лапароскопически-открытый подход. Послеоперационные осложнения наблюдались в 2,7% случаев и ограничивались осложнениями I–II степени по классификации Clavien–Dindo. Летальных исходов зарегистрировано не было. Полностью лапароскопический подход ассоциировался с более коротким сроком госпитализации и более быстрым послеоперационным восстановлением.

Одновременные абдоминальные хирургические вмешательства, выполняемые при лапароскопической холецистэктомии, представляются безопасной и эффективной хирургической тактикой у правильно отобранных пациентов. Полученные результаты позволяют предположить, что данный подход может снизить необходимость повторной анестезии и госпитализации, обеспечивая потенциальные клинические и социально-экономические преимущества. Для подтверждения полученных результатов необходимы дальнейшие проспективные многоцентровые исследования с применением сравнительного статистического анализа.

Ключевые слова: *лапароскопическая холецистэктомия; симультанные операции; желчнокаменная болезнь; абдоминальные хирургические вмешательства; малоинвазивная хирургия; хирургические исходы.*

ORIJINAL MƏQALƏ

AÇIQ GİRİŞ (OPEN ACCESS)

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UROLOGICAL PRESENTATIONS TO A PEDIATRIC SURGERY OUTPATIENT CLINIC: CHARACTERISTICS, DIAGNOSES, AND CLINICAL DECISIONS

Atilla Şenaylı*, Sevgi Ulusoy Tangül²

Abstract

Aim: To evaluate the demographic characteristics, diagnostic patterns, presenting complaints, and clinical decision-making processes of children presenting with urological complaints to a pediatric surgery outpatient clinic. This retrospective study included 156 children who presented with urological complaints. Patients with non-urological ICD-10 codes or whose families declined consent were excluded. Data on age, sex, presenting complaints, ICD-10 diagnoses, laboratory investigations, follow-up intervals, and final clinical decisions were analyzed. Statistical analyses included the Mann–Whitney U test, Kruskal–Wallis test, chi-square and Fisher’s exact tests, and multinomial logistic regression. Analyses were performed using *jamovi* 2.7.32.0 statistics package. The study was conducted in accordance with the Declaration of Helsinki, and informed consent was obtained from the parents or legal guardians. Results of the 156 patients, 115 (73.7%) were male and 41 (26.3%) were female. Median admission age was significantly lower in males (3 years, IQR 2–5) than in females (8 years, IQR 5–12) ($p < 0.001$). The most frequent diagnoses were N39.0 (36.5%), N39.9 (31.4%), and R39.8 (14.7%). Presenting complaints differed significantly by sex ($p < 0.001$), mainly due to penile and circumcision-related problems in boys. Dysuria occurred at similar rates in both sexes. Clinical decisions showed no significant association with the initial ICD-10 diagnosis ($p = 0.756$). Multinomial logistic regression indicated that laboratory investigations, rather than age or sex, were more strongly associated with subsequent management decisions. Boys predominate among pediatric urological presentations and present at a younger age than girls. Initial outpatient diagnoses are largely symptom-based and non-specific. Clinical decisions appear to be guided more by investigation results and individual clinical course than by the preliminary diagnostic code. Age- and sex-sensitive evaluation strategies may improve the efficiency of outpatient pediatric urological care.

Keywords: Pediatrics; Urology; Outpatients; Urinary Tract Infections; Clinical Decision-Making

INTRODUCTION

Pediatric urology encompasses disorders of the urinary tract and genital organs from infancy through adolescence. These conditions range from urinary tract infections to congenital genital anomalies

and may influence both physical health and psychosocial development. Common problems encountered in this age group include urinary tract infections, hypospadias, phimosis, and enuresis. Early recognition and appropriate management



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of urinary tract infection are essential to prevent complications such as pyelonephritis (1). Urinary tract infections may also occur frequently in children without underlying bladder dysfunction (1-3).

The literature consistently indicates that the pattern of urological complaints in children varies according to age and sex. Congenital anomalies predominate in infancy and early childhood, whereas infectious and functional disorders become more prominent during school age. The epidemiological profile of adolescents may further differ from that of younger children (4). Functional conditions such as nocturnal enuresis affect 15–20% of children around the age of five and decline progressively with advancing age.

Understanding the distribution of these problems by age and sex is important because it directly influences diagnostic pathways and treatment strategies. Epidemiological evaluations also contribute to assessing disease burden and comparing the cost-effectiveness of different management approaches (5). In addition, a systematic initial evaluation by the physician facilitates appropriate multidisciplinary involvement when required (5). The epidemiology of pediatric urological disorders is further shaped by geographical and socioeconomic factors.

In the present study, we analyzed data from children presenting to a pediatric surgery outpatient clinic with urological complaints. We aimed to characterize the demographic

profile, identify the ICD-10 diagnoses assigned at the first visit, and examine the relationships between age, sex, presenting complaints, and subsequent clinical decisions. We examined age differences by sex and the patterns of initial evaluation that influence diagnostic and therapeutic pathways.

METHODS

This retrospective study was conducted on pediatric patients who presented to the pediatric surgery outpatient clinic with urological complaints. Medical records of all children evaluated during the study period were reviewed. Patients were included if their primary ICD-10 diagnosis was classified as urological. Exclusion criteria were;

- (1) an ICD-10 code that was not related to the urinary or genital system and
- (2) refusal of the family to provide informed consent for the use of anonymized clinical data.

The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from the parents or legal guardians of all participating children prior to data extraction and analysis. Ethically, patient identities were anonymized. Ethical approval numbered GOKAEK-2414_2024.12.04_256 was obtained from the University Hospital Non-Interventional Clinical Research Ethics Committee.

Demographic data (age at admission and sex), presenting complaints, ICD-10 diagnoses (primary and subsequent), laboratory and imaging investigations ordered, outpatient follow-up intervals, and final clinical decisions (observation/normal, follow-up, prescription, circumcision, antireflux treatment, or surgical recommendation) were extracted from the electronic medical records. Continuous variables were summarized as median and

Yazışma üçün əlaqə:

Atilla Şenaylı*, Sevgi Ulusoy Tangül²

Yozgat Bozok University, Department of Pediatric Surgery, Yozgat, Türkiye,

*For correspondence E-mail: atillasenayli@gmail.com

interquartile range (IQR). Categorical variables were presented as frequencies and percentages.

Comparisons of continuous variables between two independent groups were performed with the Mann–Whitney U test. Comparisons across more than two groups were performed with the Kruskal–Wallis test. Associations between categorical variables were assessed with the chi-square test or Fisher’s exact test (with Monte Carlo simulation when appropriate). Multinomial logistic regression was used to evaluate the independent effects of age, sex, and laboratory testing on final clinical decisions, with “normal/observation” serving as the reference category. Model fit was assessed by deviance, Akaike information criterion (AIC), and McFadden’s pseudo- R^2 . A two-sided p -value < 0.05 was considered statistically significant.

All statistical analyses were performed using the jamovi 2.7.32.0 package.

RESULTS

A total of 156 children who presented to the pediatric surgery outpatient clinic with urological complaints were included in the analysis. Of these, 115 (73.7%) were male and 41 (26.3%) were female. The median admission age was 3 years (interquartile range [IQR]: 2–5) among males and 8 years (IQR: 5–12) among females (Table 1). Female patients were significantly older than male patients (Mann–Whitney $U = 1064$, $p < 0.001$).

The most frequent ICD-10 diagnoses were N39.0 (urinary tract infection) and N39.9 (disorder of urinary system, unspecified). N39.0 accounted for 40 (35%) of male and 17 (41%) of female diagnoses, while N39.9 accounted for 29 (25%) of male and 20 (49%) of female diagnoses. Other diagnoses were distributed at low frequencies across both sexes (Table 1).

Table 1. Demographic characteristics and ICD-10 diagnosis distribution by sex

Characteristic	Male (N = 115)	Female (N = 41)
<i>Admission age, median (IQR)</i>	3 (2, 5)	8 (5, 12)
Diagnosis, n (%)		
D41.9	1 (0.9)	0 (0)
F98.0	1 (0.9)	2 (4.9)
J22	1 (0.9)	0 (0)
N13.3	1 (0.9)	0 (0)
N35	2 (1.7)	0 (0)
N39	3 (2.6)	0 (0)
N39.0	40 (35)	17 (41)
N39.8	1 (0.9)	0 (0)
N39.9	29 (25)	20 (49)
N47	1 (0.9)	0 (0)
N48.1	1 (0.9)	0 (0)
Q53	1 (0.9)	0 (0)
Q54	1 (0.9)	0 (0)
Q64.9	1 (0.9)	0 (0)
R31	1 (0.9)	0 (0)
R32	0 (0)	1 (2.4)
R39	5 (4.3)	0 (0)
R39.8	22 (19)	1 (2.4)

Z00.1	1 (0.9)	0 (0)
F93.8	2 (1.7)	0 (0)

Data are presented as median (Q1, Q3) or n (%).

The official designations and clinical explanations of the principal ICD-10 codes encountered in the cohort are summarized in Table 2. Most presentations clustered

under symptomatic codes (particularly R39.8 and related R-group codes) rather than structural congenital anomalies (Q-group codes)

Table 2. ICD-10 codes identified in the study and corresponding clinical explanations

ICD-10 Code	Official designation	Clinical explanation
Q64.9	Congenital malformation of urinary system, unspecified	Congenital urinary-tract anomalies that cannot be further classified
R31	Hematuria, unspecified	Macroscopic or microscopic blood in the urine
R32	Unspecified urinary incontinence	Involuntary urine leakage
R33	Retention of urine	Incomplete bladder emptying or inability to void
R39	Other symptoms and signs involving the urinary system	Other specific lower-urinary-tract symptoms
R39.8	Other and unspecified symptoms and signs involving the urinary system	Urogenital findings not classifiable under the preceding categories
Z00.1	Routine child health examination	Routine pediatric health supervision performed in the outpatient setting
F93.8	Other childhood emotional disorders	Psychophysiological or emotional states that may accompany urological complaints (e.g., enuresis)

Statistical evaluation of the relationships among sex, age, and diagnosis is presented in Table 3. No significant association was found between sex and diagnostic category ($\chi^2 = 25.0$, $df = 19$, $p = 0.162$; Fisher's exact test $p = 0.085$).

Admission age differed markedly by sex (Mann–Whitney $U = 1064$, $p < 0.001$) but showed only a borderline, non-significant trend across diagnostic groups (Kruskal–Wallis $\chi^2 = 28.3$, $df = 19$, $p = 0.078$).

Table 3. Statistical comparisons of age and sex with clinical diagnoses

Variable	Test	Statistic	df	p-value	Clinical interpretation
Sex $\times$ diagnosis distribution	Chi-square (χ^2)	25.0	19	0.162	No statistically significant association between diagnostic groups and sex ($p > 0.05$)
	Fisher's exact test	—	—	0.085	Distribution remains homogeneous after accounting for sparse cells ($p > 0.05$)
Admission age	Mann–Whitney U	1064	—	<0.001	Admission age differs highly significantly by sex ($p < 0.001$)
	Kruskal–Wallis (χ^2)	28.3	19	0.078	Age distribution across diagnostic groups shows a borderline trend but is not significant ($p > 0.05$)

Total sample size $N = 156$.

Median age was significantly higher in girls than in boys (Mann–Whitney $U = 1064$, $p < 0.001$). Diagnostic profiles did not differ significantly by sex (Fisher's exact $p = 0.085$). Age distribution across ICD-10 categories was likewise non-significant (Kruskal–Wallis $p = 0.078$).

Presenting complaints stratified by sex are shown in Table 4. Overall complaint distribution differed highly significantly

between sexes (Fisher's exact test, $p < 0.001$). External genitalia-related complaints accounted for 47 (40.9%) of male presentations and were absent among females. Dysuria occurred at nearly identical frequencies in both sexes (24.3% of males, 24.4% of females). Systemic or general complaints were substantially more common among girls (41.5%).

4. Distribution of presenting complaints by sex

Presenting complaint	Male n (%)	Female n (%)	Total n (%)	Statistical analysis
<i>Dysuria</i>	28 (24.3)	10 (24.4)	38 (24.4)	
<i>Enuresis and related disorders</i>	7 (6.1)	3 (7.3)	10 (6.4)	
<i>Infection and urine changes</i>	20 (17.4)	8 (19.5)	28 (17.9)	
<i>Penile and circumcision-related complaints</i>	47 (40.9)	0 (0.0)	47 (30.1)	Fisher's exact test
<i>Stenoses</i>	8 (7.0)	2 (4.9)	10 (6.4)	$p < 0.001^*$
<i>Congenital and structural anomalies</i>	3 (2.6)	1 (2.4)	4 (2.6)	(Monte Carlo simulation)
<i>Labial fusion (female-specific)</i>	0 (0.0)	1 (2.4)	1 (0.6)	
<i>Other systemic/general complaints</i>	2 (1.7)	17 (41.5)	19 (12.2)	
Total	115 (100.0)	41 (100.0)	156 (100.0)	

$p < 0.05$ indicates a highly statistically significant difference.

The temporal relationship between outpatient follow-up intervals and the assignment of second (Diagnosis 2) and third (Diagnosis 3) diagnoses is summarized in Table 5. Both Diagnosis 2 ($\chi^2 = 391$, $df = 256$, $p < 0.001$) and Diagnosis 3 ($\chi^2 = 356$, $df = 84$, $p < 0.001$) were strongly associated with follow-up

timing. Most patients required neither a second (83.9%, $n = 131$) nor a third (87.1%, $n = 136$) diagnostic code. Same-day encounters predominantly retained the original symptomatic codes, whereas longer follow-up intervals occasionally incorporated functional or chronic codes (e.g., constipation-related K59).

Table 5. Matrix of second and third diagnoses according to outpatient follow-up interval

Follow-up interval	Diagnosis 2 (n = 156)	Diagnosis 3 (n = 156)	Clinical tendency
<i>Same-day (SAME)</i>	No additional diagnosis: 49 Acute symptomatic codes: 18	No additional diagnosis: 63 Acute clinical codes: 4	Same-day visits largely remained limited to the index symptom
<i>Early (1–2 months)</i>	No additional diagnosis: 11 Follow-up codes: 2	No additional diagnosis: 13	New organic pathology rarely added at early review

		Routine surveillance: 0	
<i>Intermediate–long</i> (3 months–5 years)	No additional diagnosis: 9 Chronic/functional codes: 3	No additional diagnosis: 10 Chronic surveillance codes: 2	Longer intervals occasionally introduced functional bowel or structural codes
<i>No follow-up / did not return (NONE)</i>	No additional diagnosis: 55 Isolated organic codes: 4	No additional diagnosis: 134 Additional codes: 2	Patients lost to follow-up rarely received tertiary diagnoses
<i>Statistical analysis</i>	Pearson $\chi^2 = 391$, df = 256, $p < 0.001^*$	Pearson $\chi^2 = 356$, df = 84, $p < 0.001^*$	Both secondary diagnostic processes are highly significantly related to timing of clinic attendance

$p < 0.05$ denotes high statistical significance. Sparse zero cells were collapsed for tabular clarity.

A multinomial logistic regression model was constructed to examine factors associated with final clinical decisions (operation recommendation, reflux treatment, circumcision, follow-up, or prescription), using “normal/observation” as the reference category. Model-fit indices and omnibus tests are shown in Table 6. The model

demonstrated moderate explanatory power (McFadden pseudo- $R^2 = 0.176$, deviance = 467.00). Omnibus likelihood-ratio tests indicated that sex ($p = 0.344$), admission age ($p = 0.137$), and laboratory-test category ($p = 0.808$) did not exert statistically significant independent main effects on final decisions.

Table 6. Multinomial logistic regression model fit and omnibus effects

<i>Model-fit index</i>	<i>Value</i>	<i>Independent variable (omnibus tests)</i>	χ^2	<i>df</i>	<i>p-value</i>
<i>Deviance</i>	467.00	Sex	16.60	15	0.344
<i>AIC</i>	677.00	Laboratory (simplified categories)	50.40	60	0.808
<i>Pseudo-R^2 (McFadden)</i>	0.176	Admission age	21.00	15	0.137

AIC: Akaike information criterion

Parameter estimates for selected contrasts are presented in Table 7. Relative to children managed expectantly, those who underwent ultrasonography alone had significantly higher odds of receiving an operation recommendation (OR = 1.28, $p < 0.001$). Children who received combined laboratory testing (ultrasonography plus

urine analysis) were substantially less likely to be assigned to simple follow-up only (OR = 0.09, 95% CI 0.01–0.55, $p = 0.009$). Neither age nor sex independently predicted operation, circumcision, follow-up, or prescription decisions after adjustment ($p > 0.05$ for all relevant contrasts).

Table 7. Selected parameter estimates from the multinomial logistic regression model (reference category: normal/observation)

<i>Clinical decision</i>	<i>Independent variable</i>	<i>Odds ratio</i>	<i>95% CI</i>	<i>p-value</i>
<i>Operation recommendation</i>	Admission age	0.85	0.46–1.54	0.581
	Sex (female vs male)	<0.01	Sparse cells	<0.001
	Laboratory (USG vs none)	1.28	1.28–1.28	<0.001

<i>Reflux treatment</i>	Admission age	1.43	0.62–3.28	0.405
	Laboratory (urine vs none)	<0.01	<0.01– <0.01	<0.001
<i>Circumcision</i>	Admission age	0.85	0.53–1.35	0.494
	Laboratory (combined vs none)	0.93	0.06–15.46	0.960
<i>Follow-up</i>	Admission age	0.94	0.79–1.12	0.479
	Sex (female vs male)	1.01	0.17–5.84	0.994
	Laboratory (combined vs none)	0.09	0.01–0.55	0.009
<i>Prescription</i>	Admission age	1.02	0.86–1.22	0.782
	Sex (female vs male)	1.77	0.31–10.20	0.524
	Laboratory (combined vs none)	0.33	0.06–2.00	0.231

Finally, the relationship between principal diagnostic categories and ultimate clinical disposition is displayed in Table 8. The three most frequent diagnoses—N39.0 (36.5%), N39.9 (31.4%), and R39.8 (14.7%)—showed comparable rates of active treatment recommendation (53–

65%). No statistically significant association was observed between diagnostic group and clinical decision (Fisher's exact $p = 0.756$). Decisions appeared to be driven more by individual clinical trajectory than by the specific ICD-10 label assigned at presentation.

Table 8. Clinical decisions according to principal diagnostic category

Diagnosis (ICD-10)	Total patients n (%)	Follow-up n (%)	Normal/control n (%)	Active treatment/recommendation* n (%)	Fisher's exact p
<i>N39.0 (urinary-tract infection)</i>	57 (36.5)	16 (28.1)	4 (7.0)	37 (64.9)	
<i>N39.9 (disorder of urinary system)</i>	49 (31.4)	18 (36.7)	5 (10.2)	26 (53.1)	
<i>R39.8 (other voiding symptoms)</i>	23 (14.7)	7 (30.4)	2 (8.7)	14 (60.9)	0.756
<i>N39 (other urinary disorders)</i>	3 (1.9)	2 (66.7)	1 (33.3)	0 (0.0)	
<i>Other urological diagnoses**</i>	24 (15.4)	9 (37.5)	1 (4.2)	14 (58.3)	
Total	156 (100)	52 (33.3)	13 (8.3)	91 (58.3)	

*Active treatment/recommendation includes operation recommendation, reflux treatment, circumcision, medical therapy, pediatric psychiatry referral, and other specific recommendations. **Rare codes (patient count < 3) were aggregated.

Overall, the clinical decision-making process in this outpatient urological cohort

was only weakly related to demographic variables or to the initial ICD-10 label and

was more closely linked to the results of targeted laboratory investigations and to the longitudinal course of symptoms.

DISCUSSION

Our findings highlight important demographic and clinical patterns in children presenting with urological complaints to a pediatric surgery outpatient clinic. Males constituted the clear majority (73.7%), and they presented at a significantly younger age than females (median 3 years versus 8 years, $p < 0.001$). This age difference is consistent with the literature, in which congenital anomalies such as hypospadias and phimosis are typically diagnosed in infancy and early childhood among boys, whereas urinary tract infections become more prominent in girls during school age (3). Similar trends have been reported in studies from our country, where urinary tract infections are more common in boys during infancy but increase substantially in girls at school age. The most frequently assigned ICD-10 codes were non-specific: N39.0 (urinary tract infection), N39.9 (disorder of the urinary system, unspecified), and R39.8 (other symptoms and signs involving the urinary system). Together, these three codes accounted for most primary diagnoses. This pattern indicates that the initial outpatient evaluation is largely symptom-oriented rather than based on a definitive structural diagnosis. Minor urinary symptoms are known to occur frequently even in otherwise healthy individuals (6). As noted by Kyrklund and colleagues, such symptoms are relatively uncommon in carefully selected healthy children aged 4–7 and 8–12 years, which may help distinguish pathological from functional complaints (6).

A highly significant difference was observed in the distribution of presenting complaints according to sex ($p < 0.001$). Penile and

circumcision-related problems accounted for approximately 41% of male presentations. In contrast, dysuria occurred at nearly identical rates in both sexes (approximately 24%). These findings align with previous observations that anatomical factors (such as a shorter urethra) increase the risk of infection in girls, while local genital problems predominate in boys (3). Interestingly, the initial ICD-10 diagnosis showed no statistically significant association with the final clinical decision (follow-up, observation, or active treatment recommendation; $p = 0.756$). Multinomial logistic regression further demonstrated that age and sex did not independently predict most clinical decisions once laboratory investigations were considered. Ordering ultrasonography or combined laboratory tests was more strongly associated with the subsequent management pathway. This suggests that, in the outpatient setting, the clinician's decision is driven more by the results of targeted investigations and the individual clinical course than by the preliminary diagnostic code assigned at the first visit.

The literature emphasizes that recurrent urinary tract infections may lead to bladder dysfunction and that symptoms such as urgency, daytime incontinence, and enuresis require careful follow-up because they may persist into adulthood (2,3,8). Upper and lower urinary tract infections have been reported to be more common in girls across age groups, while incontinence and enuresis tend to be more frequent in younger children (2). Our data are consistent with these patterns, although the predominance of non-specific coding limited more granular analysis of functional disorders.

Taken together, the results support the multidisciplinary nature of pediatric urology and underline the value of a structured

initial evaluation. Early genital examination and screening for congenital anomalies remain particularly relevant in young boys, whereas infection prevention strategies and assessment for bladder dysfunction are more pertinent in school-age girls. In recent years, technological advances have further increased the importance of timely diagnosis, as minimally invasive and endourological techniques now allow earlier intervention and may help prevent long-term complications (9).

Limitations

The main limitation of the study is the relatively modest sample size ($n = 156$), which restricts the generalizability of the findings. Because the data were obtained from a single outpatient clinic, regional bias cannot be excluded. The retrospective design limits causal inference. In addition, the text-based nature of presenting complaints may have introduced categorization inaccuracies. Future prospective, multicenter studies with larger cohorts would help validate these patterns and allow more detailed analysis of functional and structural subgroups.

CONCLUSION

This retrospective study demonstrated that boys constitute most children presenting with urological complaints to a pediatric surgery outpatient clinic and that they present at a significantly younger age than girls. The most frequently assigned diagnoses were non-specific codes (N39.0, N39.9, and R39.8), reflecting a predominantly symptom-based approach at the first visit. Presenting complaints differed markedly by sex, mainly because of penile and circumcision-related problems in boys, whereas clinical decisions were more closely related to laboratory investigations and individual clinical course than to the initial diagnostic label.

These findings reinforce the importance of age- and sex-sensitive evaluation pathways. Larger multicenter studies are needed to confirm the observed patterns and to refine outpatient referral and management strategies in pediatric urology.

REFERENCES

1. Shaikh N, Morone NE, Bost JE, Farrell MH. Prevalence of urinary tract infection in childhood: a meta-analysis. *Pediatr Infect Dis J*. 2008 Apr;27(4):302-8. doi: 10.1097/INF.0b013e31815e4122. PMID: 18316994.
2. Salö M, Nejstgaard MC, Hambræus M, Graneli C, Börjesson A, Hagelsteen K, Stenström P. Sex and age differences in lower urinary tract dysfunction in healthy children. *Acta Paediatr*. 2021 Sep;110(9):2618-2626. doi: 10.1111/apa.15959. Epub 2021 Jun 30. PMID: 34050977.
3. Hellström A, Hanson E, Hansson S, Hjälmås K, Jodal U. Association between urinary symptoms at 7 years old and previous urinary tract infection. *Arch Dis Child*. 1991 Feb;66(2):232-4. doi: 10.1136/adc.66.2.232. PMID: 2001110; PMCID: PMC1792822.
4. Jesus LE, Dekermacher S, Frota J, Bragança JJ, Anderson KM. Adolescents and adults in pediatric urology clinics. *J Pediatr Urol*. 2014 Jun;10(3):408-11. doi: 10.1016/j.jpuro.2013.06.009. Epub 2013 Jul 21. PMID: 23880326.
5. Miller DC, Saigal CS, Litwin MS. The demographic burden of urologic diseases in America. *Urol Clin North Am*. 2009 Feb;36(1):11-27, v. doi: 10.1016/j.ucl.2008.08.004. PMID: 19038632; PMCID: PMC2614213.

6. Kyrklund K, Taskinen S, Rintala RJ, Pakarinen MP. Lower urinary tract symptoms from childhood to adulthood: a population based study of 594 Finnish individuals 4 to 26 years old. J Urol. 2012 Aug;188(2):588-93. doi: 10.1016/j.juro.2012.04.016. Epub 2012 Jun 15. PMID: 22704114.
7. Vaz GT, Vasconcelos MM, Oliveira EA, Ferreira AL, Magalhães PG, Silva FM, Lima EM. Prevalence of lower urinary tract symptoms in school-age children. Pediatr Nephrol. 2012 Apr;27(4):597-603. doi: 10.1007/s00467-011-2028-1. Epub 2011 Oct 4. PMID: 21969094.
8. Akil IO, Ozmen D, Cetinkaya AC. Prevalence of urinary incontinence and lower urinary tract symptoms in school-age children. Urol J. 2014 Jul 8;11(3):1602-8. PMID: 25015605.
9. Cobellis G. Urology in Pediatrics. Children (Basel). 2022 Mar 2;9(3):337. doi: 10.3390/children9030337. PMID: 35327709; PMCID: PMC8947070.

PEDIATRİK CƏRRAHIYYƏ AMBULATORİYASINA UROLOJİ MÜRACİƏTLƏR: XÜSUSİYYƏTLƏR, DIAQNOZLAR VƏ KLİNİK QƏRARVERMƏ

Atila Şenaylı, Sevgi Ulusoy Tangül*

Yozqat Bozok Universiteti, Uşaq cərrahiyyəsi kafedrası, Yozqat, Türkiyə

** Yazışma üçün E-mail: atillasenayli@gmail.com*

Xülasə

İşin məqsədi: pediatrik cərrahiyyə ambulatoriyasına uroloji şikayətlərlə müraciət edən uşaqların demoqrafik xüsusiyyətlərini, diaqnostik yanaşmalarını, əsas müraciət şikayətlərini və klinik qərarvermə proseslərini qiymətləndirmək. Bu retrospektiv tədqiqata uroloji şikayətlərlə müraciət edən 156 uşaq daxil edilmişdir. Qeyri-uroloji ICD-10 kodları olan və ya ailələri tədqiqatda iştirak etməyə razılıq verməyən pasiyentlər tədqiqatdan xaric edilmişdir. Yaş, cins, müraciət şikayətləri, ICD-10 diaqnozları, laborator müayinələrin nəticələri, müşahidə intervalları və yekun klinik qərarlar barədə məlumatlar təhlil edilmişdir. Statistik təhlillərə Mann–Whitney U testi, Kruskal–Wallis testi, χ^2 testi, Fisher-in dəqiq testi və multinomial logistik regressiya daxil edilmişdir. Təhlillər *jamovi* 2.7.32.0 statistik proqram paketindən istifadə edilməklə aparılmışdır. Tədqiqat Helsinki Bəyannaməsinin prinsiplərinə uyğun şəkildə həyata keçirilmiş və valideynlərdən və ya qanuni nümayəndələrdən məlumatlandırılmış razılıq alınmışdır. 156 pasiyentin 115-i (73,7%) oğlan, 41-i (26,3%) qız olmuşdur. Müraciət zamanı median yaş oğlanlarda (3 yaş, IQR 2–5) qızlarla müqayisədə (8 yaş, IQR 5–12) əhəmiyyətli dərəcədə aşağı olmuşdur ($p < 0,001$). Ən çox rast gəlinən diaqnozlar N39.0 (36,5%), N39.9 (31,4%) və R39.8 (14,7%) olmuşdur. Müraciət şikayətləri cinsə görə əhəmiyyətli dərəcədə fərqlənmişdir ($p < 0,001$); bu fərq əsasən oğlanlarda penis və sünnətlə əlaqəli problemlərlə bağlı olmuşdur. Dizuriya hər iki cinsdə oxşar tezlikdə müşahidə edilmişdir. Klinik qərarlar ilə ilkin ICD-10 diaqnozu arasında statistik cəhətdən əhəmiyyətli əlaqə aşkar edilməmişdir ($p = 0,756$). Multinomial logistik regressiyanın nəticələri göstərmişdir ki, sonrakı müalicə və idarəetmə qərarları ilə yaş və cinsdən daha çox laborator müayinələrin nəticələri əlaqəlidir. Pediatrik uroloji müraciətlər arasında oğlanlar üstünlük təşkil edir və onlar qızlarla müqayisədə daha erkən yaşda müraciət edirlər. İlkin ambulator diaqnozlar əsasən simptomlara əsaslanır və spesifik xarakter daşımır. Klinik qərarların ilkin diaqnostik koddan daha çox müayinələrin nəticələri və pasiyentin fərdi klinik gedişinə əsasən qəbul edildiyi görünür. Yaş və cins xüsusiyyətlərini nəzərə alan qiymətləndirmə strategiyalarının tətbiqi ambulator pediatrik uroloji yardımın səmərəliliyini artırma bilər.

Açar sözlər: *Pediatric; Urology; Ambulatory patients; Urinary tract infections; Clinical decision*

УРОЛОГИЧЕСКИЕ ОБРАЩЕНИЯ В АМБУЛАТОРНУЮ КЛИНИКУ ДЕТСКОЙ ХИРУРГИИ: ХАРАКТЕРИСТИКИ, ДИАГНОЗЫ И КЛИНИЧЕСКИЕ РЕШЕНИЯ

Атилла Шенайлы*, Севги Улусой Тангюль

Кафедра детской хирургии, проф. д-р, Университет Йозгат Бозок, Йозгат, Турция
Кафедра детской хирургии, доц. д-р, Университет Йозгат Бозок, Йозгат, Турция

* Для корреспонденции E-mail: atillasenayli@gmail.com

Резюме

Цель исследования - оценить демографические характеристики, диагностические подходы, основные жалобы при обращении и процессы принятия клинических решений у детей, обращающихся в амбулаторное отделение детской хирургии с урологическими жалобами. В данное ретроспективное исследование были включены 156 детей, обратившихся с урологическими жалобами. Пациенты с неурологическими кодами ICD-10, а также пациенты, родители или законные представители, которых отказались от участия, были исключены из исследования. Были проанализированы данные о возрасте, поле, жалобах при обращении, диагнозах по ICD-10, результатах лабораторных исследований, интервалах наблюдения и окончательных клинических решениях. Статистический анализ включал U-критерий Манна-Уитни, критерий Краскела-Уоллиса, критерий χ^2 , точный критерий Фишера и мультиномиальную логистическую регрессию. Анализ проводился с использованием статистического программного пакета *jamovi версии 2.7.32.0*. Исследование было проведено в соответствии с принципами Хельсинкской декларации; от родителей или законных представителей было получено информированное согласие.

Из 156 пациентов 115 (73,7%) были мальчиками и 41 (26,3%) — девочками. Медианный возраст при обращении был значительно ниже у мальчиков (3 года, межквартильный диапазон [IQR] 2–5 лет), чем у девочек (8 лет, IQR 5–12 лет) ($p < 0,001$). Наиболее часто встречающимися диагнозами были N39.0 (36,5%), N39.9 (31,4%) и R39.8 (14,7%). Жалобы при обращении статистически значимо различались в зависимости от пола ($p < 0,001$), главным образом вследствие проблем, связанных с половым членом и обрезанием у мальчиков. Дизурия встречалась с одинаковой частотой у представителей обоих полов. Статистически значимой связи между клиническими решениями и первоначальным диагнозом по ICD-10 выявлено не было ($p = 0,756$). Мультиномиальная логистическая регрессия показала, что результаты лабораторных исследований были более тесно связаны с последующими решениями по ведению пациентов, чем возраст или пол.

Среди обращений детей с урологическими жалобами преобладают мальчики, причём они обращаются за медицинской помощью в более раннем возрасте, чем девочки. Первоначальные амбулаторные диагнозы в значительной степени основываются на симптомах и имеют неспецифический характер. По-видимому, клинические решения в большей степени определяются результатами диагностических исследований и индивидуальным течением заболевания, чем предварительным диагностическим кодом. Использование стратегий обследования с учётом возраста и пола может повысить эффективность амбулаторной урологической помощи детям.

Ключевые слова: *педиатрия; урология; амбулаторные пациенты; инфекции мочевыводящих путей; принятие клинических решений.*

EVALUATION OF MORPHOFUNCTIONAL CHANGES OF THE LIVER IN DIABETIC HEPATOPATHY

Ibrahimova G.H.^{1*}, Talishinskaya M.B.¹, Sultanova G.M.¹, Ibrahimova Sh.H.², Seyidova İ.Z.³, Ahmadova R.R.⁴, Mehtiyeva A.Y.⁵

Abstract

The article presents information about the changes in markers of impaired metabolism in the liver (alanine aminotransferase – ALT, aspartate aminotransferase – AST, gamma-glutamyltransferase – GGT, lactate dehydrogenase – LDH) in blood and lymph, lymph flow rate (LFR), as well as histomorphological structures of the liver in experimental diabetes mellitus induced in rabbits by alloxan. Experiments were carried out on 14 rabbits of both sexes. The rabbits used in the study were divided into two groups: the control group consisted of 6 rabbits, and the experimental group consisted of 8 rabbits. Diabetic symptoms were detected 5 days after alloxan injection. For analysis, blood samples were taken from the heart cavity, and lymph from the thoracic duct. The operation was performed under the anesthesia. In both the control and experimental groups of rabbits, blood and lymph were taken to detect glucose level, markers of metabolic disturbances in the liver, LFR, as well as the histomorphological characteristics of the liver were studied. The studied parameters were measured in the 8 rabbits of the second experimental group on the 5th, 15th, and 30th days after alloxan administration. It was determined that the increase in markers of impaired metabolism in the liver in both blood and lymph, as well as the histomorphological changes developing in the liver, were accompanied by a decrease in lymph flow rate.

Keywords: *Diabetic hepatopathy, ALT, AST, GGT, and LDH, lymph.*

INTRODUCTION

Diabetes mellitus (DM) is a rapidly spreading disease worldwide, characterized by long-term complications, and remains one of the serious medical and social problems. According to the World Health Organization, in 2021, the number of people with diabetes worldwide exceeded 500 million, and it is projected that this indicator will reach 700 million by 2030 [1, 2, 3]. Diabetes is also spreading rapidly in Azerbaijan. In 2024, the total number of registered diabetic patients in the unified

electronic registry of medical institutions under the Administration of the Regional Medical Divisions was reported to be 322,353 [4, 5].

One of the organs most affected by DM is the liver. The liver plays a key role in regulating glucose homeostasis. During persistent hyperglycemia, end-products of non-enzymatic glycosylation accumulate in the body, including hepatocytes. This leads to damage of both intracellular and extracellular structures, a decrease in NADPH levels,



an acceleration of oxidative stress, and increased conversion of glucose to sorbitol [6, 7, 8, 9, 10, 11]. As a result of the disruption of the integrity of hepatocyte membranes, enzymes such as ALT, AST, GGT, and LDH are released into the blood and lymph. This is considered an indicator of liver damage. Although significant progress has been made in studying the histomorphological changes in the liver and the alterations in the levels of enzymes considered indicators of liver damage in the blood in diabetes mellitus, however, there is limited information about such changes in the lymph under conditions of persistent hyperglycemia [12, 13, 14, 15]. It should be noted that a significant portion of the lymph in the thoracic duct originates from the liver, and lymphatic fluid can provide more detailed information about metabolic processes occurring in tissues [6, 17, 18]. Although the role of the lymphatic system in the pathogenesis of liver failure is of great importance, its role during diabetes mellitus has not been sufficiently investigated [19, 20, 21, 22].

experimentally induced diabetes mellitus in rabbits using alloxan injection, to determine the lymph flow rate, and to investigate histomorphological changes in the liver.

The aim of this research was to comparatively study the levels of markers impaired metabolism in the liver (ALT, AST, GGT, LDH) in the blood and lymph during

MATERIALS AND METHODS

The study was carried out on 14 rabbits of the "Chinchilla" breed, weighing between 1.8 and 2.3 kg. The rabbits used in the experiments were divided into two groups: the control group included 6 animals, and the experimental group included 8. In the experimental group, diabetes mellitus was induced in rabbits by intraperitoneal administration of 5% alloxan monohydrate solution at a dose of 100 mg/kg. Diabetic symptoms were observed 5 days after the injection. Blood samples were obtained from the cardiac cavity and lymph samples from the thoracic duct. All procedures were carried out under anesthesia. For this purpose, 8 mg/kg of Calypsol and 0.15 ml/kg of 1% Dimedrol solution were injected intravenously. In the control group, blood and lymph samples were collected from six rabbits, and the levels of liver function-related enzymes (ALT, AST, γ -GT, LDH) as well as LFR were determined. In addition, the histomorphological structures of the liver were studied. The same parameters were assessed in the experimental group of 8 rabbits on 5th, 15th, and 30th days after alloxan administration. For histomorphological evaluation, liver tissues were fixed using 10% neutral buffered formalin or Bouin's solution. Subsequently, the specimens were dehydrated through a graded ethanol series, embedded in paraffin, sectioned, and stained for histological examination.

Yazışma üçün əlaqə:

İbrahimova G.H.^{1*}, Talışinskaya M.B.¹, Sultanova G.M.¹, İbrahimova Sh.H.², Seyidova İ.Z.³, Ahmadova R.R.⁴, Mehtiyeva A.Y.⁵

¹ Department of Pathological Physiology, Azerbaijan Medical University, Baku, Azerbaijan

² Department of Allergology and immunology, Azerbaijan Medical University, Baku, Azerbaijan

³ Department of Physiotherapy and rehabilitation, Azerbaijan Medical University, Baku, Azerbaijan

⁴ Research Institute of Living Systems of the Ministry of Science and Education of the Republic of Azerbaijan

⁵ Department of Radiology, Azerbaijan Medical University, Baku, Azerbaijan

*For correspondence E-mail:

doctor.ibrahimova@gmail.com

The numerical data obtained during the course of the study were statistically analyzed in accordance with current recommendations. Statistical analyses were carried out using the method of variation analysis and with the help of the 'IBM Statistics SPSS-26' software. For the comparison of variation series, the non-parametric Mann-Whitney U test was also used.

RESULTS AND DISCUSSION

During the study, the levels of enzymes characterized the diabetic hepatopathy (ALT, AST, γ -GT, LDH), LFR, and the histomorphological structures of the liver were investigated in the blood and lymph of rabbits with alloxan-induced diabetes mellitus over a period of 30 days and compared with control values. The obtained results are presented in the tables below (Table 1).

Table 1. Dynamics of changes in the indicators determined in the blood of animals during alloxan-induced experimental diabetes

Parameters	Control group	Days after administration of alloxan monohydrate solution M (min-max)		
		5	15	30
Glucose mmol/l	4,79 (4,60-5,01)	6,25 (4,91-6,87)*	10,15 (9,48-11,05)*	12,11 (11,45-13,03)*
ALT IU/L	40,43 (39,20-41,64)	46,01 (45,38-46,66)	53,1 (52,02-54,03)	60,41 (59,02-61,03)
AST IU/L	23,84 (23,64-24,07)	27,40 (26,39-28,41)*	35,51 (34,73-36,38)*	43,57 (42,68-44,57)*
QGT IU/L	5,6 (5,3-6,3)	7,5 (6,1-8,1)*	8,8 (7,5-9,0)*	12,2 (11,5-13,1)
LDH IU/L	170,6 (169,1-171,0)	190,9 (189,0-192,1)	210,1 (209,6-211,6)*	230,5 (229,7-231,4)*

*Note: The * symbol indicates the statistical significance of the difference between the experimental and control group parameters.*

As seen from Table 1, after alloxan administration, the levels of blood glucose and the liver function-assessing enzymes (ALT, AST, GGT, LDH) gradually increased throughout the experiment ($p < 0,001$).

The dynamics of changes in the parameters determined in the lymph are in the same direction as in the blood (Table 2).

Table 2. Dynamics of changes in the parameters determined in the lymph of animals with alloxan-induced diabetes mellitus

Parameters	Control group	Days after administration of alloxan monohydrate solution M (min-max)		
		5	15	30
Glucose level mmol/l	5,2 (4,2-6,2)	7,4 (5,9-8,7) *	11,5 (10,7-12,6)*	13,3 (12,4-14,4)*
ALT IU/L	45,3 (44,8-46,8)	57,8 (56,2-58,5)	63,3 (62,5-64,1)*	69,7 (68,8-70,9)*
AST IU/L	27,9 (11,1-12,5)	36,8 (35,9-37,8)	45,3 (44,8-46,8)*	51,8 (50,7-52,0)
GGT IU/L	8,7 (7,8-9,0)	13,4 (12,4-14,4)	17,9 (16,7-18,1)*	24,1 (23,8-25,8)
LDH IU/L	190,8 (189,8-191,5)	222,4 (221,5-223,6)	240,3 (239,3-241,5)*	263,9 (262,1-263,4)*
LFR (ml.min/kg)	1,224 (1,180-1,270)	1,203 (1,160-1,240)	1,013 (0,850-1,150)	0,813 (0,650-0,950)

*Note: The * symbol indicates the statistical significance of the difference between the experimental and control group parameters.*

As seen from Table 2, after alloxan administration, the levels of glucose and liver function-assessing enzymes (ALT, AST, GGT, LDH) in the lymph gradually increased ($p < 0.001$), and the lymph flow rate decreased throughout the experiment (Table 2).

As seen from the presented tables, in animals with induced diabetes mellitus, an increase in the activity of ALT, AST, GGT, and LDH in blood and lymph was observed throughout the experiment compared to the control values. This tendency was more pronounced in the lymph than in the blood. In rabbits with alloxan-induced diabetes

mellitus, the increased markers of metabolic disorders in the liver (ALT, AST, GGT, LDH) in blood and lymph are explained by the acceleration of hepatocyte cytolysis under persistent hyperglycemia [23, 24, 25].

During the experiment, histomorphological changes developing in the liver were studied on the 5th, 15th, and 30th days after alloxan administration. On the 5th day of alloxan-induced diabetes mellitus, dystrophic changes were observed in hepatocytes around the periportal vessels (Figure 1).



Figure 1. Histomorphological changes in the liver on the 5th day of alloxan-induced diabetes mellitus. Hematoxylin and eosin (H&E) staining. Magnification: $\times 206$.

On the 15th day of diabetes mellitus, edema of the connective tissue in the periportal area of the liver is observed (Figure 2).

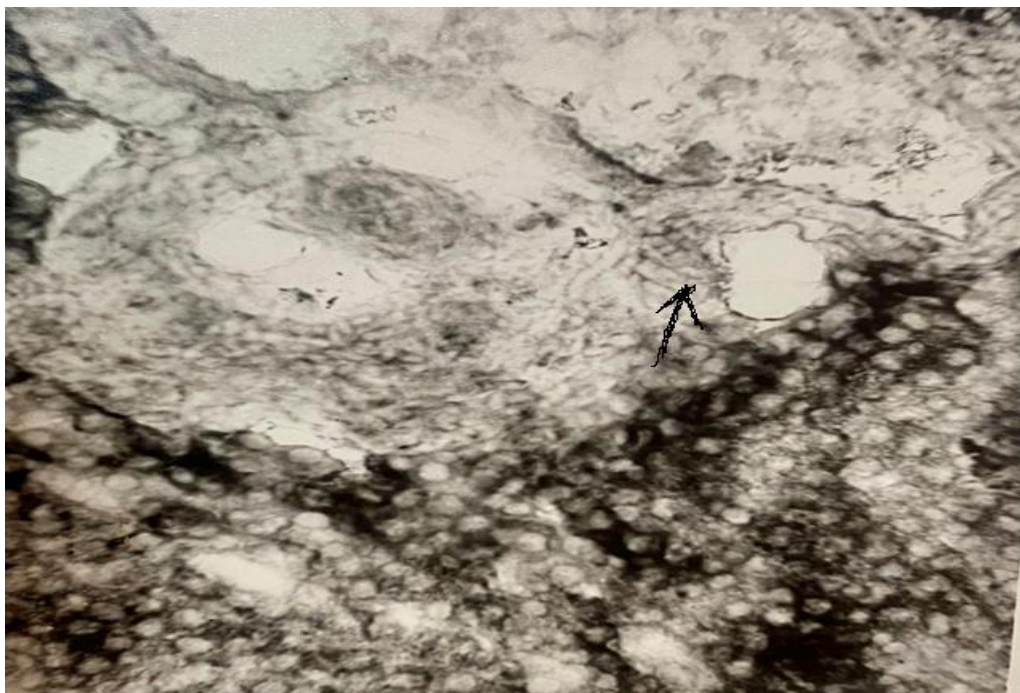


Figure 2. Histomorphological changes in the liver on the 15th day of alloxan-induced diabetes mellitus. Hematoxylin and eosin (H&E) staining. Magnification: $\times 206$.

On the 30th day of alloxan-induced diabetes mellitus, destructive and necrotic changes developed in the liver parenchyma (Figure 3).

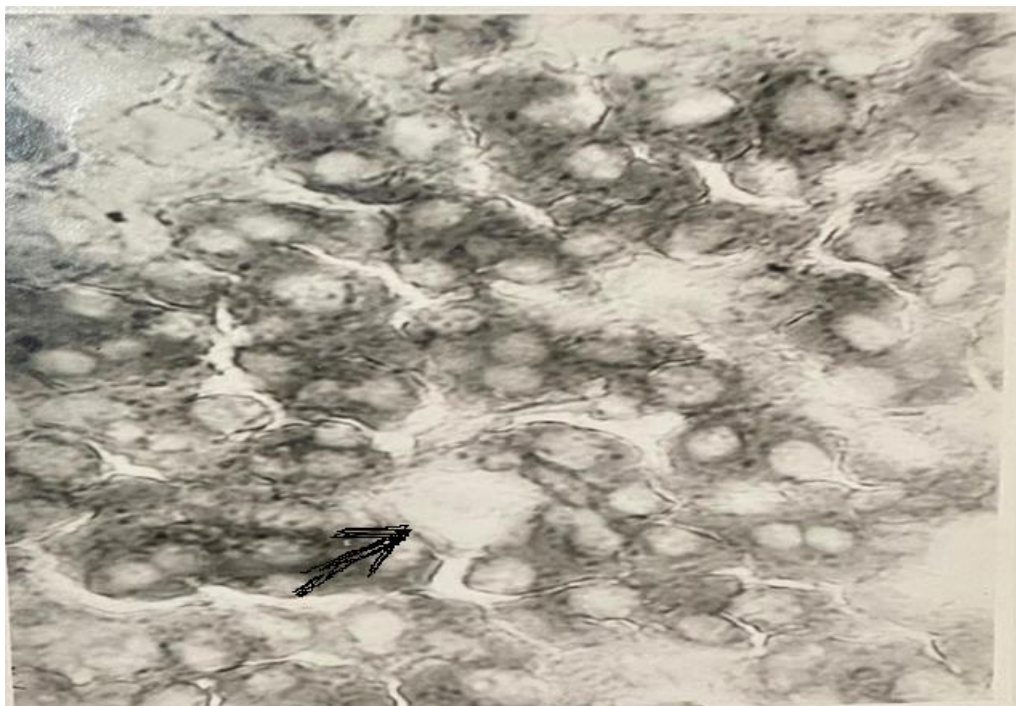


Figure 3. Histomorphological changes in the liver on the 30th day of alloxan-induced diabetes mellitus. Hematoxylin and eosin (H&E) staining. Magnification: $\times 206$.

Based on the information presented above, we conclude that during diabetes mellitus, enzymes released from damaged hepatocytes enter the bloodstream and lymphatic system. The increase of these enzymes in both blood and lymph, along with the histomorphological changes occurring in the liver, is considered the development of diabetic hepatopathy, one of the long-term complications of diabetes mellitus [26, 27, 28, 29]. The decrease in lymph flow rate slows the removal of accumulated toxic products from tissues, further deepening the histomorphological changes that are developing in the liver [30, 31, 32, 33].

CONCLUSION.

In alloxan-induced diabetes mellitus, the increased markers of metabolic disorders in the liver (LDH, AST, ALT, GGT) in both blood and lymph, along with the progressive deepening of histomorphological changes in the liver, were accompanied by a decrease in lymph flow rate.

REFERENCES

1. Sun H., Saeedi P., Karuranga S., et.al. IDF Diabetes Atlas: Global, regional and country level diabetes prevalence estimates for 2021 and projections for 2045., *Diabetes Res Clin Prac.* 2022, Jan;183:109119. doi: 10.1016/j.diabres. 2021. 109 119. Epub 2021 Dec 6., <https://pubmed.ncbi.nlm.nih.gov/34879977/>
2. Paschou S. A., Papadopoulou-Marketou N. et.al. On Type 1 Diabetes Mellitus Pathogenesis, *Endocr Connect* 2018, Jan, 7(1), p.38-46. PMID: 29191919, PMCID: PMC5776665, DOI: 10.1530/EC-17-0347, <https://pubmed.ncbi.nlm.nih.gov/29191919/>
3. Abu N. A., Lim C. B., Nor N. S. M. Glycogenic Hepatopathy in Children with Poorly Controlled Type 1 Diabetes Mellitus, *Clin Pediatr Endocrinol*, 2021, 30(2), p.93-97. PMID:

- 33867669,PMCID: PMC8022034, DOI: 10.1297/cpe.30.93,https://pubmed.ncbi.nlm.nih.gov/33867669/
4. Abdulla A. Number of diabetics in Azerbaijan announced, Administration of the Regional Medical Divisions, 2024
 5. Alieva T.T. Changes in prevalence of diabetes mellitus in the Republic of Azerbaijan in 1994-2012 and prospects for 2030, *Kazan Medical Journal*, Home Archives 2014, Vol 95, No 4. DOI: https://doi.org/10.17816/KMJ1845, https://kazanmedjournal.ru/kazanmedj/article/view/1845?utm_source=chatgpt.com
 6. Berzigotti A., Tsochatzis E., Boursier J., et.al. EASL Clinical Practice Guidelines on Non-invasive Tests for Evaluation of Liver Disease Severity and Prognosis, Update. *J. Hepatol*, 2021, Volume 75, Issue 3, September p. 659-689. https://www.sciup.org/reference/referencpapers?referenceid=4041224
 7. Jessica E B F Lima , Natalia C S Moreira, Elza T Sakamoto-Hojo Mechanisms underlying the pathophysiology of type 2 diabetes: from risk factors to oxidative stress, metabolic dysfunction, and hyperglycemia, *Mutat Res Genet Toxicol Environ Mutagen*, 2022, Feb-Mar:874-875:503437. PMID: 35151421 DOI: 10.1016/j.mrgentox.2021.503437 https://pubmed.ncbi.nlm.nih.gov/35151421/
 8. Jiang S., Tang X., Wang K. et.al. Hepatic functional and pathological changes of type 1 diabetic mice in growing and maturation time, *J Cell Mol Med.*, 2019, 23(8), p. 5794–5807. DOI: 10.1111/jcmm.14504, https://doi.org/10.1111/jcmm.14504, https://pubmed.ncbi.nlm.nih.gov/31222979/
 9. Najafova V.N. Non- invasive radiodiagnosis of hepatic fibrosis in diabetic patients, *Azerbaijan Tibb Jurnalı*, Bakı, 2024, №2, p.97-103. DOI: https://doi.org/10.34921/amj.2024.80.94.002, http://azmedjournal.com/index.php/amz/article/view/461
 10. Dadashova A., Aliyeva G., et.al. Hyperhomocysteinemia in pediatric β -thalassemia: links to vitamin cofactor deficiencies and oxidative stress, *Clin Exp Pediatr*, 2025, Jul 8,PMID:40776617. https://pubmed.ncbi.nlm.nih.gov/40776617/
 11. Rahimova R.R. Study of matrix metalloproteinase activity in patients with autoimmune thyroiditis, *Ukr.Biochem.J*, 2022, Volume 94. Issue, p. 51-56. http://ukrbiochemjournal.org/2022/07/study-of-matrix-metalloproteinase-activity-in-patients-with-autoimmune-thyroiditis.html
 12. de Vries M., Westerink J., Kaasjager K.H.A.H., de Valk H.W. Prevalence of nonalcoholic fatty liver disease (NAFLD) in patients with type 1 diabetes mellitus: a systematic review and meta-analysis, *J Clin Endocrinol Metab.*,2020, 105(12), p. 3842–3853. DOI: 10.1210/clinem/dga575?https://pubmed.ncbi.nlm.nih.gov/32827432/
 13. Shiful I., Sadaqur R., et.al. Prevalence of elevated liver enzymes and its association with type 2 diabetes: A cross-sectional study in Bangladeshi adults, *Endocrinol Diabetes Metab*, 2020, Feb 12;3(2):e00116. doi: 10.1002/edm2.116, PMID: PMC7170449 PMID: 32318634https://pmc.ncbi.nlm.nih.gov/articles/PMC7170449/#:~:text=In%20this%20study%2C%2061.2%25%20of,higher%20in%20subjects%20having%20diabetes

14. Rafaqat S., Sattar A., Khalid A. et.al. Role of liver parameters in diabetes mellitus – a narrative review, *Punjab, Endocrine regulations*, 2023, vol. 57, 200–220, doi:10.2478/enr-2023-0024, https://www.lvrach.ru/2021/12/15438168?utm_source=chatgpt.com, https://www.sav.sk/journals/uploads/02261650EndoReg_2023_57_08_Rafaqat.pdf
15. Bueverov A.O., Zilov A.B. Liver damage in type 1 diabetes mellitus, *Russian Journal of Gastroenterology, Hepatology, Coloproctology*, 2021, Tom 31, № 2. <https://doi.org/10.22416/1382-4376-2021-31-2-7-13>.
16. Mandal A., Bhattarai B., Kafle P. et.al. Elevated Liver Enzymes in Patients with Type 2 Diabetes Mellitus and Non-alcoholic Fatty Liver Disease, *National Library of Medicine*, 2018, Nov 23, 10(11): p. 3626. PMID: PMC 6347442 PMID:30697502, <https://pmc.ncbi.nlm.nih.gov/articles/PMC6347442/>, <https://pubmed.ncbi.nlm.nih.gov/30697502/>
17. Asada S., Kawaratani H., Mashitani T. et.al. Glycogenic hepatopathy in type 1 diabetes mellitus, 2018, *Intern Med* 57, 1087–1092. PMID: 29279489, PMID: PMC5938497, DOI: 10.2169/internalmedicine.9490-17, <https://pubmed.ncbi.nlm.nih.gov/29279489/>
18. Rzayev Z.I., Akhundov I.T., İbrahimli Sh. F., İslamzade F.I. The influence of endolymphatic drug therapy on the functional indexes in patients with lower extremities diabetic angiopathy and diabetic foot syndrome. *Azerbaijan Tibb Jurnalı*, Bakı, 2024, №1, 96-102, DOI: 10.34921/amj.2024.1.015.
19. Abboud W., Abdulla S., Al Zaabi M., Moufarrej R. Young Man with Hepatomegaly: A Case of Glycogenic Hepatopathy, *Case Reports Hepatol*, 2018, Apr 15:6037530. PMID: 29850300, PMID: PMC5925151, DOI: 10.1155/2018/6037530, <https://pubmed.ncbi.nlm.nih.gov/29850300/>
20. Noroozi Karimabad M, Khalili P., Ayoobi F. et.al. Serum liver enzymes and diabetes from the Rafsanjan cohort study, *BMC Endocr. Disord*, 2022, 12, 22(1), 127. doi: 10.1186/s12902-022-01042-2, PMID: 35549705, PMID: PMC9102258, DOI: 10.1186/s12902-022-01042-2, <https://pubmed.ncbi.nlm.nih.gov/35549705/>
21. Yaru Bi , Yang Yang , Xiaojie Yuan , et.al. Association between liver enzymes and type 2 diabetes: a real-world study, *Front Endocrinol (Lausanne)*, 2024, Feb 20, 15, 1340604. PMID: PMC 1091 3017 PMID:38444589, <https://pmc.ncbi.nlm.nih.gov/articles/PMC10913017/>, <https://pubmed.ncbi.nlm.nih.gov/38444589/>
22. Delli Bovi A.P., Marciano F. et.al. Oxidative Stress in Non-alcoholic Fatty Liver Disease, An Updated Mini Review. Review article, *Front. Med.*, 26 February, Sec. Gastroenterology, 2021, Vol.8. <https://www.frontiersin.org/journals/medicine/articles/10.3389/fmed.2021.595371/full>
23. Li Y, Wang J, Han X, Hu H. et.al. Serum alanine transaminase levels predict type 2 diabetes risk among a middle-aged and elderly, Chinese population. *Ann Hepatol* 2019, 18, p. 298–303. <https://pubmed.ncbi.nlm.nih.gov/31040092/>
24. Wu X, Xu J. Relationship between aspartate aminotransferase to alanine aminotransferase ratio and risk of incident diabetes mellitus in a Chinese population, *Second Affiliated Hospital &*

- Yuying Children's Hospital of Wenzhou Medical University, Research Square. 2022, DOI: <https://doi.org/10.21203/rs.3.rs-1369408/v1>
25. Wang Y, Wu T, et.al. Relationship between serum gamma-glutamyl transferase level and impaired fasting glucose among, Chinese community-dwelling adults: a follow-up observation of 6 years. *Metab Syndr Relat Disord* 19, 2021, 100–106. <https://pubmed.ncbi.nlm.nih.gov/33170087/>
 26. Kong M, Liu C et.al. Higher level of GGT during mid-pregnancy is associated with increased risk of gestational diabetes mellitus, *Clin Endocrinol* 2018, 88, p.700–705. <https://pubmed.ncbi.nlm.nih.gov/29385633/>
 27. Kashtl GJ, Abed BA et.al. A comparative study to determine LDH enzyme levels in serum samples of women with breast cancer and women with breast cancer and type 2 diabetes mellitus, *J Med Chem Sci* 2023, 6, 883–890
 28. Talishinskaya M.B., İbrahimova G.H., Panahova S.R., et.al. Diabetic hepatopathy as a manifestation of the late complications of diabetes mellitus, *ATU Journal* (2025), Volume 5 №2, p.23-33, DOI: <https://doi.org/10.28942/atuj.v5i2y2025.131>
 29. Talishinskaya M.B., İbrahimova G.H., Mehdiyev T.V. Correction of markers of diabetic osteopathy in blood and lymph, *ATJ*, 2025, №2, 177-181. DOI: <https://doi.org/10.34921/amj.2025.2.028>
 30. Ahmed F. W., Kirupakaran T., Quante M., Smith A. Hepatomegaly, Elevated Hepatic Enzymes, and Bridging Fibrosis in Patients with Type 1 Diabetes Mellitus, *Cureus*, 2021, Apr 13, 13(4), e14446. PMID: 33996309, PMCID: PMC8114958, DOI: 10.7759/cureus.14446, <https://pubmed.ncbi.nlm.nih.gov/33996309/>
 31. Dmour HH, Khreisat EF, et.al. Assessment of lactate dehydrogenase levels among diabetic patients treated in the outpatient clinics at King Hussein Medical Center, Royal Medical Services, Jordan. *Med Arch*, 2020, 74, 384–386. <https://pubmed.ncbi.nlm.nih.gov/33424095/>
 32. Bancroft JD, Gamble M. *Bancroft's Theory and Practice of Histological Techniques*. 8th ed. Elsevier; 2019.
 33. Suvarna SK, Layton C, Bancroft JD. *Bancroft's Theory and Practice of Histological Techniques*. 9th ed. Elsevier; 2024.

DIABETİK HEPATOPATİYA ZAMANI QARACİYƏRİN MORFOFUNKSIONAL DƏYİŞİKLİKLƏRİNİN QIYMƏTLƏNDİRİLMƏSİ

İbrahimova G.H.^{1*}, Talışinskaya M.B.¹, Sultanova G. M.¹, İbrahimova Ş.H.², Seyidova İ.Z.³ Əhmədova R.R.⁴, Mehtiyeva A. Y.⁵

¹ Azərbaycan Tibb Universitetinin, Patoloji fiziologiya kafedrası, Bakı, Azərbaycan

² Azərbaycan Tibb Universitetinin, Allergologiya və immunologiya kafedrası, Bakı, Azərbaycan

³ Azərbaycan Tibb Universitetinin, Fizioterapiya və reabilitasiya kafedrası, Bakı, Azərbaycan

⁴ *Azərbaycan Respublikası Elm və Təhsil Nazirliyinin Canlı Sistemlərin Tədqiqi İnstitutu*⁵ *Azərbaycan Tibb Universitetinin, Radiologiya kafedrası, Bakı, Azərbaycan** *Yazışma üçün əlaqə E-mail: doctor.ibrahimova@gmail.com*

Xülasə

Məqalədə dovşanlarda alloksan vasitəsilə modelləşdirilmiş şəkərli diabet zamanı qaraciyərdə metabolizmin pozulmasının markerlərinin (alaninaminotransferaza – ALT, aspartataminotransferaza – AST, qamma qlutamilttransferaza – QGT, laktatdehidrogenaza – LDH) qanda və limfada dəyişiklikləri, limfanın axın sürəti (LAS), eləcə də qaraciyərin histomorfoloji xüsusiyyətləri haqqında məlumat verilmişdir. Eksperimental tədqiqatlar hər iki cinsə aid olan 14 baş dovşan üzərində aparılmışdır. Tədqiqatda istifadə edilmiş dovşanlar 2 qrupa bölünmüşdür. Kontrol qrupuna 6 və təcrübə qrupuna 8 dovşan daxil edilmişdir. Dovşanlarda alloksan inyeksiyasından 5 gün sonra diabetik simptomlar aşkarlanmışdır. Analiz üçün qan ürək boşluğundan, limfa isə döş limfa axacağından alınmışdır. Əməliyyat anesteziya altında aparılmışdır. Həm kontrol, həm də təcrübə qruplarında olan dovşanlardan qan və limfa alınmış şəkərin səviyyəsi, qaraciyərdə metabolizmin pozulmasının markerləri, LAS, eləcə də qaraciyərin histomorfoloji xüsusiyyətləri öyrənilmişdir. Öyrənilən göstəricilər ikinci təcrübə qrupuna daxil edilmiş 8 dovşanda alloksan yeritdikdən sonra 5-ci, 15-ci və 30-cu gün təyin edilmişdir. Müəyyən olunmuşdur ki, həm qanda, həm də limfada qaraciyərdə metabolizmin pozulmasının markerlərinin artması və qaraciyərdə inkişaf edən histomorfoloji dəyişikliklər limfanın axın sürətinin azalması ilə müşayiət olunmuşdur

Açar sözlər: *diabetik hepatopatiyası və onun markerləri, limfa*

ОЦЕНКА МОРФОФУНКЦИОНАЛЬНЫХ ИЗМЕНЕНИЙ ПЕЧЕНИ ПРИ ДИАБЕТИЧЕСКОЙ ГЕПАТОПАТИИ

Ибрагимова Г.Г.^{1}, Талышинская М.Б.¹, Султанова Г.М.¹, Ибрагимова Ш.Г.², Сеидова И.З.³, Ахмадова Р.Р.⁴, Мехтиева А.Ю.⁵*

¹*Кафедра патологической физиологии Азербайджанского медицинского университета, Баку, Азербайджан*

²*Кафедра аллергологии и иммунологии Азербайджанского медицинского университета, Баку, Азербайджан*

³*Кафедра физиотерапии и реабилитации Азербайджанского медицинского университета, Баку, Азербайджан*

⁴*Институт Исследования Живых Систем Министерства Науки и Образования Азербайджанской Республики, Баку*

⁵*Кафедра Радиологии Азербайджанского медицинского университета, Баку, Азербайджан*

* *Для корреспонденции E-mail: doctor.ibrahimova@gmail.com*

Резюме

В статье представлены данные об изменениях маркеров нарушения метаболизма в печени (аланинаминотрансфераза — АЛТ, аспартатаминотрансфераза — АСТ, гамма-глутамилтрансфераза — ГГТ, лактатдегидрогеназа — ЛДГ) в крови и лимфе, о скорости

лимфотока (СЛТ), а также о гистоморфологических особенностях печени при аллоксан-индуцированном сахарном диабете у кроликов. Экспериментальные исследования были проведены на 14 кроликах обоего пола. Животные были разделены на 2 группы: контрольную (6 кроликов) и экспериментальную (8 кроликов). Диабетические симптомы были выявлены на 5-й день после инъекции аллоксана. Для анализа брали кровь из полости сердца, а лимфу — из грудного лимфатического протока. Операции проводились под анестезией. У кроликов обеих групп исследовали уровень глюкозы, маркеры нарушения метаболизма печени, скорость лимфотока, а также гистоморфологические особенности печени. У животных экспериментальной группы показатели определялись на 5-й, 15-й и 30-й дни после введения аллоксана. Установлено, что повышение маркеров нарушения метаболизма печени как в крови, так и в лимфе, а также развивающиеся гистоморфологические изменения в печени сопровождалось снижением скорости лимфотока.

Ключевые слова: *диабетическая гепатопатия и её маркеры, лимфа*

MORPHOMETRIC ANALYSIS OF BLOOD VESSELS OF THE LARGE INTESTINE IN NORMAL AND EXPERIMENTAL CONDITIONS: CLINICAL APPLICATIONS IN GASTROENTEROLOGY

Babayeva R.E.

Abstract

In the present study, the morphometric characteristics of the blood vessels of the large intestine in humans under normal conditions, as well as in experimental animal models, were examined. Using light microscopy techniques, the diameters, lengths, densities, and cross-sectional areas of arterioles, precapillaries, capillaries, postcapillaries, and venules were measured. Normative data revealed regional variations in vascular distribution within the colon, while experimental findings demonstrated morphological changes in vessels during hemorrhage and perfusion disturbances. The obtained data provide valuable insights into the functional state of the vascular system of the large intestine, broaden the understanding of ischemic and post-ischemic pathogenesis, and contribute to the advancement of diagnostic and therapeutic strategies in clinical gastroenterology.

Keywords: *Large intestine, blood vessels, morphometry, arteriole, experimental study, clinical gastroenterology*

INTRODUCTION

The digestive process consists of sequential stages characterized by the formation of the food bolus, the production of chyle, and the absorption of substances

into the blood and lymph. This system plays a crucial role in maintaining homeostasis in the organism, and the biological meaning of its functions is related to the formation of blood plasma. Since the nutrients entering the blood and lymph are foreign to the organism, each food intake represents one of the main challenges to homeostasis [1].

In modern times, comprehensive investigation of the vascular bed of the digestive system organs remains an urgent problem. Only precise information about the state of blood flow can provide an understanding of the morphological

Yazışma üçün əlaqə:

Babayeva R.E.

Azerbaijan Medical University, Department of Human Anatomy and Medical Terminology

*For correspondence E-mail:
ramilababayeva18@gmail.com



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mechanisms regulating intramural circulation [2,3]. According to literature data, the gastrointestinal organs receive a large portion of the cardiac output, thereby contributing to the maintenance of metabolism. This process is evidenced by vascular reactions occurring during increased nutrient absorption. The blood circulation of the gastrointestinal organs also supports the protective mechanisms of the mucosa after the intake of toxins or harmful substances and is necessary to ensure adequate perfusion of vital organs during stress. Under certain physiological (exercise) and pathological (hemorrhage) conditions, a sharp decrease in blood flow in the gastrointestinal organs is observed [2].

In recent years, advances in angiological research, microsurgery, endoscopy, clinical lymphology, and gastroenterology have enabled more detailed studies of colonic vascularization. The investigation of the morphological features of colonic blood vessels is important not only from a theoretical perspective but also for improving diagnostic and therapeutic strategies in clinical gastroenterology.

Aim: The aim of this study is to analyze the morphometric parameters of colonic blood vessels in humans under normal conditions and in experimental animals, to determine their structural organization and functional state, and to evaluate the potential applications of these findings in improving diagnostic and therapeutic strategies in clinical gastroenterology.

MATERIALS AND METHODS

The study analyzed preparations obtained from cadavers of both sexes aged 20–70 years, in which vascular occlusions and stenoses had been identified. Experimental investigations were conducted on 12 dogs of both sexes, weighing between 10.0 and 18.0 kg (9

animals in the main group and 3 in the control group). The housing and euthanasia of animals were performed in accordance with Directive 2010/63/EU of the European Parliament and the Council on the protection of animals used for scientific purposes [4].

To achieve the stated objectives, the experimental part of the study employed a universal method based on classical impregnation techniques (intravascular Ranvier–Coyer and immersion Bilshovsky–Gross methods). This method was developed by I.I. Markov (Certificate of Authorship No. 1919100, dated September 8, 1990) and had been previously tested in studies of microvascular blood flow in laboratory animals [5,6].

Histological sections were examined using microscopic techniques. Prior to staining, paraffin sections were placed in 96% ethanol for 20–30 minutes to remove residual fat. The deparaffinized sections were rinsed in distilled water, stained with Ehrlich's hematoxylin solution for 2–5 minutes, washed again in distilled water, and subsequently in running tap water for 3–5 minutes. Differentiation was performed for 1–2 seconds in 1% hydrochloric acid solution prepared in 70° ethanol. Sections were then washed in running water for 30 minutes with frequent changes. The prepared slides were mounted with cover glasses and examined under a light microscope.

For Van Gieson staining, Weigert's iron hematoxylin solution and a mixture of picric acid with fuchsin were used. Pre-prepared sections were immersed in Weigert's hematoxylin for 2–5 minutes, rinsed in distilled water for 10 minutes, and subsequently placed in the picrofuchsin solution for 2–3 minutes. The sections were cleared in a carbol-toluol solution

and mounted in Canada balsam for microscopic evaluation.

RESULTS AND DISCUSSION

New data have been obtained regarding the histostructures of the longitudinal layer

of the muscular coat. It was revealed that this layer is not continuous along the circumference of the intestine but consists of separate segments (Fig.1).

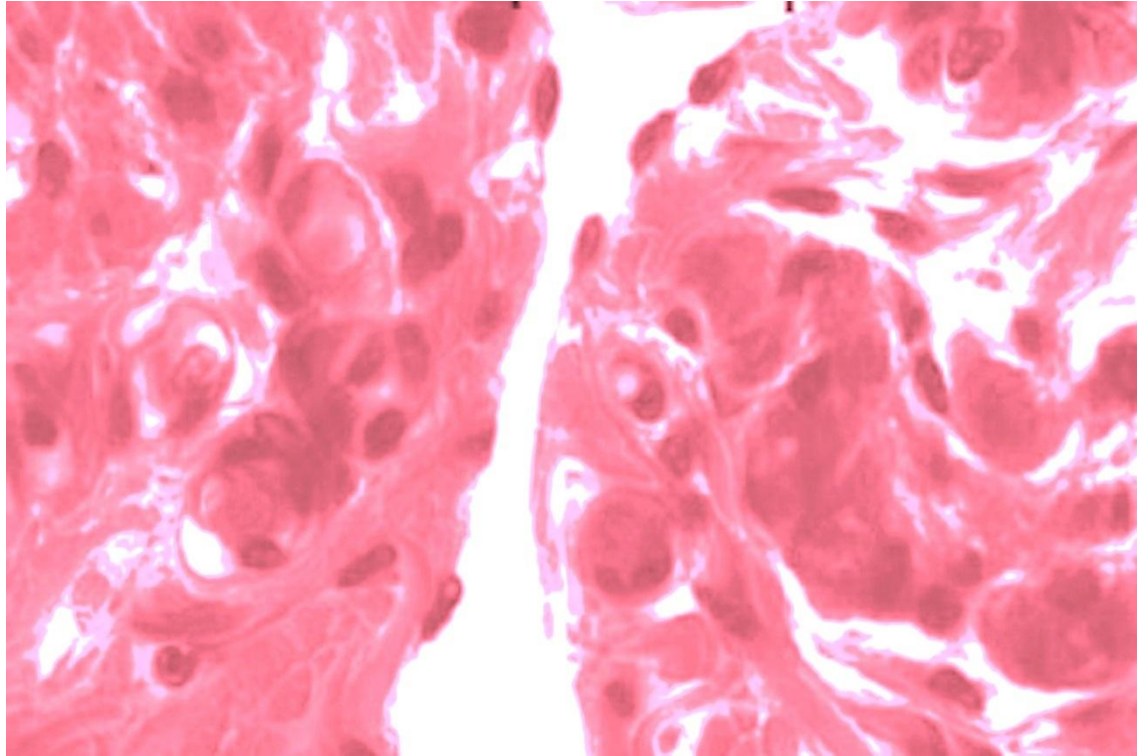


Fig 1. Cross-section of the longitudinal layer of the muscular coat of the large intestine. Hematoxylin-eosin staining. Magnification ×400

In preparations, the number of these segments varied from 8 to 16, with the distance between individual segments ranging from 25 to 525 μm , and their length from 300 to 1200 μm . The intersegmental gaps along the intestinal circumference were recorded to be arranged in a clockwise direction, indicating that the longitudinal layer of the muscular coat moves in a spiral manner.

At the mesenteric border of the intestine, the intersegmental gaps of the longitudinal layer are constant and always coincide with portions of the circular layer of the muscular coat. In this way, muscular “gates,” or rather muscular “tunnels,” are formed, through which straight arteries

enter the submucosal base, while straight veins are directed toward the mesentery. The straight vessels pass through the muscular coat at angles ranging from 65° to 850°.

Under physiological conditions, the area of intersegmental gaps along the intestinal perimeter constitutes between 1.98% and 4.77% of the ring area. This means that during intestinal peristalsis, the conductivity of the vascular bed may both increase and decrease by up to 2.5 times. Arteries passing through the muscular “gates” divide into two branches of equal diameter, forming the main submucosal vascular plexus of the intestine. In cross-sections, the relationship of the

longitudinal layer of the muscular coat with arteries, arterioles, veins, venules,

lymphatic vessels, and capillaries traversing it is clearly visible (Fig. 2).

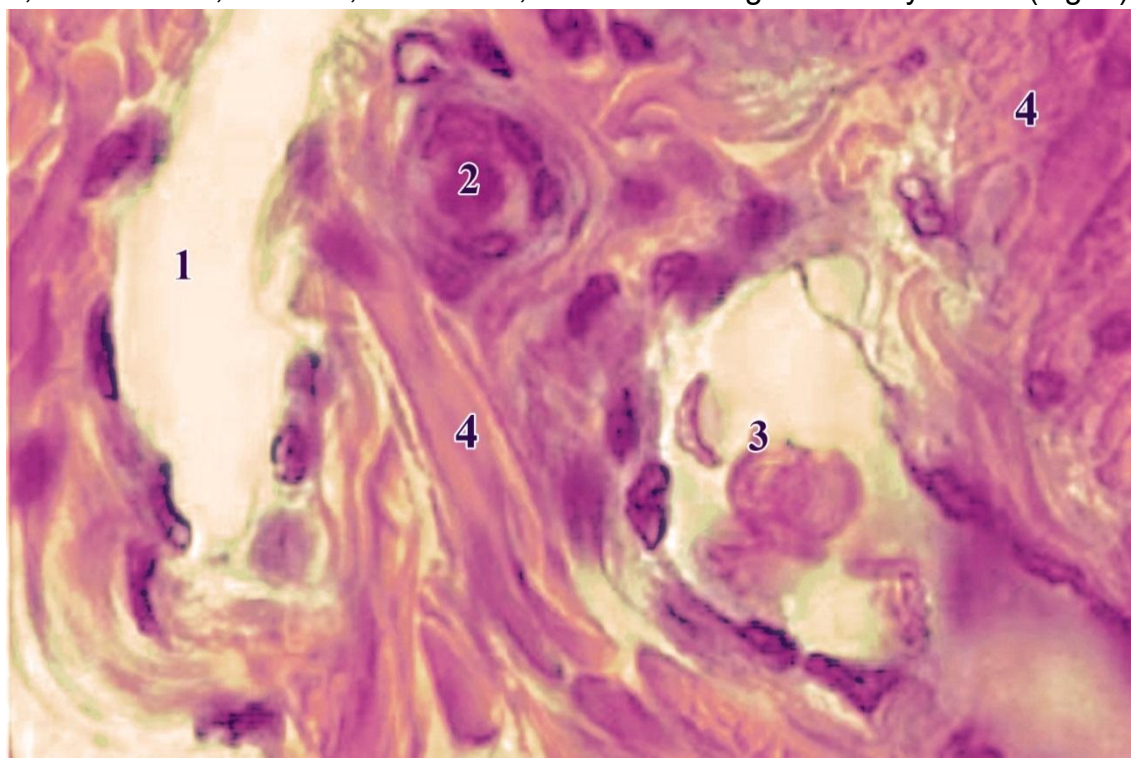


Fig 2. Cross-section of the longitudinal layer of the muscular coat of the large intestine. Staining by the Van Gieson method. Magnification $\times 400$. Note: 1) Lymphatic capillary; 2) Arteriole; 3) Venule; 4) Muscular coat.

The functional range of these vessels (from complete compression between muscular segments to maximal dilation) demonstrates the influence of the longitudinal muscular layer on the vascular lumen. Notably, there is no loose connective tissue between the walls of lymphatic vessels and capillaries and the muscular segments.

Thus, the results of studying the vasculature of the human large intestine under normal conditions show that the extra-organ arteries supplying blood to this organ (celiac trunk, superior and inferior mesenteric arteries) are of the muscular-elastic type. Their walls exhibit clearly defined external and internal elastic membranes. The adventitia is wide, containing numerous blood and lymphatic microvessels as well as nerve elements.

This layer is connected with surrounding organs through fascial beds. According to our findings, the arteries located between the folds of the intestinal mesentery lack fascial tissue beds. However, they are surrounded by loose connective tissue and are bound to both layers of the peritoneum by collagen fibers.

We have obtained new data regarding the histostructures of the longitudinal layer of the muscularis. It was revealed that this layer is not continuous along the circumference of the intestine but consists of separate segments.

The results of experimental studies on the morphological structures of blood circulation in the large intestine demonstrated that, following blood withdrawal, angiograms showed a reduction in the diameter of the main trunk of the superior mesenteric artery to 2.5–

3.0 mm (prior to blood withdrawal, the diameter was 4.0–5.0 mm). The duration of the arterial phase increased to 11.2 s (before blood withdrawal – 1.5 s). Portal pressure decreased from 6.4 mmHg to 3.8 mmHg, while blood flow in the superior mesenteric artery decreased from 294 ml/min to 90 ml/min.

In the study of injected histological preparations, the most significant changes

in the microvascular bed were detected in those regions of the intestinal wall with shared blood supply. Moreover, these areas are located mesoperitoneally and are therefore functionally inactive. In the microvascular bed of the submucosal base of these regions, signs of impaired vascular permeability were identified (Fig.3), with a shift of the gradient toward both arterial and venous microvessels.

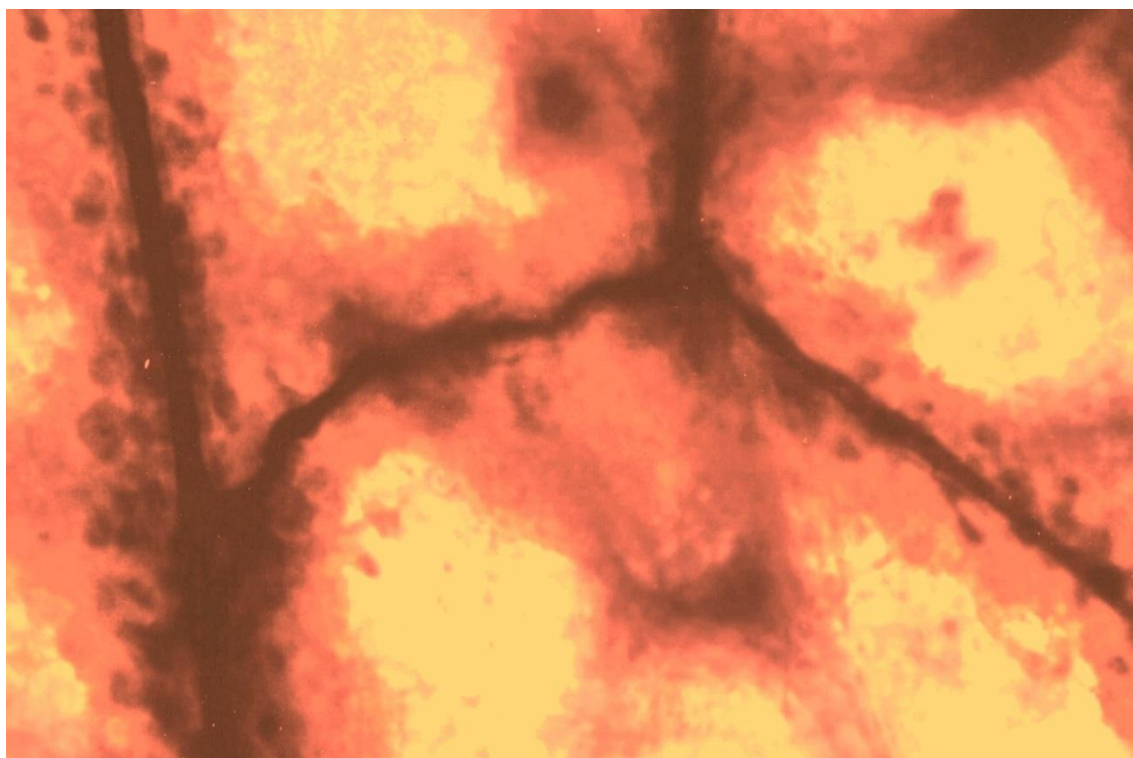


Fig 3. Impairment of vascular permeability in the arterioles of the submucosal base of the ascending colon in the experimental animal. Intravascular impregnation by the Ranvier–Coyer method. Magnification $\times 200$.

Loss of blood plasma was accompanied by hemoconcentration changes. However, the formation of thrombi in venules was not observed. Along the length of numerous arterioles, a sharp reduction in diameter was recorded, indicating cessation of

bleeding within them. The diameter of venules, on the other hand, was reduced in a fragmentary manner, characterized by alternating constricted zones resembling peristaltic waves (Fig.4).

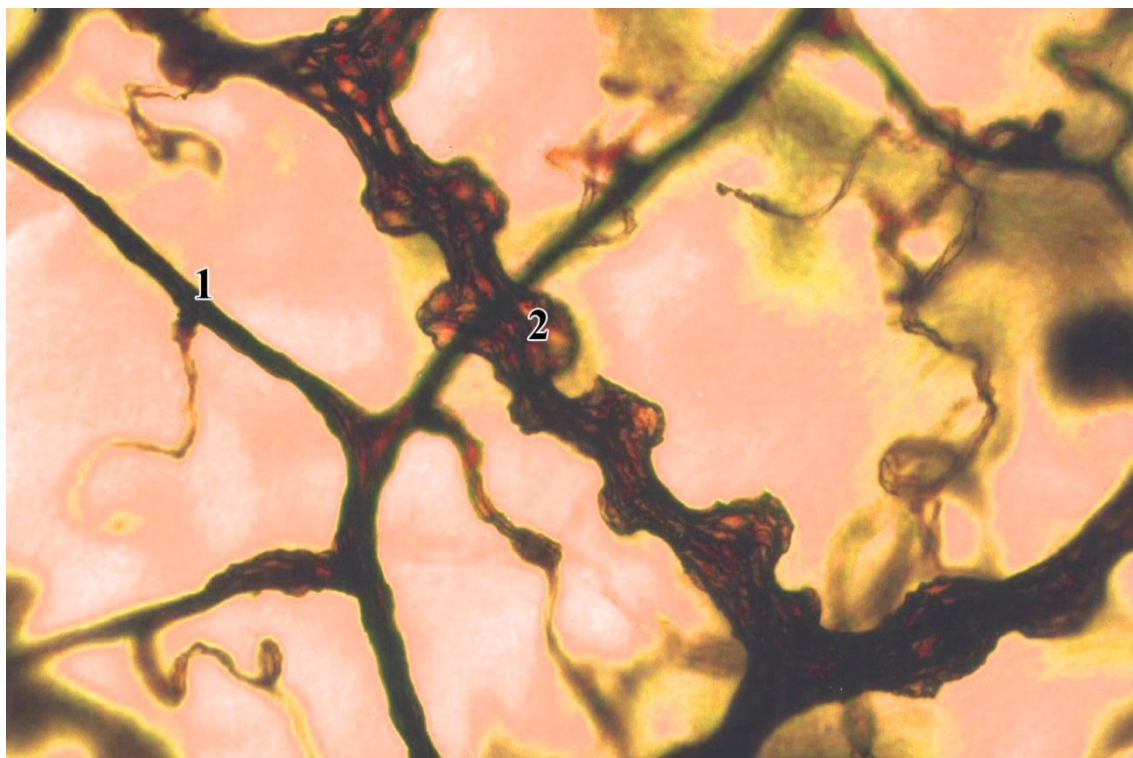


Fig 4. Spasm of arterioles and venules in the submucosal base of the ascending colon of the experimental animal. Intravascular impregnation by the Ranvier–Coyer method. Magnification $\times 200$.

Note: 1) Arteriole; 2) Venule.

Extravasation in the microvessels of the muscularis was less pronounced, and bleeding within them remained minimal throughout the experiment. Extravascular and rheological changes, as well as alterations in blood flow within the microvessels of the muscularis, were also less evident during the study. The superior mesenteric artery differs from other major arteries by the range of its vascularization. With impaired perfusion of the intestinal

wall's microcirculatory bed, the velocity of blood flow in the superior mesenteric artery decreases, and the diameter of its main trunk becomes reduced.

Data characterizing the microcirculatory bed of the greater omentum indicate that a significant volume of blood plasma leaks beyond its boundaries into the peritoneal cavity. The structural organization of these vessels resembles the glomerular capillary network of the renal corpuscle (Fig. 5).

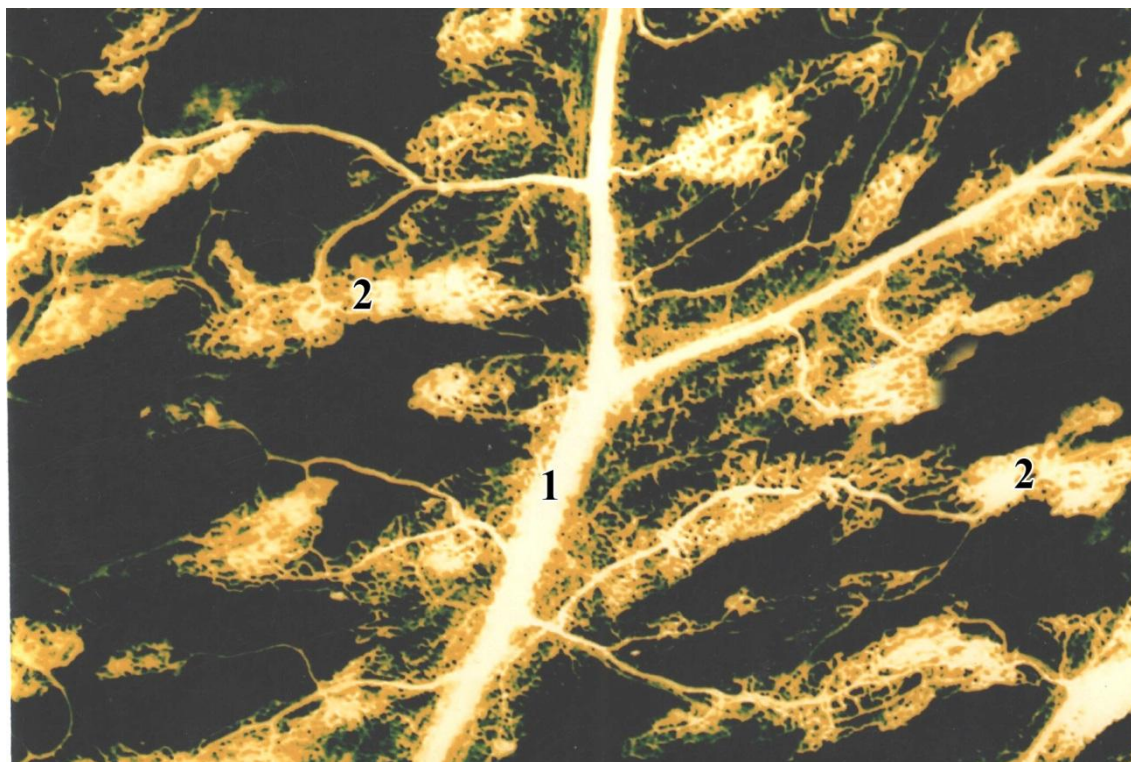


Fig 5. Microvascular bed of the greater omentum in the experimental animal. Intravascular impregnation by the Ranvier–Coyer method. Micropreparation trace. Magnification $\times 600$. Note: 1) Arteriole; 2) Vascular glomeruli.

Thus, the results of experimental investigation into the morphological structures of blood circulation in the large intestine demonstrate that, following blood withdrawal, angiograms reveal a reduction in the diameter of the main trunk of the superior mesenteric artery and an increase in the duration of the arterial phase.

In the study of injected histological preparations, the most significant changes in the microvascular bed were detected in those regions of the intestinal wall with shared blood supply. This provides grounds to consider the greater omentum as a donor of peritoneal fluid or even as the “third kidney” of mammals. In the microvascular bed of the submucosal base of these regions, signs of impaired vascular permeability were identified.

Loss of blood plasma was accompanied by hemoconcentration changes. However, the formation of thrombi in venules was not observed. Along the length of numerous

arterioles, a sharp reduction in diameter was recorded, indicating cessation of bleeding within them. The diameter of venules, on the other hand, was reduced in a fragmentary manner, characterized by alternating constricted zones resembling peristaltic waves.

CONCLUSION

The results of this study demonstrate that morphometric analysis of the vascular system of the large intestine is of great importance for evaluating its functional state both under normal conditions and in experimental settings. Normative parameters reveal the distributional characteristics of vessels in different segments of the intestine, while experimental models allow the observation of morphological changes in vessels during hemorrhage and perfusion disturbances.

The findings obtained:

- Expand knowledge on the pathogenesis of ischemic and post-ischemic changes [7].
- Provide opportunities for improving diagnostic and therapeutic strategies in clinical gastroenterology [8].
- Indicate that monitoring morphometric parameters of the microcirculatory bed may serve as a reliable marker for early detection of perfusion disorders [9,10].
- Show that this approach can be integrated into both experimental translational research and clinical practice through the application of endoscopic and angiological methods [11,12,13].

Thus, morphometric analysis of the vascular system of the large intestine is distinguished not only by its theoretical relevance but also by its practical applicability in clinical gastroenterology, providing a reliable foundation for future translational research.

REFERENCES

1. Golperin YuM, Lazarev PI. Digestion and homeostasis. Moscow: Ripol Klassik; 2020. 303 p.
2. Granger DN, Holm L, Kvietys P. The gastrointestinal circulation: physiology and pathophysiology. *Compr Physiol*. 2015;5(3):1541–83.
3. Xu H, Mao C, Liu J, et al. Microanatomic study of the vascular and duct system of the submandibular gland. *J Oral Maxillofac Surg*. 2011;69(4):1103–7.
4. Directive 2010/63/EU of the European Parliament and of the Council on the protection of animals used for scientific purposes. St. Petersburg; 2012. 48 p.
5. Markov II, Petrov ES, Markova VI. Universal method for selective identification of argyrophilic structures. *Morphological Bulletin*. 2016;(1):114–7.
6. Babayeva RE. Morphological features of neurons innervating different parts of the large intestine. *World Med Biol*. 2021;(2):163–7. doi:10.26724/2079-8334-2021-2-76-163-167.
7. Keskin N, Bamac B, Cakir O, Colak T, Ors A, Barut C. Morphometric analysis of the inferior mesenteric artery using magnetic resonance angiography. *Bratisl Med J*. 2025;126(12):2107–15.
8. Gastrointestinal Endoscopy. Morphometric analysis of the blood supply to the stalk of pedunculated colon polyps. *Gastrointest Endosc*. 2013;77(3):455–62.
9. Slavu I, Filipoiu F, Tulin R, et al. Arterial vascularization of the colon: a guide to surgical resection. *J Mind Med Sci*. 2024;11(2):145–53.
10. Mareev GO, Mareev OV, Kapralov SV, Klimenko GA, Kalyuta TY. Laser Doppler flowmetry in the diagnosis of intestinal ischemia severity. *Russ Open Med J*. 2025;14(1):e0101.
11. Dickson K, Malitan H, Lehmann C. Imaging of the intestinal microcirculation during acute and chronic inflammation. *Biology (Basel)*. 2020;9(12):418.
12. Haas G, Fan S, Ghadimi M, et al. Different forms of tumor vascularization and their clinical implications: vessel co-option in colorectal cancer liver metastases. *Front Cell Dev Biol*. 2021;9:612774.
13. Ortenzi M, Carsetti A, Picchetto A, et al. Implementation of intraoperative procedures to prevent anastomotic leak in low anterior rectal resections: novel

technique to evaluate anastomotic
vascularization. Surg Endosc.

2025;39(4):1123–31.

NORMADA VƏ EKSPERIMENTDƏ YOĞUN BAĞIRSAĞIN QAN DAMARLARININ MORFOMETRIK ANALIZI: KLINİK GASTROENTEROLOGİYADA TƏTBİQ IMKANLARI

Babayeva R.E.

Azərbaycan Tibb Universiteti. İnsan anatomiyası və tibbi terminologiya kafedrası

** Yazışma üçün əlaqə E-mail: ramilababayeva18@gmail.com*

Xülasə

Bu tədqiqatda normada insanın və eksperimentdə heyvanların yoğun bağırsağının qan damarlarının morfometrik xüsusiyyətləri öyrənilmişdir. Mikroskopik metodlarla aparılan analizlər nəticəsində arteriola, prekapillyar, kapillyar, postkapillyar və venulaların diametrləri, uzunluqları, sıxlığı və köndələn kəsik sahələri müəyyən edilmişdir. Normativ göstəricilər bağırsağın müxtəlif şöbələrində damarların paylanma xüsusiyyətlərini aşkar etmiş, eksperimentdə isə qanaxma və perfuziya pozğunluqları zamanı damarların morfoloji dəyişiklikləri izlənməmişdir. Əldə olunan nəticələr yoğun bağırsağın damar sisteminin funksional vəziyyətini qiymətləndirmək, qan dövrəni pozulması ilə bağlı dəyişikliklərin patogenezi haqqında məlumatları genişləndirmək və klinik gastroenterologiyada diaqnostika və müalicə strategiyalarını təkmilləşdirmək üçün əhəmiyyətlidir.

Açar sözlər : *Yoğun bağırsağ, qan damarları, morfometriya, mikrosirkulyator yataq, arteriola, kapillyar, venula, eksperiment, klinik gastroenterologiya*

МОРФОМЕТРИЧЕСКИЙ АНАЛИЗ СОСУДОВ ТОЛСТОГО КИШЕЧНИКА В НОРМЕ И В ЭКСПЕРИМЕНТАЛЬНЫХ УСЛОВИЯХ: КЛИНИЧЕСКОЕ ЗНАЧЕНИЕ ДЛЯ ГАСТРОЭНТЕРОЛОГИИ

Бабаева Р.Э.

Азербайджанский медицинский университет, Кафедра анатомии человека и медицинской терминологии

** Для корреспонденции E-mail: ramilababayeva18@gmail.com*

Резюме:

В настоящем исследовании изучены морфометрические характеристики сосудов толстого кишечника у человека в нормальных условиях, а также в экспериментальных моделях на животных. С использованием микроскопических методов измерялись диаметры, длина, плотность и площадь поперечного сечения артериол, прекапилляров, капилляров, посткапилляров и венул. Нормативные данные выявили региональные различия в распределении сосудов в пределах толстого кишечника, тогда как экспериментальные результаты продемонстрировали морфологические изменения сосудов при кровотечении и нарушениях перфузии. Полученные данные предоставляют ценные сведения о функциональном состоянии сосудистой системы толстого кишечника, расширяют понимание ишемического и постишемического патогенеза и способствуют совершенствованию диагностических и терапевтических стратегий в клинической гастроэнтерологии.

Ключевые слова: *толстый кишечник, кровеносные сосуды, морфометрия, артериола, экспериментальное исследование, клиническая гастроэнтерология*

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MODERN MICROBIOLOGICAL AND ECOLOGICAL CHARACTERISTICS OF THE ASHIGBAYRAMLI RESERVOIR

Ansarova A.H.¹, Abaszade Z.A.², Guliyeva R.N.², Jafarova U.T.², Seyidbeyli M.I.², Mammadli Kh.Sh.³, Mammadova N.Ch.¹

Abstract

For the long-term and efficient use of one of Azerbaijan's major reservoirs, the quantitative assessment of microorganisms in its water and bottom sediments is of great importance. These include total microbial counts, specific physiological groups, and coliform-enterobacteria. The Ashigbayramli Reservoir was established in 1951 in the Ismayilli district of the Republic of Azerbaijan. Its primary purpose is to provide water supply to several settlements, including the districts of Goychay and Aghsu, as well as irrigation of agricultural lands. The floristic composition of dominant phytoplankton groups, their primary production, and the destruction of organic matter were studied seasonally throughout 2013. Microbiological investigations included determination of the total number of microorganisms in water and bottom sediments, as well as saprophytic and physiological groups of bacteria. A sanitary-hydrobiological assessment of water quality was conducted based on bacterial group abundance.

Keywords: Primary production, bacterioplankton, *E. coli*, coliform-enteric groups, saprophytes, physiological groups, destruction.

INTRODUCTION

In modern conditions of water resource utilization, the study of reservoirs is

essential for identifying alternative water sources, preventing anthropogenic eutrophication, and improving water-use efficiency. The main source of water supply to the Ashigbayramli Reservoir is a river. Water flow is directed through a special canal into the natural (old) channel of the Devabatan River and then into the reservoir basin via the Agrichay River—first into Ashigbayramli and subsequently through a dam-sluice system into the lower basin, the Yekakhana Reservoir. The reservoir covers an area of 0.8 km² with a

Yazışma üçün əlaqə:

Ansarova A.H.¹, Abaszade Z.A.², Guliyeva R.N.², Jafarova U.T.², Seyidbeyli M.I.², Mammadli Kh.Sh.³, Mammadova N.Ch.¹

1 Azerbaijan Medical University, Department of Medical biology and Genetics

2 Azerbaijan Medical University, Department of Normal physiology

3 Reference Clinical Laboratory, Baku, Azerbaijan

*For correspondence E-mail: abaszade.zumrud@mail.ru



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total water volume of 5 million m³. Since the feeding river also serves as a freshwater source for domestic use and flows through densely populated areas, and because the reservoir itself is exposed to various anthropogenic impacts, assessment of its sanitary-hydrobiological and ecological-microbiological condition is necessary.

MATERIALS AND METHODS

To determine the annual dynamics of water microbiota and bottom sediment vegetation, studies were conducted seasonally during 2013.

Four sampling stations covering the entire reservoir area were established. Sampling was performed according to planned routes and fixed observation sites.

Water samples for microbiological analysis were collected using Sorokin's method, and hydrobiological-hydrochemical samples were obtained using a Knudsen bathometer. Bottom sediments were collected using a tube corer.

Major biogenic elements, certain heavy metals, and other pollutants were measured using a Palintest THM Digital device, while dissolved oxygen was determined with a Milwaukee MW 600 instrument. Primary phytoplankton production and organic matter destruction

were calculated using the G.G. Vinberg method.

Microbiological analyses and culturing procedures were carried out according to the methodological guidelines of V.I. Romanenko, S.I. Kuznetsov, A.G. Rodina, and S.N. Vinogradsky. Oil hydrocarbon- and phenol-degrading bacteria were determined by the Voroshilova-Dianova method.

During the study period, 1,897 water and bottom sediment samples were collected, and 5,310 microbiological analyses were performed. All numerical results were statistically processed.

RESULTS

The Ashigbayramli Reservoir is shallow, with a maximum depth not exceeding 2.5m near the dam area. Due to its canal-type characteristics, thermal stratification does not form, and the reservoir rarely freezes. Water transparency is relatively high (0.8–1.2 m). Erosion processes are weak because the shores consist mainly of rocky and gravel substrates. The central and dam-adjacent areas contain silty-sandy sediments. Under moderate climatic conditions and sufficient nutrient availability (mineral nitrogen 1.3 mg/L and phosphates 0.09 mg/L), phytoplankton and phytobenthos develop from early spring.

Table1 Floristic composition of phytoplankton in Ashigbayramli reservoir

№ p/p		Branches Taxa	Taxa		
			Spring	Summer	Autumn
1		Cyanophuta	13	6	17
2		Euglenophuta	1	2	1
3		Dinophuta	3	4	2
4		Bacillarophuta	8	12	4
5		Chlorophuta	5	10	8
6		Chrysophuta	1	2	2

As can be seen from Table 1, a total of 101 species of phytoplankton are recorded in the reservoir, among which blue-green and green diatom taxa are almost identical and predominate. Based on the fact that representatives of green and blue-green species predominate in phytoplankton throughout the year, it can be assumed that with increasing anthropogenic pressure on the ecosystem, bloom and death events and water poisoning with phytoncides and other metabolic products are possible. During the development of phytoplankton, the temperature factor determines its qualitative composition. Thus, when the water temperature is up to

10-12°C, taxa of the diatom division predominate in the algal flora, and as the water warms, an increase in green and blue-green vegetation is observed. In addition, samples were collected from surface water layers to determine the amount of primary photosynthetic production of phytoplankton and its dominant divisions (qualitative composition). Due to the non-freezing of the reservoir and the sufficient number of bright sunny days even in winter, phytoplankton photosynthesis and the destruction of organic matter in the water are observed here in all seasons of the year (Table 2).

Table 2 Primary phytoplankton production and destruction (organic matter) (mg O₂/l. day)

Location	Winter		Spring		Summer		Autumn	
	II ¹	D	II ²	D	II ³	D	II ⁴	D
1	0,1	0,3	0,6	2,1	1,30	3,0	0,3	2,4
2	0,2	0,4	0,9	2,4	1,70	2,8	0,8	2,3
3	0,3	0,8	1,3	2,6	2,10	3,3	1,4	2,9
4	0,4	0,9	2,0	3,2	3,20	3,8	2,3	3,0
Medium	0,25	0,60	1,2	2,50	2,10	3,10	1,20	2,2

PP – Primary production;

D – Decomposition of organic matter

As can be seen, the minimum of phytoplankton production is observed in winter and the maximum in summer. The characteristic indicators of organic matter destruction, the average seasonal destruction data are almost 2 times higher than the initial production. The increase in the average annual destruction value compared to the photosynthetic production of phytoplankton is apparently due to the

introduction of allochthonous organic matter with river waters, the main source of which is considered to be wastewater from settlements located in the catchment area. It should be noted that saprophytic (heterotrophic) bacteria react very sharply to the presence of easily mineralized organic matter. The results of our study are presented in Table 3.

Table 3 Total number of microorganisms (million/ml) in water and silt-soil (billion/g) in Ashigbayramli reservoir by season, amount of saprophytic bacteria in water (thousand/ml) and aerobic and anaerobic cellulose-decomposing, free nitrogen-fixing, denitrifying and sulfating bacteria in water and silt-soil.

Type of analysis	Station	Winter		Spring		Summer		Autumn	
		In the water	In the mud	In the water	In the mud	In the water	In the mud	In the water	In the mud
Total microbial count	1	1,4	0,6	2,4	1,6	3,6	1,8	2,1	1,9
	2	0,6	1,3	3,2	2,1	2,1	2,7	1,9	2,1
	3	0,8	1,3	1,6	3,0	2,9	3,3	2,0	2,6
	4	0,9	1,4	2,0	3,4	3,2	3,4	4,6	3,0
Saprophytic bacteria	1	1,0	0,9	3,0	2,2	4,2	2,7	3,7	1,7
	2	1,2	1,2	5,0	2,6	3,2	3,2	2,2	2,4
	3	1,3	1,4	3,2	3,2	2,9	3,3	2,3	2,6
	4	0,9	1,5	10,1	3,6	3,6	4,6	1,8	3,0
Cell-absorbing aerobes	1	10	100	1000	100	100	100	1000	10
		10	100	100	100	100	100	1000	10
	2	10	10	1000	1000	100	1000	1000	100
		10	100	100	1000	100	1000	1000	1000
	3	10	10	1000	1000	100	1000	100	100
		10	100	100	1000	100	1000	100	100
	4	10	10	1000	1000	100	1000	100	100
		10	100	100	1000	100	1000	100	1000
Nitrogen-fixing (aerobes)	1	0,0	3,0	16,0	10	270	0	250	123,0
	2	0,0	7,0	67,0	10,0	294	10,0	270	118,0
	3	0,0	5,0	44,0	10,0	110,0	10,0	190	110,0
	4	0,0	8,0	33,0	0,0	40,0	10,0	110	104,0
Sulphating	1	0,0	0,0	0,0	0,0	10,0	66,0	10,0	60,0
	2	0,0	0,0	5,0	11,0	10,0	106,0	100	116,0
	3	0,0	16,0	3,0	34,0	100	260,0	100	26,0
	4	0,0	24,0	7,0	46,0	100	320,0	100	67,0
Denitrifier	1	0,0	0,0	10,0	100	100	1000	1000	100
	2	0,0	0,0	10,0	100	100	1000	100	1000
	3	0,0	10,0	100	1000	100	1000	100	1000
	4	0,0	10,0	100	1000	100	1000	100	1000

The table shows that the total number of microorganisms in water and bottom sediments varies between 0.6 million/ml and 1.4-3.4 billion/g, respectively. Such fluctuations in the number of microflora in time and space depend primarily on the ambient temperature and the availability of

energy material. In particular, saprophytic bacteria are a kind of biological indicator in this regard. The maximum number of saprophytic bacteria, noted in Tables 2 and 3, coincides with the maximum rate of primary production and destruction of organic matter. Since the water depth in

the reservoir is shallow, the mineralization of organic matter in the water column does not have time to be completely oxidized, and its relatively complex components settle to the bottom. Therefore, there is a significant difference in the qualitative composition of colonies of saprophytic bacteria grown in water and bottom sediments. It is characteristic that at one time S.I. Kuznetsov (1952; 1970) proved that in reservoirs the easily digestible components of proteinaceous organic substances are mineralized primarily by non-spore forms of saprophytic bacteria, while the heavier compounds of the substrate are absorbed by spore-forming bacteria. Based on the above, it can be noted that in the Ashigbayramli reservoir, non-spore-forming saprophytic bacteria cultures grown from water samples constitute an average of 68-76 percent, and in bottom sediments - 40-51 percent. It was also determined that the number of saprophytic bacteria in the water of the Ashigbayramli reservoir varies greatly depending on the season compared to the bottom sediments. As can be seen in Table 3, if the difference in the number of saprophytic bacteria between the minimum (winter) and maximum (summer) indicators in the water column is equal to 3, then in bottom sediments it is higher - 6-7 times. Also, there are no noticeable deviations in the distribution of the total number of microorganisms in water and silt-soil both by reservoir sections and by seasons. Therefore, it can be assumed that the Ashigbayramli reservoir has optimal conditions for self-purification processes, since homothermia and oxygen saturation are observed in the reservoir in all seasons.

DISCUSSION

It is characteristic that the widespread distribution of aerobic forms of bacteriocenosis among physiological groups of bacteria is more noticeable in the water and bottom sediments of the reservoir. As can be seen in Table 3, in all seasons of the year the number of aerobic cellulose-decomposing bacteria in the water exceeds the bottom layer by 10 times, while in the bottom sediments the difference in the number of aerobic-anaerobic bacteria is almost not noticeable. Free-living aerobic nitrogen-fixing bacteria (genus *Azotobacter*) are observed in relatively high numbers in areas where higher aquatic plants develop and in warm seasons when their maximum number exceeds 290/ml. Another free-living nitrogen-fixing genus, the anaerobic *Clostridium pasterianum*, is not widespread in the reservoir. It is not observed in most water samples, and in soils it does not exceed 110-120/g. There is a rather noticeable difference in the number and distribution of sulfate-reducing and denitrifying bacteria, which are anaerobes. As can be seen in Table 3, the main biotopes of both bacterial groups are bottom sediments, but the number of sulfate-reducers is on average 4-5 times less than that of denitrifiers. Based on the fact that for optimal development and physiological and biochemical activity of sulfatizers, there must be a sufficient amount of organic matter, mineral salts of sulfates and sulfites, and a lack of oxygen (Kuznetsov, 1959), it can be assumed that the conditions in reservoirs are not suitable for the active activity of these bacteria and there is no danger of poisoning hydrobionts with hydrogen sulfide and deterioration of the quality of the water in them. Since there are settlements, farms

and local industrial facilities in the basin of the Ashigbayramli reservoir, he is on a summer expedition to the reservoir and the river. Arpachai determined the number of coli groups, phenol and hydrocarbon

oxidizing bacteria (table 4). The presence of phenol-hydrocarbon oxidizing bacteria in the water in the amount of 1000/ml is, of course, due to technogenic factors of anthropogenic origin.

Table 4 Number of phenol, hydrocarbon, oxidizing and coliform bacteria groups in water (1 ml) of Arpachay and Ashigbayramli reservoirs (August, 2013).

Stations	FR ¹	C/h	Coli bacteria
Arpachay River (above the city)	10	10	32
Collector (downtown)	1000	1000	360
Estuarii (source of a reservoir)	1000	1000	123
Water reservoir 1	1000	1000	55
Water reservoir 2	100	100	34
Water reservoir 3	100	100	28
Water reservoir 4	100	100	14

1. Note: FD – phenol decomposition;

2. HOA – hydrocarbon oxidizing agent.

The results of determining the number of coli-enteric bacteria are very indicative. As can be seen from Table 4, the amount of coli group in the water above the Arpachay River settlement area is only 32/ml, and in the settlement area (collector) it increases more than 10 times. At the same time, the number of E. coli in the water along the flow path from the point of maximum pollution to the reservoir (10-11 km) decreases by 5-6 times. However, the presence of E. coli in the river itself, in the reservoir, indicates that the water is polluted with feces, the main sources of which are populated areas.

CONCLUSION

Thus, without conducting the above-mentioned studies in the context of seasons and factors, and without determining the course of substance circulation in reservoirs, it is impossible to correctly assess and predict, from a scientific and applied perspective, the sanitary-hydrobiological safety of water collected in reservoirs for use in

households, agriculture, industry, and processing farms. The primary production of phytoplankton, which is considered the basis of the balance of organic matter in reservoirs, and the amount of destroyed substrates were studied together. Biological productivity, the degree of self-purification of waters, anthropogenic eutrophication and its ecological significance were studied. It became clear that the waters in the reservoirs created in the Kura and Araz rivers, which are severely polluted in Georgia and Armenia, are polysaprobic and autoeutrophic.

REFERENCES

1. Ansarova A.H., Salmanov M.A. Ecological monitoring of the Lower Kura. Works of the Azerbaijan National Committee of the Marine Biodiversity Association. I issue, Baku, 2002, pp. 52-57
2. Ansarova A.H. Ecological microbiology of the Lower Kura. "Modern problems of

the Caspian Sea" // Az ETBTI 90th anniversary, International scientific conf. mat. Astrakhan, 2003, pp. 49-54

3. Ansarova A.H. Ecological assessment of organic pollution of the Lower Kura. Scientific-methodological conf. Baku, 2004, pp. 81-83

4. Salmanov M.A. Ecology for everyone (the issue of our waters). Baku, 2008, 350 p.

5. Newton R.J., et al. (2021). Bacterioplankton and biological productivity in freshwater ecosystems. *Nature Reviews Microbiology*, 19: 593–609. (WoS/Scopus)

6. Ansarova A.H. Modern problems of human ecology, Baku-2021, 495 p.

7. Huseynova N., Aliyev R. (2022). Hydrobiological assessment of the Lower Kura River under anthropogenic stress. *Water*, 14: 2543. (Scopus/WoS)

8. Wang Y., et al. (2022). Formation of microbiological regimes in artificial reservoirs. *Journal of Hydrology*, 610: 127881. (Scopus/WoS)

9. Ансарова А.Г. Современные проблемы экологии человека. Баку-2021, 495 с.

10. Mammadov V., et al. (2023). Fisheries ecology and environmental change in the Kura River Basin. *Journal of Applied Ichthyology*, 39: 112–124. (Scopus/WoS)

11. Chen Q., et al. (2022). Cultivation strategies for heterotrophic aquatic microorganisms. *Frontiers in Microbiology*, 13: 879654. (Scopus/WoS)

12. Li M., et al. (2025). Hydrological regulation effects on deltaic ecosystems and biogeochemical fluxes. *Nature Water*, 3: 112–124. (WoS/Scopus)

13. Huseynova N., Aliyev R. (2023). Regulatory factors and macrophyte succession in large reservoirs of the Kura River Basin. *Aquatic Botany*, 187, 103640. (Scopus/WoS)

14. Huseynova N., Mammadov V. (2023). Reservoir morphometry and water quality monitoring in the Kura Basin. *Journal of Hydrology*, 621, 129604. (Scopus/WoS)

15. Mammadov V., Aliyev R., Gasanov E. (2024). Hydrofauna assessment of the Lower Kura River and Mingachevir Reservoir. *Journal of Applied Ichthyology*, 40, 112–124. (Scopus/WoS)

AŞIQBAYRAMLI SU ANBARININ MÜASİR MİKROBİOLOJİ VƏ EKOLOJİ XÜSUSİYYƏTLƏRİ

Ənsərova A.H.¹, Abaszadə Z.Ə.^{2*}, Quliyeva R.N.², Cəfərova Ü.T.², Seyidbəyli M.İ.², Məmmədli X.Ş.³, Məmmədova N.Ç.¹

¹Azərbaycan Tibb Universiteti, Tibbi biologiya və Genetika kafedrası

²Azərbaycan Tibb Universiteti, Normal fiziologiya kafedrası

³Referens Klinik Laboratoriya, Bakı, Azərbaycan

* Yazışma üçün E-mail: abaszade.zumrud@mail.ru

Xülasə

2013-cü ilin mövsümü üçün dominant fitoplankton qruplarının floristik tərkibi, onların ilkin istehsalı və üzvi maddələrin məhv edilməsi öyrənilmişdir. Mikrobioloji tədqiqatlar mikroorqanizmlərin ümumi sayının, saprofitlərin sayının, su və dib çöküntülərinin bakteriyalarının fizioloji qruplarının təyini

əhatə etmişdir. Suyun sanitar hidrobioloji qiymətləndirilməsi coli bakterial qruplarına əsasən aparılmışdır.

Açar sözlər: *İlkin istehsal, bakterioplankton, bağırsaq çubuqları-coli entero qrupu, saprofit, fizioloji qruplar, məhv edilməsi.*

СОВРЕМЕННЫЕ МИКРОБИОЛОГИЧЕСКИЕ И ЭКОЛОГИЧЕСКИЕ ХАРАКТЕРИСТИКИ ВОДОХРАНИЛИЩА АШИГБАЙРАМЛИ

Ансарова А.Г.¹, Абасзаде З.А.^{2*}, Кулиева Р.Н.², Джафарова У.Т.², Сейидбейли М.И.², Маммадли Х.Ш.³, Мамедова Н.Ч.¹

¹Азербайджанский Медицинский Университет, Кафедра Медицинской биологии и Генетики

²Азербайджанский Медицинский Университет, Кафедра Нормальной физиологии

³Клиническая Лаборатория Реферанс, Баку, Азербайджан

* Для корреспонденции E-mail: abaszade.zumrud@mail.ru

Резюме

Изучены по сезонам 2013 года флористический состав доминирующих групп фитопланктона, его первичная продукция и деструкция органического вещества. Микробиологические исследование охватили определение общей численности микроорганизмов, количества сапрофитных, физиологических групп бактерий воды и донных отложений. Дана санитарно-гидrobiологическая оценка воды по данным коли групп бактерий.

Ключевые слова: *Первичная продукция, бактериопланктон, кишечные палочки – коли-энтерогруппы, сапрофитные, физиологические группы, деструкция.*

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DIAGNOSTIC AND MANAGEMENT CHALLENGES IN AR-NEGATIVE AIS-LIKE PHENOTYPES: A CASE SERIES

Jafarli I.^{1*}, Huseynova I.²

Abstract

We report on three pediatric patients (18-35 months) presenting with features of a 46, XY disorder of sex development (DSD) with an androgen insensitivity syndrome (AIS)-like phenotype, including variable presentations of chordee, penoscrotal transposition, hypospadias-associated anomalies, and undescended testes. These cases were initially suspected of having Partial Androgen Insensitivity Syndrome (PAIS) based on clinical presentation; however, no structural Müllerian, renal, or anorectal anomalies were identified.

Genetic evaluation included targeted AR gene sequencing and broader DSD panels. Endocrine evaluation included basal and stimulated testosterone, gonadotropins, and testicular function markers (AMH, inhibin B). Immunohistochemistry (IHC) was performed on gonadal and scrotal tissue biopsies to assess androgen receptor (AR) expression patterns. Clinical management followed a multidisciplinary team approach, emphasizing conservative surveillance, longitudinal endocrine monitoring, and individualized developmental support, consistent with frameworks established in recent pediatric surgical literature [1]. All three patients tested negative for pathogenic variants in AR and standard DSD panel genes, with baseline hormonal profiles showing normal-to-low-normal responses to hCG stimulation. IHC analysis in Case 1 revealed atypical AR expression, characterized by weak, variable positivity in preputial and scrotal epithelial cells, with significant AR-negative regions. Management remained conservative; early gonadectomy was strictly avoided, and elective reconstructive surgeries (such as chordee correction and orchidopexy) were deferred to allow for spontaneous pubertal progression and further phenotypic assessment.

These cases illustrate the diagnostic challenges of "AR-negative" DSD, where an AIS-like phenotype persists despite the absence of identifiable genetic mutations. The presence of atypical AR expression patterns suggests potential receptor dysfunction or undiscovered regulatory mechanisms. A flexible, multidisciplinary management strategy is incorporating psychological support, genetic counselling, parental informed consent, and the deferral of non-urgent irreversible surgeries is essential to provide comprehensive, patient-centered care [2].

Key words: disorders of sex development, Partial Androgen Insensitivity Syndrome, AR gene, immunohistochemistry, whole-exome sequencing

INTRODUCTION

Disorders of Sex Development (DSD) represent a heterogeneous group of

congenital conditions where chromosomal, gonadal, or anatomical sex development is atypical. Among these, 46,XY DSD caused



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by androgen resistance clinically termed Partial Androgen Insensitivity Syndrome (PAIS) is a major diagnostic consideration for infants presenting with ambiguous genitalia and external virilization defects [3]. Classically, the suspicion of PAIS triggers genetic sequencing of the AR gene. However, clinical literature indicates that less than 30% of boys with phenotypes suggestive of PAIS harbor a detectable AR mutation [4]. This diagnostic gap leaves clinicians with "AR-negative" cases where the clinical and hormonal profile suggests androgen resistance, yet the molecular etiology remains elusive. Furthermore, ethical standards in paediatric surgery require strict adherence to institutional review board (IRB) oversight and explicit parental informed consent, particularly when evaluating delicate congenital variations.

MATERIALS AND METHODS

Clinical Presentation and Diagnostic Findings

The three patients presented between 18 and 35 months of age. Clinical features varied per individual case, encompassing localized genital anomalies without structural Müllerian, renal, or anorectal involvement. Maternal androgen exposure was ruled out in all instances.

Baseline and Stimulated Hormonal Profile:

To assess testicular function and clarify the biochemical basis of suspected androgen resistance, baseline and human chorionic gonadotropin (hCG)-stimulated hormone levels were evaluated. Basal luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone fell within age-appropriate prepubertal ranges. Post-hCG stimulation testing demonstrated appropriate testosterone increments alongside preserved serum Anti-Müllerian Hormone (AMH) and inhibin B levels, confirming functional testicular tissue without evidence of gonadal dysgenesis.

Genetic Analysis: Sequencing of the AR gene and extended panel testing for DSD-related genes were negative for pathogenic variants across all three patients.

Immunohistochemistry: Analysis of Case 1 revealed atypical AR expression, with variable, weak positivity in the prepuce and scrotal samples, and significant regions of AR-negativity.

The presence of a clinical AIS-like phenotype without an identified genetic cause highlights the limitations of current targeted gene sequencing and the potential role of undiscovered molecular pathways or regulatory region abnormalities [4].

Yazışma üçün əlaqə:

Jafarli I.^{1*}, Huseynova I.²

¹Chelsea and Westminster Hospital, UK

²Institute of Molecular Biology and Biotechnologies, Azerbaijan

*For correspondence E-mail: ilhama.jafarli@nhs.net

Table 1. AR-Negative Inconclusive Cases

Cases	Age (months)	Clinical Presentation	Hormonal Profile (Basal hCG Stimulated)	Gene Sequencing	Immunohistochemistry	Management
1	18	Abnormal scrotal skin attachment, chordee, widened penoscrotal distance	LH: normal; FSH: normal; T: normal prepubertal rise post-hCG; AMH/Inhibin B: normal	No mutations in AR or extended DSD panel genes	Atypical AR expression: 30% of epithelial cells in prepuce/scrotal skin showed weak, variable positivity. ~2% of epidermal cells showed strong nuclear positivity. Scrotal samples were AR-negative except for an isolated sebaceous gland.	Deferred early reconstructive surgery Planned future chordee correction/penile degloving if indicated Genetic counseling and formal parental consent Longitudinal MDT endocrine surveillance
2	24	Meatal stenosis, webbed penis, scrotal transposition	LH: normal; FSH: normal; T: adequate response post-hCG; AMH/Inhibin B: normal	Negative for AR mutations	Not performed (insufficient tissue biopsy)	Deferred non-urgent surgery Serial growth and endocrine monitoring Enrolled in longitudinal MDT review

Management and Therapeutic Strategy

A conservative management approach was adopted, with emphasis on careful longitudinal follow-up under institutional ethical guidelines and parental informed consent. The patients underwent regular endocrine monitoring, including serial hormonal evaluations to measure hypothalamic-pituitary-gonadal axis function. Growth velocity and pubertal development were monitored through periodic clinical assessments to evaluate ongoing physical maturation.

Surgical strategy explicitly avoided early gonadectomy, as histological evaluation

and hormonal markers confirmed functional, non-dysgenetic testes with no elevated malignancy risk. Elective reconstructive procedures (such as chordee correction, penoscrotal transposition repair, and orchidopexy) were intentionally postponed to allow for spontaneous physical development and to support shared decision-making as the child matures [5]. In addition, comprehensive psychosocial support was provided to the families to facilitate coping, address concerns related to the diagnosis, and prepare for future discussions

regarding gender identity and long-term well-being.

DISCUSSION

The challenge of managing AR-negative DSD lies in the unpredictability of long-term outcomes. While patients with genetically confirmed PAIS may show higher rates of complications like gynecomastia, the prognosis for those without identified mutations is less clear [4].

The use of immunohistochemistry (IHC) for AR remains a useful but supplementary tool. As seen in Case 1, patchy or weak nuclear expression does not necessarily confirm a functional defect but suggests potential receptor dysregulation. Future diagnostics may increasingly rely on Whole-Exome Sequencing (WES) to uncover novel genetic factors, as recent studies have identified causal variants in previously "idiopathic" cases through expanded research-based sequencing [6]. Recent advances in next-generation sequencing (NGS), particularly whole-exome sequencing (WES), have substantially expanded the spectrum of genes implicated in AIS-like phenotypes, facilitating the identification of pathogenic variants in genes involved in androgen biosynthesis, androgen signaling pathways, gonadal development, and other molecular mechanisms that may mimic classical AIS [7].

Some studies demonstrated that incorporating NGS into the diagnostic evaluation of patients with DSD significantly improves molecular diagnostic yield compared with conventional sequential testing [7]. Their findings emphasize that patients with an AIS-like presentation but negative AR testing frequently receive alternative molecular diagnoses through broader gene panel or exome sequencing, allowing for more accurate disease classification and personalized clinical

management. Similar research highlighted the evolving role of WES as an important diagnostic tool in unresolved DSD cases, particularly when the phenotype does not clearly correspond to a single genetic disorder [6]. The authors emphasized that identifying the underlying molecular defect not only refines diagnosis but also improves genetic counseling, recurrence risk assessment, and multidisciplinary clinical decision-making.

CONCLUSION

The recognition of AR-negative AIS-like phenotypes has important implications for patient management beyond establishing a molecular diagnosis. Accurate genetic and hormonal characterization contributes to individualized decisions regarding gonadal surveillance, endocrine treatment, fertility counselling, and psychosocial support. Furthermore, long-term outcome studies have reinforced the importance of integrating genetic diagnosis with careful multidisciplinary evaluation when making decisions regarding sex assignment and lifelong management. Another study emphasized that gender-related outcomes in individuals with DSD depend on multiple factors, including the underlying diagnosis, hormonal function, psychosocial environment, and ongoing clinical support [8]. Consequently, establishing a precise molecular diagnosis through comprehensive genomic testing provides a stronger foundation for patient-centered counselling and shared decision-making. Collectively, these findings support the routine integration of comprehensive genomic approaches, including WES, into the evaluation of patients with AR-negative AIS-like phenotypes to improve diagnostic accuracy, optimize long-term management, and advance understanding of the complex genetic architecture of DSD.

REFERENCES

1. Ishii T. Clinical practice guidelines for the management of differences of sex development in Japan. *Endocr J*. 2026;73(2):115-130. doi:10.1507/endocrj.EJ25-0542.
2. Jafarli I, John B, Fahmy M, Syed M, Mishra P, Huseynova I. Diagnosis and management of androgen insensitivity syndrome in children. *Glob J Pediatr Neonatal Care*. 2023;4(2). doi:10.33552/GJPNC.2023.04.000584.
3. Batista RL, Costa EMF, Rodrigues AS, Gomes NL, Faria JA, Nishi MY, Arnhold IJP, Domenice S, Mendonca BB. Androgen insensitivity syndrome: a review. *Arch Endocrinol Metab*. 2018;62(2):227-235. doi:10.20945/2359-3997000000031.
4. Lucas-Herald A, et al. The long-term outcome of boys with partial androgen insensitivity syndrome and a mutation in the androgen receptor gene. *J Clin Endocrinol Metab*. 2016;101(11):4419-4427.
5. Marino L, Messina A, Acierno JS, et al. Testosterone-induced increase in libido in a patient with a loss-of-function mutation in the AR gene. *Endocrinol Diabetes Metab Case Rep*. 2021;2021(1):EDM-21-0031. doi:10.1530/EDM-21-0031.
6. Tenenbaum-Rakover Y, et al. The evolving role of whole-exome sequencing in the management of disorders of sex development. *Endocr Connect*. 2021;10(4): R112-R123.
7. Hughes LA, McKay-Bounford K, Webb EA, Dasani P, Clokie S, Chandran H, et al. Next generation sequencing (NGS) to improve the diagnosis and management of patients with disorders of sex development (DSD). *Endocr Connect*. 2019;8(2):100-110. doi:10.1530/EC-18-0376.
8. Sharma S, et al. Long-term outcomes of gender decisions in differences of sex development: a national multicenter study. *J Indian Assoc Pediatr Surg*. 2025;30(1):45-52. doi:10.4103/JIAPS.JIAPS_451_25.

AR-MƏNFI ANDROGENLƏRƏ QISMƏN HƏSSASLIQ SİNDROMUNA BƏNZƏR FENOTİPLƏRDƏ DIAQNOSTIKA VƏ MÜALİCƏ PROBLEMLƏRİ: KLİNİK HALLAR SERİYASI

Cəfərli İ.^{1*}, Hüseynova İ.^{2*}¹ Çelsi və Vestminster Xəstəxanası, Böyük Britaniya² Molekulyar Biologiya və Biotexnologiyalar İnstitutu, Bakı, Azərbaycan* Yazışma üçün E-mail: ilhama.jafarli@nhs.net

Xülasə

Biz 46,XY cinsiyyət inkişafı pozğunluğunun xüsusiyyətlərini nümayiş etdirən, 18–35 aylıq üç pediatrik pasiyent haqqında məlumat veririk. Pasiyentlərdə androgenlərə qismən həssaslıq sindromuna bənzər fenotip müşahidə olunmuşdur. Klinik təzahürlərə müxtəlif dərəcələrdə xordeya, penoskrotal transpozisiya, hipospadiya ilə əlaqəli anomaliyalar və kriptorxizm daxil olmuşdur. Bu hallar ilkin olaraq klinik təzahürlərə əsasən *Androgenlərə Qismən Həssaslıq Sindromu* kimi qiymətləndirilmişdir. Lakin Müllərian strukturlarında, böyrəklərdə və anorektal nahiyədə heç bir struktur anomaliya aşkar edilməmişdir.

Bu hallar “AR-mənfi” cinsiyyət inkişafı pozğunluğunun diaqnostik çətinliklərini nümayiş etdirir. Belə hallarda müəyyən edilə bilən genetik mutasiyalar olmadığı halda androgenlərə qismən həssaslıq sindromuna bənzər fenotipin davam etməsi müşahidə olunur. AR ekspressiyasının atipik nümunələrinin mövcudluğu reseptor disfunksiyasının və ya aşkar edilməmiş tənzimləyici mexanizmlərin mümkünlüyünü göstərir. Kompleks və pasiyentyönümlü tibbi yardımın təmin edilməsi üçün psixoloji dəstək, genetik məsləhətləşmə, valideynlərin məlumatlandırılmış razılığı və təcili olmayan, geri dönməz cərrahi müdaxilələrin təxirə salınmasını əhatə edən çevik, multidissiplinar yanaşma vacibdir.

Açar sözlər: cinsi inkişaf pozğunluqları; androgenlərə qismən həssaslıq sindromu; AR geni; immunohistokimya; tam ekzom sekvenslənməsi.

ДИАГНОСТИЧЕСКИЕ И ТЕРАПЕВТИЧЕСКИЕ ПРОБЛЕМЫ ПРИ AR-НЕГАТИВНЫХ ФЕНОТИПАХ, СХОДНЫХ С СИНДРОМОМ НЕЧУВСТВИТЕЛЬНОСТИ К АНДРОГЕНАМ: СЕРИЯ КЛИНИЧЕСКИХ СЛУЧАЕВ

Джафарли И.^{1*}, Гусейнова И.²

¹ Больница Челси и Вестминстера, Великобритания

² Институт молекулярной биологии и биотехнологий, Азербайджан

* Для корреспонденции E-mail: ilhama.jafarli@nhs.net

Резюме

Мы представляем три клинических случая у детей в возрасте от 18 до 35 месяцев с признаками нарушения полового развития 46, XY и фенотипом, сходным с синдромом нечувствительности к андрогенам. Клинические проявления включали различные варианты хордэ, пеноскротальной транспозиции, аномалий, ассоциированных с гипоспадией, а также крипторхизмом. Первоначально на основании клинической картины у этих пациентов предполагался синдром частичной нечувствительности к андрогенам (СЧНА). Однако структурных аномалий мюллеровых структур, почек или аноректальной области выявлено не было.

Эти случаи демонстрируют сложности диагностики нарушений полового развития с «отрицательным AR»-статусом, при котором фенотип, напоминающий синдром нечувствительности к андрогенам, сохраняется несмотря на отсутствие выявляемых генетических мутаций. Наличие атипичных паттернов экспрессии AR указывает на возможную дисфункцию рецептора или существование ещё не выявленных регуляторных механизмов. Для обеспечения комплексной, ориентированной на пациента медицинской помощи необходим гибкий мультидисциплинарный подход, включающий психологическую поддержку, генетическое консультирование, информированное согласие родителей и отсрочку проведения необязательных необратимых хирургических вмешательств.

Ключевые слова: нарушения полового развития; синдром частичной нечувствительности к андрогенам; ген AR; иммуногистохимия; полное экзомное секвенирование.

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DEVELOPMENT OF THE FIRST DEPARTMENT OF CHILDREN'S DISEASES FROM 1924 TO THE PRESENT DAY

Baylarov R.O., Huseynova S.A., Rasulova L.T., Namazova B.A., Huseynova I.I.

The Department of Pediatrics, founded in 1924 by Professor O.A. Kornitsky at the N. Narimanov Children's Hospital, was the first pediatric department at the Medical Faculty of Azerbaijan University. Initially, the department had three wards with 20-25 beds and a laboratory room. In addition, the department gained the right to teach fifth-year students at Semashko Hospital on infectious children's diseases. At first, training was conducted at N.Narimanov Hospital: in the infant clinic and the University Children's Outpatient Clinic for consultations for children aged 2-3 years. During this period, the department's activities were focused on the following areas: a) nutritional disorders in infants and young children, b) discorrelative forms of

growth and development of the child's body - rickets, diathesis, c) diseases of the blood and hematopoietic organs in early childhood. Classes for fourth and fifth-year students were held according to the plan at the University Outpatient Clinic and Polyclinic. During this period, the department also conducted active scientific work. From 1926 to 1930, the department's employees made numerous reports both in Baku and at the All-Union Congresses of Pediatricians (Moscow, May 1927). Journals on the study of early childhood, Moscow (Volume IV, No. 4, 1926) were also published. In 1927, Professor A.O. Karnitsky published a textbook for students and doctors entitled "Child Development and Features of Its Periods". Under the leadership of Professor A.O. Karnitsky and together with the employees of the University Children's Clinic and doctors of the N.Narimanov Hospital, the "Baku Society of Pediatricians" was created. In 1930-1935, B.P. Brukhansky worked as the head of the department. He was also the director of the N.K.Krupskaya Institute for the Protection of Mothers and Children. In 1935, Professor Z.A. Huseynbekov was elected head of the department. However, in the late 1930s, Z. Huseynbekov was

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Baylarov R.O., Huseynova S.A., Rasulova L.T., Namazova B.A., Huseynova I.I.
Azerbaijan Medical University, Department of I Children's diseases



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subjected to repression and was arrested on false charges. Therefore, in 1940, the leadership of the department was entrusted to Professor C.G. Stein. Scientific research conducted in the 1930s was mainly devoted to the problem of rheumatism and regional pathology (especially malaria). In 1938, the Department of Pediatrics was transferred to the 70-bed pediatric department of the Black City Clinical Hospital No. 2. There were clinical and biochemical laboratories, an auditorium for lectures, and rooms for practical classes. Several employees of the department were mobilized into the army during the war. The head of the department, Prof. S.G. Stein, was awarded the title of "Honored Scientist" on May 11, 1942, along with several medical specialists (S.I. Velikhan, I.S. Ginzburg, V.A. Tarnogradsky) for his impeccable work and service in the field of medicine. During his leadership, the basis of scientific research work at the department was rheumatism, malaria and rickets.

Released from prison in 1956, Z.Huseyinbeyov was re-elected as head of the department and worked in this position until 1959. As a deputy of the Baku City Council, he actively participated in the political and social life of the city, and at the same time carried out large-scale scientific and pedagogical and medical work.

In 1959, this department was headed by Associate Professor N. Karimova, and in 1965-1970, by Professor A.A. Namazova. Under the leadership of Professor A.A.Namazova, the Republican Cardiorheumatology Center was created in 1965-1970. In addition to rheumatism, the clinical features of vascular dystonias in children were also practiced in this center. From 1970 to 1992, the Azerbaijan Medical University, Faculty of Medical and Preventive Medicine was located at the 2nd Clinical Hospital named after E.Efendiyev,

the 7th Children's Infectious Diseases Hospital and the 2nd Children's Polyclinic. During this period, the department was headed by Professor N.A. Huseynova, who graduated from the N.I. Pirogov Moscow Medical Institute in 1948. A talented and versatile scientist, N.A. Huseynova always devoted a lot of time to the development of innovations and the improvement of teaching and methodological work in the department she headed for 22 years.

Pediatric gastroenterology became the main focus of the department. The study of diseases of the gastrointestinal and hepatobiliary systems began in the early 1970s, when endoscopic, bacteriological and ultrasound examination methods were first used to diagnose and verify the spread of lesions of the gastrointestinal mucosa. This led to the introduction of scientifically based treatment methods. A dispensary observation system for sick children was created and organized. Epidemiological studies of gastrointestinal diseases among more than 20,000 schoolchildren revealed their high prevalence (22%) and structure, identified the characteristics of regional pathology and identified a group of risk factors for their development.

N.A. Huseynova's unusual way of thinking and remarkable working capacity allowed the department to conduct advanced research not only in gastroenterology, but also in other relevant areas of pediatrics.

From 1992 to 2017, the department was headed by Professor N.D. Guliyev for 25 years.

In the last ten years, the research topics of the department have included "Clinical and paraclinical parallels in the syndrome of impaired intestinal absorption in early childhood" and "Study of the clinic and pathogenesis of chronic hepatitis in children", of exceptional importance in the study of these topics has been the wide

application of new biochemical, bacteriological, immunological and instrumental research methods in clinical practice. New effective treatment methods for diseases of the gastrointestinal system have been developed and introduced into daily practice, which has significantly changed the treatment strategies of pediatricians, increased the effectiveness of treatment for sick children and sharply reduced the number of relapses. The medical and social significance of the proposed preventive methods for the preclusion of diseases of the gastrointestinal system is great. It has become known that in children, as in adults, gastrointestinal diseases rarely occur as a single disease and syntropy is observed.

Since 1994, the main base of the Pediatrics Department has been transferred to the K.Y. Farajova Research Pediatrics Institute. This has expanded the possibilities for conducting research on issues such as breastfeeding and hypogalactia. Hypogalactia is one of the main problems, since the lack of breast milk is a global problem in pediatrics. New information on the etiology, prevention and treatment of hypogalactia has been published in the press as teaching aids, presented at conferences, and presented in the "Health" program of Azerbaijan Television.

Large-scale work is being carried out to provide practical assistance to the population, especially refugees.

For the multifaceted activity of the pediatrician, scientist, teacher, organizer, some employees of the department are highly appreciated by the health, state and government: Professor Huseynova N.A., in 1984 with the "Healthcare Excellence" badge, in 1986 with the "Labor Veteran" medal, in 1990 with the Honored Doctor of Azerbaijan, in 2010 with the "Progress Medal", Professor N.J. Guliyev received the

honorary title of Honored Scientist in 2000. In 2007, he was awarded the "Progress" medal, and in 2007 he was elected president of the Turkish World Pediatricians Association. Associate Professor S.A. Huseynova was awarded the honorary title of "Honored Doctor" by the President of the Republic of Azerbaijan İlham Aliyev in 2011. From 2016 to the present day, the department continues its scientific, practical, and pedagogical activities as the Department of Pediatrics of the Faculty of Medical and Preventive Medicine. From 2017 to 2023, Professor S.Sh. Hasanov served as the head of the department. The main research areas of the department remained topical issues of neonatology and pediatric gastroenterology.

With the tireless attention and support of the President of the Republic of Azerbaijan I.H. Aliyev, the opening of the Educational-Therapeutic Clinic in 2010 allowed our department to conduct classes with patient demonstrations both at the K.Farajova Scientific Research Institute of Pediatrics and at the TTK.

Currently, the department is located at the K. Farajova Scientific Research Institute of Pediatrics, Educational-Therapeutic Clinic, Educational Surgery Clinic and AMU building No. 5. Since 2023, Professor R.O. Baylarov has been heading the department. In addition to pedagogical activities, the staff of the department continues to conduct active research in the fields of neonatology, nephrology, and gastroenterology.

Since 1970, 53 candidate and 4 doctoral dissertations have been defended at the department under the leadership of professors N.A. Huseynova, N.J. Guliyev, S.Sh. Hasanov and R.O. Baylarov.

As a result of the research, publications in the form of articles, educational and methodological manuals and presentations have been published at conferences and

congresses held both in our republic and abroad.

Research conducted in the fields of pediatric gastroenterology and neonatology forms the basis for the creation of monographs and textbooks.

Prof. N.A. Huseynova, prof. N.J. Guliyev, assoc. prof. S.A. Huseynova, assoc. prof. S.A. Abiyeva, assoc. prof. R.H. Rzayeva, assoc. prof. A.M. Hasanov, assoc. prof. Z.S. Talyshly have written the textbooks "Childhood Gastroenterology" and "Dietary Therapy in Pediatrics"; Prof. N.J. Guliyev, Prof. S.M. Gasimova, Assoc. Prof. U.A. Aliyeva are the authors of the textbook "Childhood diseases (theoretical knowledge and practical skills)".

Prof. R.O. Baylarov, Prof. A.G. Hasanov, U.K. Hajiyeva, Assoc. Prof. F.M. Mammadova are the authors of the textbook "Childhood diseases" (Part I).

Prof. G.M. Nasrullayeva is the author of several textbooks and educational and methodological manuals for students, including the book "Recurrent respiratory diseases in children". She is also the author of the textbooks "Immunology"; "Childhood diseases"; "Clinical Immunology" (2009 and 2011); Primary and Secondary Immunodeficiency teaching methodical manual and Congenital Immune Pathology textbook, as well as the textbooks "Childhood diseases" and "Clinical Immunology" in English.

Prof. S.Z.Garayeva is the author of the monograph "Physical Development and Health Status of Early Childhood" (2022); the textbook "Childhood Diseases" (2014). Also, the employees of the department, Assoc. Prof. I.Sh. Mahmudov, Assoc. Prof. U.A. Aliyeva, Assoc. Prof. B.A. Babayeva, Assoc. Prof. A.B. Huseynova, "Acute non-specific diseases of the bronchopulmonary system in children"; Assoc. Prof. I.Sh. Mahmudov, Assoc. Prof. U.A. Aliyeva,

Assoc. Prof. G.M. Hasanguliyeva, "Pathologies of the digestive system in children"; Prof. S.Sh. Hasanov, Assoc. Prof. I.Sh. Mahmudov, Assistant Prof. N.O.Mollayeva, Assistant Prof. F.B.Amrahova, "Basic principles of emergency medical care provided to children in various pathologies"; Assoc. Prof. U.A. Aliyeva, Assoc. Prof. I.Sh. Mahmudov, "Congenital functional diseases of the cardiovascular system in children. Also Assoc. Prof. U.A. Aliyeva, Assoc. Prof. I.Sh. Mahmudov are the authors of the textbooks "Congenital heart defects, examination methods and treatment".

Department staff: prof. G.M.Nasrullayeva, Assoc. Prof. N.A.Bayramova, prof.S.Z.Garayeva, Assoc. Prof. S.A.Huseynova, Assoc. Prof. A.M.Hasanov have repeatedly successfully delivered reports at international conferences.

Some of our employees, who defend the position of pediatric education and at the same time participate in the improvement of pediatric services, are members of international medical societies.

Prof.G.M.Nasrullayeva is a corresponding member of the International EcoEnergy Academy, a corresponding member of the Russian Academy of Natural Sciences, a member of EAACI (European Academy of Allergy and Clinical Immunology), a member of the British Immunological Society, a member of ESID (European Society of Immune Deficiency), a member of the Azerbaijan Allergy and Immunology Society.

Assoc. Prof. N.A. Bayramova is a member of the Early Childhood Development Scientific Committee of the International Pediatric Association, the editorial board of the Turkish Archives of Pediatrics journal, the International Guide for Monitoring Child Development International Committee of

the International Development Pediatrics Association. She is the deputy editor of the Azerbaijan Medical Association Journal. She is the Secretary General of the Central Asian Pediatrics Union. She is the Chairman of the Scientific Research Working Group of the Azerbaijan Pediatric Society, a member of the Azerbaijan Pediatrics Association and the scientific staff of the Scientific and Educational Center.

The teaching staff of the department honors and remains faithful to the traditions of the pediatric community, has earned the love and respect of students.

The head of the teaching department, Assistant Prof. B.A. Namazova, was awarded the title of "Teacher of the Year" of the Azerbaijan Medical University in 2024, and Assoc. Prof. N.A. Bayramova in 2025.

In addition, during decades of scientific and pedagogical work, the employees of the department have trained a large number of talented scientists and practicing doctors. The great diligence of N.A. Huseynova has allowed the department to conduct innovative research not only in gastroenterology, but also in other relevant areas of pediatrics.