ISSN: 0067-2904

Exploring the Role of *miRNA-9* and *HLA-Class I* Expression in Burn-Induced Vitiligo: The Interplay of Immune Response and Bacterial Infections

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Received: 19/1/2025 Accepted: 4/5/2023 Published: 30/4/2026

Abstract

Vitiligo is a complex autoimmune disorder characterized by the progressive loss of melanocytes, driven by a combination of genetic predisposition and immune dysfunction. This study explores the interaction between bacterial infections and immune activation in burn patients with vitiligo. Blood samples from 45 vitiligo patients and 45 burn patients were analyzed for *HLA-class I* and *miRNA-9* expression using two-step qPCR, key regulators of immune response and melanocyte activity. Swabs from burn patients' skin ulcers were cultured to assess bacterial involvement in immune dysregulation. Results revealed significant immune imbalance, with HLA-class I overexpressed (fold change 29.98) and miRNA-9 downregulated (fold change 0.46), indicative of heightened immune activity. Bacterial cultures identified *Pseudomonas aeruginosa* (45.0%), *Staphylococcus aureus* (40.0%), and other species. HLA-class I upregulation was more pronounced in burn patients (95%) compared to vitiligo patients (65%), potentially linking inflammation or bacterial colonization to immune dysregulation. The findings suggest inflammation, potentially influenced by bacterial colonization, may exacerbate immune dysregulation and contribute to vitiligo onset, with *miRNA-9* playing a regulatory role in the expression of *HLA-ClassI.*

Keywords: Vitiligo, *HLA-Class I, miR-9,* Autoimmune diseases, Bacterial infections.

استكشاف دور تعبير miRNA-9 و HLA-Class I في البهاق الناتج عن الحروق: التفاعل بين الاستجابة المناعية والعدوى البكتيرية

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الخلاصة

البهاق هو اضطراب مناعي ذاتي معقد يتميز بفقدان تدريجي للخلايا الميلانينية نتيجة للتفاعل بين الاستعداد الجيني والخلل في وظيفة الجهاز المناعي. تهدف هذه الدراسة إلى استكشاف العلاقة بين العدوى البكتيرية وتنشيط الجهاز المناعي لدى مرضى الحروق المصابين بالبهاق. تم تحليل عينات دم من 45 مريضًا بالبهاق و45 مريضًا بالحروق لدراسة تعبير جينات *HLA-class I* و *miRNA-9*باستخدام تقنية qPCRذات المرحلتين، وهما منظمين رئيسيين لاستجابة المناعة ووظيفة الخلايا الميلانينية. كما تم زراعة مسحات من تقرحات جلد مرضى الحروق لفحص دور العدوى البكتيرية في اضطراب المناعة.اظهرت النتائج

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اختلالًا كبيرًا في التوازن المناعي، حيث زاد تعبير *HLA-class I* (تغير الطي 29.98) وانخفض تعبير *miRNA-9*(تغير الطي 0.46) بنفس القدر لدى مرضى الحروق والبهاق، مما يشير إلى نشاط مناعي مفرط مشترك في الحالتين. وكشفت الزراعة البكتيرية عن انتشار *الزائفة الزنجارية* (45.0%)، *المكورات العنقودية الذهبية* (40.0%)، وأنواع أخرى مثل *الإشريكية القولونية* (5.0%)، *الكلبسيلة* (25.0%)، و*المعوية البرازية* .(10.0%)كان ارتفاع تعبير *HLA-class I* أكثر وضوحًا لدى مرضى الحروق (95%) مقارنة بمرضى البهاق (65%)، مما يشير إلى احتمال تأثير الالتهاب أو الاستعمار البكتيري على اضطراب المناعة.تشير النتائج إلى أن الالتهاب، الذي قد يتأثر بالاصابات البكتيرية ، يمكن أن يسهم في اضطراب المناعة المرتبط بظهور البهاق، مع دور تنظيمي محتمل لـ*miRNA-9*.

1. Introduction

Vitiligo is a dermatological disorder marked by the depletion of melanocytes, leading to diminished pigmentation and distinct chalky-white patches[1]. Although it does not hinder physical functioning, vitiligo profoundly affects patients mentally because of its apparent manifestations, resulting in stress, self-consciousness, and social shame. Patients often experience depression, anxiety, and social isolation Triggers may include sunburn, pregnancy, skin injuries, and psychological stress [2].

A systematic review and modeling study published in The Lancet Public Health in 2024 demonstrated the global lifetime prevalence of vitiligo to be roughly 0.36%, impacting around 28.5 million individuals globally. The study indicated a higher incidence in adults (0.67%) than in children (0.24%) and noted regional disparities, with Central Europe and South Asia exhibiting the highest rates of 0.52% [3]. Vitiligo impacts both genders uniformly throughout various ethnic groups. Nonetheless, certain research indicates a marginally elevated frequency in females attributed to autoimmune vulnerability and aesthetic considerations[4].

Vitiligo falls under two categories: segmental vitiligo (SV) and non-segmental vitiligo (NSV). NSV comprises several subtypes, including generalized vitiligo, acrofacial vitiligo, and universal vitiligo, each with different clinical features and therapeutic issues [5].
Vitiligo is a multifactorial disease with complex causes involving the interplay of genetic, environmental, and immune factors. The precise mechanisms are not fully understood; however, variables such as stress, trauma, and chemical exposure may interfere with normal biological components, instigating autoimmune reactions in genetically predisposed individuals [6]. The course of vitiligo entails the loss of melanocytes, resulting in depigmented patches. Several theories elucidate melanocyte destruction, predominantly focusing on autoimmune and oxidative stress pathways, which are universally acknowledged as significant contributions[7].Innate immunity is crucial in vitiligo as it connects oxidative stress to immune responses. Crucial cells, including NK cells and dendritic cells, recognize stressed melanocytes, thereby triggering immunological responses against them.

Vitiligo patients exhibit elevated cytokines and increased genes associated with innate immunity. Damage-associated chemicals such as HSP70 and HMGB1, produced by stressed melanocytes, stimulate immunological responses. The NLRP3 inflammasome, together with increased IFN-α from dendritic cells and IFN-γ from ILC1 cells, plays a role in the first death of melanocytes [8,9]. In vitiligo, the adaptive immune system targets melanocytes. CD8+ T lymphocytes specifically target melanocytes in the skin, aided by chemokines that enhance inflammation. Regulatory T cells, which typically modulate immune responses, are diminished in vitiligo, permitting CD8+ cells to persist in their assault. Tissue- resident memory T cells (TRM) persist in the skin, facilitating chronic inflammation and disease

recurrence. B cells contribute by generating antibodies against melanocytes, signifying a wider immunological participation in vitiligo [10].

Genetic research confirms that vitiligo has an autoimmune basis, with about 85% of associated genes linked to inflammation and cell death .This study focuses on two genes *HLA class I* and *miRNA-9.* The *HLA class I* genes, essential for the immunological response, are situated on the short arm of chromosome 6, precisely between locations 6p21.31 and 6p21.32 .This area includes multiple highly polymorphic genes, such as *HLA-A, HLA-B, and HLA-C*[11] . These main products are essential for delivering antigens to T cell receptors, hence enabling adaptive immunity [12]. The expression of *HLA class I* genes is crucial in the etiology of vitiligo. The interaction between *HLA class I* expression and immune cell activation facilitates the autoimmune assault on melanocytes . *HLA-A02:01* is significantly linked to the susceptibility to vitiligo and other autoimmune diseases, such as urticarial [13]. Among *HLA-class I* alleles, owing to its function in presenting melanocyte antigens to cytotoxic T lymphocytes. A transcriptional regulator associated with this allele amplifies its expression, augmenting immune recognition and perhaps leading to melanocyte loss in vitiligo [14]. Other alleles, such as *HLA-DRB1 04* highly related to rheumatoid arthritis [15]. *MicroRNA-9* (miR-9) is a diminutive, non-coding RNA that modulates gene expression by attaching to target mRNAs' 3′-untranslated region (UTR), resulting in mRNA destruction or translational repression[16, 17]In humans, miR-9 is processed from three precursors (miR-9-1, -2, and -3) located on chromosomes 1, 5, and 15, respectively .*miR-9* is regarded as one of the miRNAs linked with oxidative stress in vitiligo . The fluctuation in *miRNA-9* gene expression significantly contributes to the etiology of vitiligo. Research demonstrates that *miR-9* is activated in patients with vitiligo, corresponding with elevated levels of long non-coding RNA. MALAT-1 ,*miRNA-9* downregulation has been linked to increased oxidative stress and apoptosis in melanocytes, leading to their loss in vitiligo lesions [19].

As many as 63.8% of people who have vitiligo exhibit the Koebner phenomenon (KP), which is a phenomena that is frequently observed in autoimmune dermatological disorders or conditions that affect the skin. It is possible that its presence indicates an increased level of disease activity, which is typically linked with a more extensive involvement of the skin and an intensification of autoimmune reactions. In patients with vitiligo, keratosis pilaris can be brought on by a wide variety of skin traumas, such as burns, bruises, or exposure to ultraviolet (UV) light.[20]. Certain bacterial infections may provoke an inflammatory response, contributing to developing vitiligo lesions through KP [20]. Burn injuries and other dermal injuries can compromise the epidermal barrier, facilitating the colonization of opportunistic microorganisms such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This colonization may result in dysbiosis, an imbalance in the skin microbiota associated with numerous autoimmune and inflammatory disorders. In *S. aureus* infections, the downregulation of TLR2 is associated with elevated levels of IL-6 and IL-10, signifying immunological dysregulation [21]. For example, P. aeruginosa utilizes mechanisms to decrease host immune responses, including the inhibition of the NLRP3 inflammasome, which is essential for successful inflammatory responses [22]. *S. aureus* synthesizes superantigens such as SEA, SEB, and TSST-1, which can elicit robust immunological responses by activating a substantial quantity of T cells. Superantigens can induce a dysregulated immune response, resulting in the depletion of melanocytes in individuals with vitiligo. Another significant role is bacterial toxins, *P. aeruginosa* and *S. aureus* disrupt host cells and enhance inflammation through toxins. Pyocyanin from *P. aeruginosa* induces oxidative stress and apoptosis, while rhamnolipids damage immune cells. S. aureus alpha-

toxins cause cell lysis and inflammation, contributing to infection and potential autoimmune effects [23].

Materials and Methods

Subjects and Sampling

This cross-sectional research was done from November 2023 to July 2024. Samples were acquired at Baghdad Teaching Hospital, a specialized medical facility for burns. The actual laboratory work was conducted in the Biotechnology Research Center at Al Nahrin University, Department of Biology, College of Science, University of Baghdad, and in private laboratories for molecular tests Ethics Committee, Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq, and the Iraqi Ministry of Health all granted their sanction for this study. CSEC/1023/0078. The study collected over 90 blood specimens from two patient groups, each with 45 samples from individuals with vitiligo and burns aged 15-60. Forty-five skin swabs were collected from burned skin ulcers.

Blood samples were collected from patients and control groups using vein puncture methods. The samples were divided into two groups to obtain serum and whole blood, which was then used to extract total genomic human RNA. The procedure involved inserting two milliliters of blood into an EDTA anti-coagulated tube to extract total genomic human RNA. Forty-five swab samples were collected from burned skin ulcers using sterile cotton swabs that had previously been moisturized with normal saline. Each swab was placed in a Cary Blair transport medium, labeled, and transported to the laboratory under refrigerated conditions within 24 hours for analysis.

Molecular analysis of the specimens

RNA extraction and concentration measurement

RNA was extracted by TRIzol method using GENEzol™ TriRNA Pure Kit (Geneaid Biotech Ltd / Taiwan). Gel electrophoresis was done using the Cleaver electrophoresis system (Cleaver / UK) to confirm that RNA was extracted. Then to confirm the RNA exists in a good concentration, the concentration of RNA was measured using Quantus™ Fluorometer (Promega / United States).

Measuring gene expression of miR-9 and HLA class-I by two-step RT-PCR.

The gene expression of *miR-9* and *HLA class-I* was quantified using a two-step RT-PCR methodology. The initial stage was the conversion of RNA to cDNA with two separate kits to assess *HLA class I* RNA and *miR-9* expression. The Accupower RT premix (cDNA) kit (Bioneer/Korea) was utilized for HLA class I RNA. This kit employs an oligo-dT primer to specifically reverse transcribe poly-A tail-containing messenger RNA (mRNA) into complementary DNA (cDNA). The cDNA synthesis was conducted at 42°C for 60 minutes to generate cDNA from RNA, followed by heat inactivation at 95°C for 5 minutes to deactivate the reverse transcriptase enzyme. The resultant cDNA was preserved at temperatures ranging from 4°C to -20°C for subsequent utilization.

The miRNA 1st strand cDNA stem loop kit (Vazyme/China) was employed for *miR-9,* specifically formulated for microRNA (miRNA) molecules devoid of poly-A tails. This kit contains a stem-loop reverse transcription primer (5'-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCATA-C-3'), which improves specificity for mature miRNAs such as *miR-9.* The two-step cDNA synthesis commenced with the elimination of genomic DNA, during which samples were incubated at 42°C for 2 minutes to eradicate contaminating genomic DNA. Subsequently, primer annealing occurred at 25°C for 5 minutes to facilitate the binding of the stem-loop primer to RNA. Reverse transcription was conducted at 50°C for 15 minutes to generate cDNA from

RNA with the RT enzyme. The reaction was concluded by inactivating the enzyme at 85°C for 5 minutes.

Quantitative real-time polymerase chain reaction (qRT-PCR) was subsequently performed. Four primer pairs were developed (designed by this study) for the HLA class I gene, the miRNA-9 gene, and their corresponding housekeeping genes, GAPDH and U6. All primer sequences utilized are mentioned in Table 1.

Table 1: qRT-PCR primers

Target gene	Forward 5' →3'	Reverse3'→ 5'
HLA	5'- CTCAACCTCCCAAGCTCAAGC-3'	5'- AGGTAAGCACGGAAAAGCCAG-3'
GAPDH	5'- GCCTTCTCCATGGTGGTGAA-3'	5'- GCACAGTCAAGGCCGAGAAT-3'
miRNA-9	5'- GCGCGTCTTTGGTTATCTAGCT-3'	5'-AGTGCAGGGTCCGAGGTATT-3'
U6	5'- GCTTCGGCAGCACATATACTAAA AT-3'	5'- CGCTTCACGAATTTGCGTGTCA T-3'

The thermocycler was set with these parameters: an initial denaturation at 95°C for 1 minute, followed by 40-45 cycles of denaturation at 95°C for 10 seconds and extension at 60°C for 30 seconds. A melting curve was produced with temperatures varying from 95°C to 60°C, incrementing by 0.5 °C every 15 seconds. Gene expression levels were quantified as fold changes using the Livak and Schmittgen described ΔΔCT method [24].

Bacterial Culturing, Isolation, and Identification

Aseptic Technique

All procedures were performed under sterile conditions, including workspace disinfection, autoclaving equipment, and flaming inoculation tools. Personal protective equipment was used throughout. Bacterial samples were collected from burned skin ulcers using sterile swabs and inoculated in Brain Heart Infusion (BHI) broth.

BHI Broth Preparation

BHI broth (HiMedia, India) was prepared by dissolving 37 g/L of medium in distilled water, autoclaved, and cooled. Samples were incubated overnight at 37°C in a shaking incubator for bacterial enrichment.

Culturing and Isolation

All media were prepared according to the manufacturer's specifications, autoclaved at 121°C for 15 minutes, and subsequently cooled to 45-50°C before being poured into sterile petri dishes. Plates were examined for contamination before use. The quadrant streak technique was applied to ensure the development of well-isolated colonies. Bacterial cultures were streaked on Mannitol Salt Agar (HiMedia, India) and MacConkey Agar (HiMedia, India) for isolation and differentiation of Gram-positive and Gram-negative bacteria. Subculturing on HiChrome Agar (TM Media, India) chromogenic media enables differentiation through colony color, which arises from the enzymatic activity of certain bacteria on chromogenic substrates,the color index of Hichrome Agar shown in Table 2. Plates were incubated at 37°C for 24-48 hours in a temperature-controlled incubator.

Table 2: Color index of Hichrome Agar

Bacterial Species	Color of Colony on HiCrome™ agar	ATCC Reference
Escherichia coli.	Pink to Purple	ATCC 25922
Enterococcus faecalis.	Blue	ATCC 29212
Staphylococcus spp.	Cream to Yellow	ATCC 25923
Pseudomonas aeruginosa.	Colorless (Green pigment may be observed)	ATCC 27853
Klebsiella pneumoniae	Bluish Purple	ATCC 700603

Data Analysis and Visualization Tools

Python library was utilized for data analysis and visualization. The Pandas library handled descriptive statistics, data preprocessing, and merging. Matplotlib library and Seaborn generated visualizations such as histograms, boxplots, scatterplots, and heat maps. Statistical tests, including the Wilcoxon signed-rank test and paired t-test, were performed using SciPy.

Results

The *HLA-Class I* gene demonstrated significant upregulation in the vitiligo and burns patient groups. The mean ΔΔCt was -1.76, corresponding to an average fold change of 29.98 (2^-ΔΔCt).

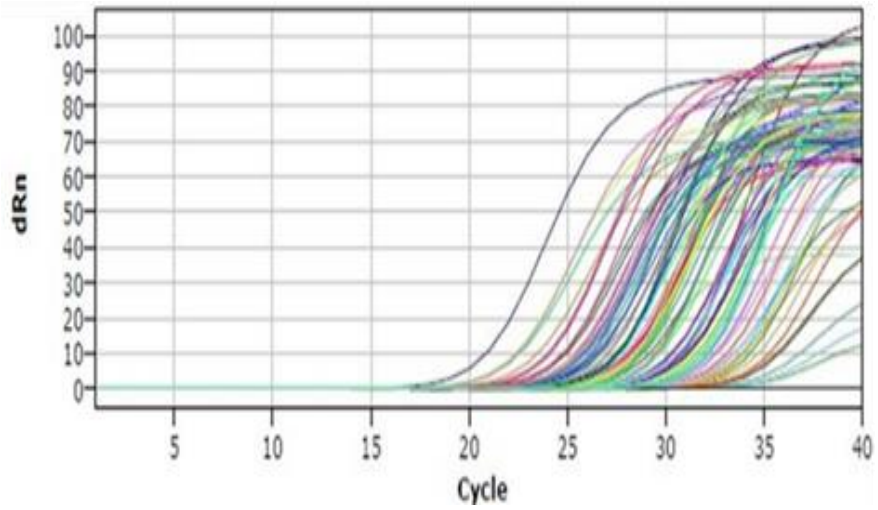


Figure 1: Ct values for housekeeping gene (*gapdh)* and gene of interest (*hla-class i*) in vitiligo and burn patient samples.

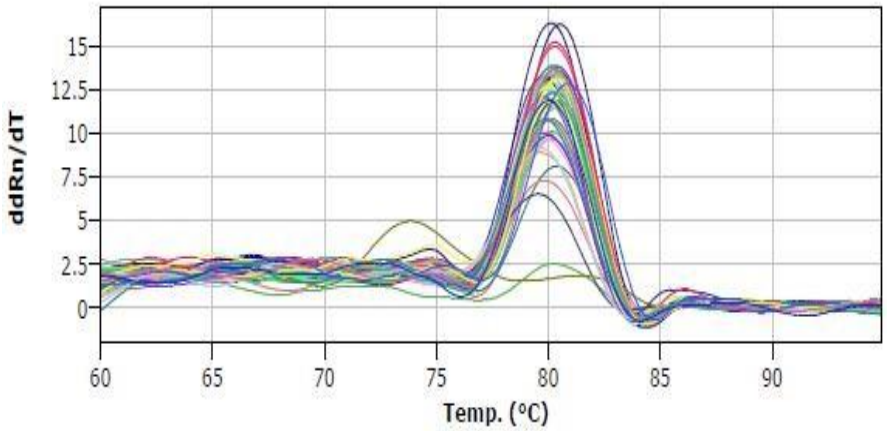


Figure 2: melting curve analysis of *hla-class i* gene amplification

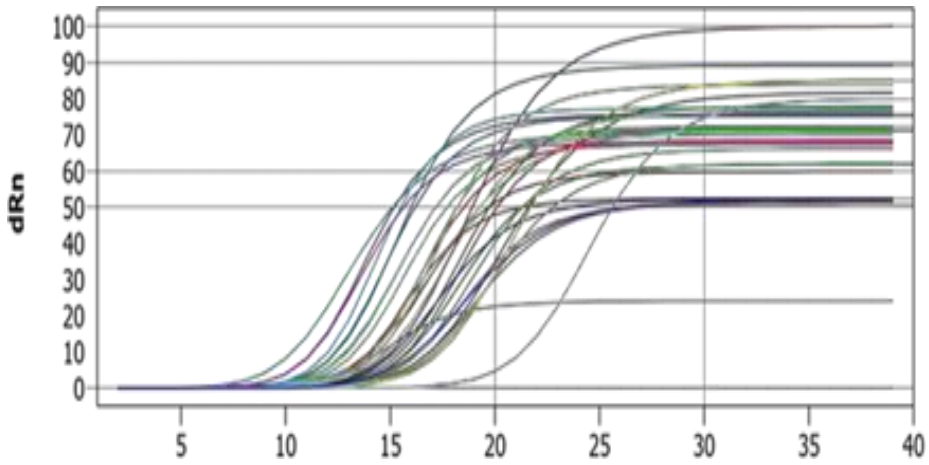
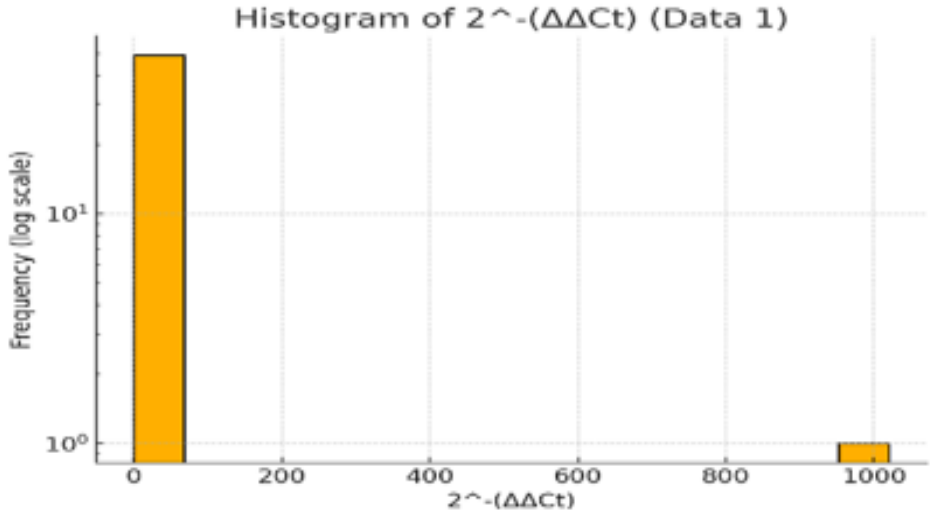


Figure 3: ct values of the *gapdh* housekeeping gene across vitiligo and burn patient samples Considerable variability was observed, with a standard deviation of 143.33 and a range spanning 0.0077 to 1019.75.



Histogram 1. Fold change distribution (2^-(δδct)) of *HLA Class-I gene* gene expression in vitiligo and burn patients)

The *miR-9* gene showed significant downregulation, with a mean ΔΔCt of 1.15 and an average fold change of 0.46 (2^-ΔΔCt).

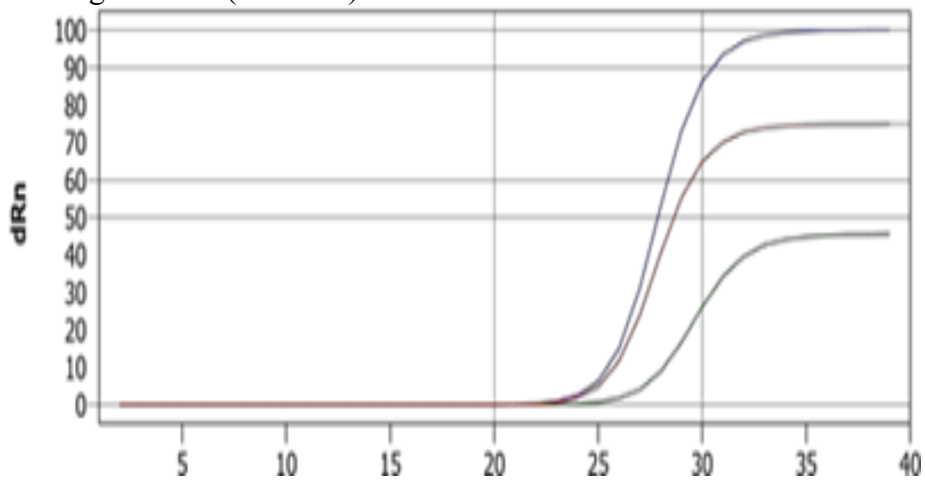


Figure 4: CT curves of *miRNA-9* from the three samples included in this investigation.

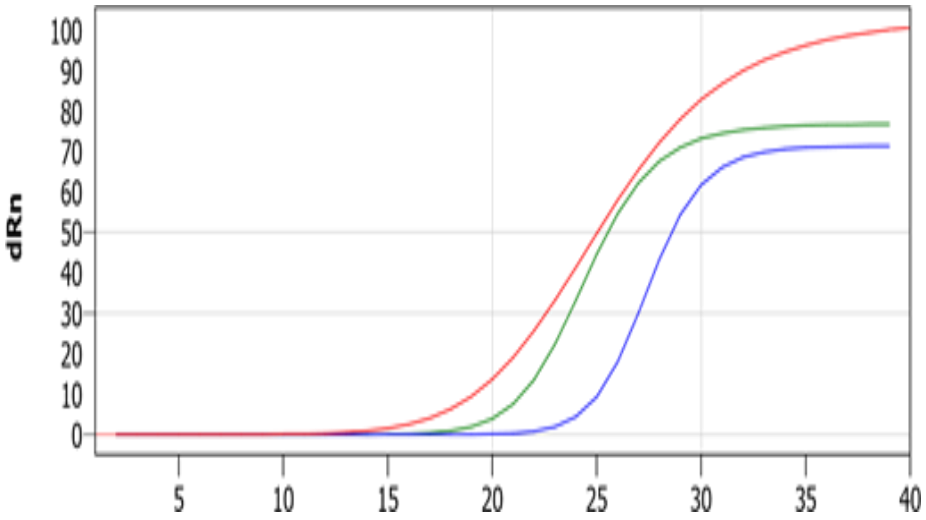
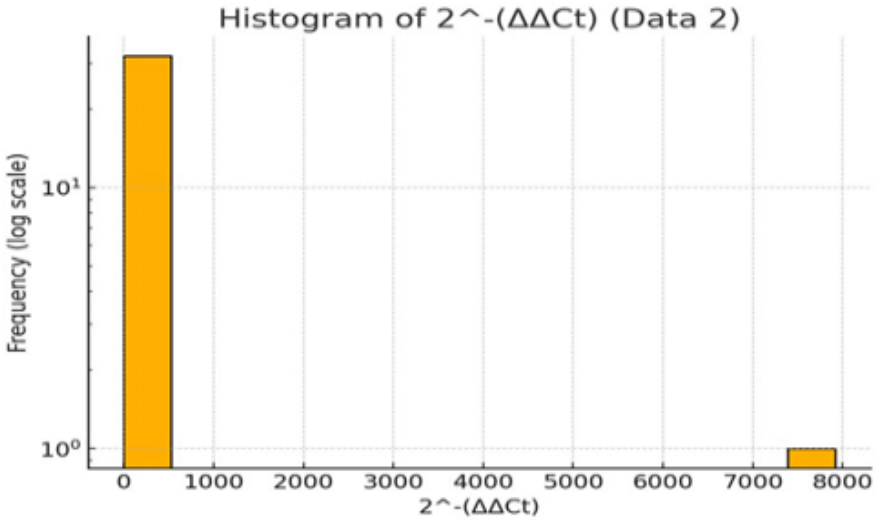


Figure 5: CT curves of *miRNA-9* from the three samples included in this investigation.

miRNA-9 gene expression also exhibited high variability, with a standard deviation of 1377.09 and a range from 0.01 to 7912.95.



Histogram 2. fold change distribution (2^-(δδct)) of *mirna-9* gene expression in vitiligo and burn patients)

Correlation analysis between *miR-9 and HLA-Class I* expression revealed weak relationships. In the vitiligo cohort, Spearman's ρ was 0.27 (p = 0.40), and Pearson's r was 0.16 (p = 0.69). In the burn cohort, Spearman's ρ was -0.35 (p = 0.30), and Pearson's r was 0.25 (p = 0.72). Linear regression yielded R-squared values of 1.6% for the vitiligo group and 1.5% for the burn group.

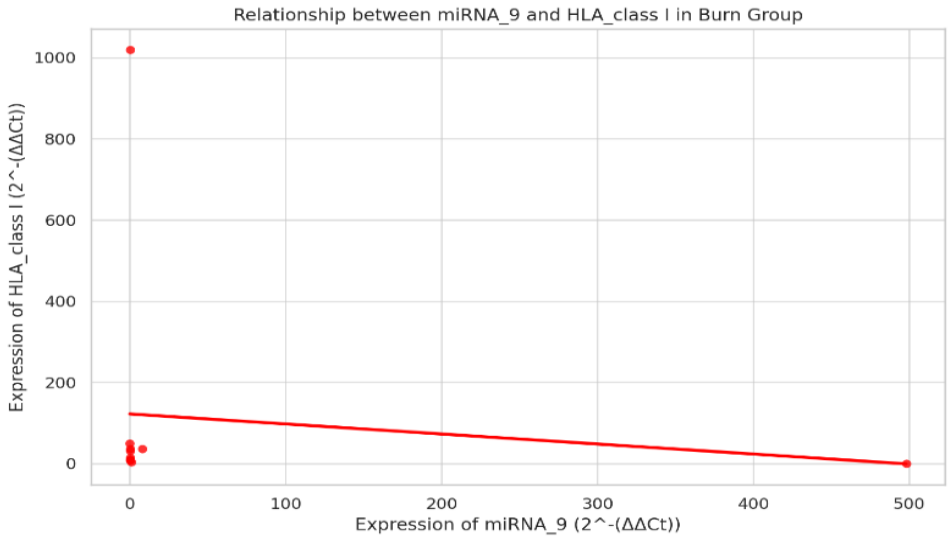


Figure 6: Describes the relationship between *mirna-9* expression and *hla class-i* expression among burns patients group.

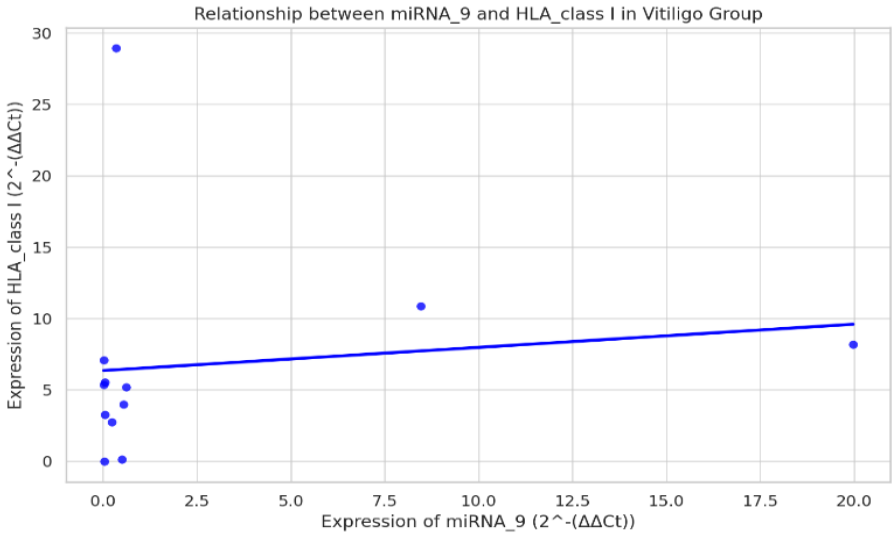


Figure 7: Describes the relationship between *miRNA-9* expression and *HLA class-I* expression among vitiligo patients group.

Bacterial species identified from swab samples included *S. aureus* (40.0%), *P. aeruginosa* (45.0%), *Escherichia coli* (5.0%), *Klebsiella* spp. (25.0%), and *Enterococcus faecalis* (10.0%).
However, statistical analysis did not reveal significant correlations between specific bacterial species and gene expression levels. *HLA-Class I* expression was upregulated in 95% of burn patients compared to 65% of vitiligo patients.

Discussion

The study's findings reveal substantial immunological dysregulation in patients with vitiligo and burn injuries, evidenced by alterations in HLA-Class I and miR-9 expression. The significant elevation of HLA-Class I indicates increased antigen presentation activity, which is essential for controlling immune responses. The mean fold change of 29.98 in its

overexpression is beneficial for acute immune reactions but might worsen autoimmunity. In vitiligo, oxidative stress and cytokines like IFN-γ, IL-4, IL-6, and IL-10 likely worsen this upregulation [25]. This increases antigen presentation on melanocytes, leading to their destruction by cytotoxic T cells, which adds to depigmentation. Burn injuries may trigger an autoimmune response by producing antigens that mimic melanocyte proteins. HLA-Class I molecules display these antigens, increasing the chance of cross-reactivity. This is when T lymphocytes mistakenly attack melanocytes along with compromised cells. Additionally, the production of damage-associated molecular patterns (DAMPs) induces the overexpression of HLA-Class I as part of a severe immunological response [26].

The variation in HLA-Class I expression, shown by a large standard deviation of 143.33 and a range from 0.0077 to 1019.75, highlights the different immunological responses among patients. This variability is likely influenced by genetic predisposition, the extent of tissue injury, and environmental factors, highlighting the complexity of immune activation in these scenarios[27]. also, numerous causes and conditions affect that, including the extent of tissue damage in burn patients and the level of oxidative stress and cytokine activation in vitiligo patients , single-cell diversity and genetic polymorphism [28] . The patterns observed are illustrated in Figures 1 and 2, while the histogram 1 further emphasizes the variability.

The inhibition of miR-9 introduces an additional aspect to the immunological dysregulation noted. The mean fold change of 0.46, along with a substantial standard deviation of 1377.09 and a broad range of 0.01 to 7912.95, indicates significant heterogeneity in miR-9 expression. The noted downregulation of miR-9 in both vitiligo and burn patients underscores its essential function in modulating inflammation, immunological responses, and melanocyte viability. Normally, miR-9 inhibits pro-inflammatory cytokines like TNF-α and IL-1β, as well as transcription factors such as NF-κB. Its downregulation removes this constraint, leading to increased immune activation. Its downregulation extends inflammation, enhances cytokine production, and diminishes the regulation of immunological responses, leading to melanocyte death [30].In burns, the liberation of damage-associated molecular patterns (DAMPs) triggers inflammatory pathways, which further inhibit miR-9 and intensify immune-mediated melanocyte injury. Chronic inflammation induced by oxidative stress, increased IFN-γ, and autoantibody generation in vitiligo maintains miR-9 suppression, establishing a lasting autoimmune milieu [31]. Furthermore, it disturbs the equilibrium between pro-inflammatory Th17 cells and regulatory T-cells (Tregs), promoting an aggressive immunological environment [32]**.** These findings illustrate the multifaceted role of miR-9 in immune regulation, particularly in its suppression of inflammatory cytokines and modulation of immune responses. The interplay between DAMP-induced inflammation in burns and chronic immune activation in vitiligo highlights a complex regulatory network that disrupts immune homeostasis. This dysregulation perpetuates a cycle of inflammation, immune sensitization, and melanocyte destruction, ultimately contributing to the onset or progression of vitiligo and depigmentation in burn patients [33].

The histogram (2) for fold changes (2^-ΔΔCt), shows a sharp skew towards low values, confirming the predominant downregulation of miR-9. However, extreme outliers on the higher end indicate significant upregulation in isolated samples. The 2^-ΔΔCt boxplot highlights significant variability in fold changes, with rare cases of upregulation amidst overall downregulation.

The diversity in miR-9 expression among patients emphasizes the influence of individual biological and environmental factors, suggesting patient-specific strategies for comprehending immune control. Figures 3, 4, and 5 illustrate these findings, while the

histogram (2) highlights the skew toward downregulation with occasional outliers. This condition fosters an environment conducive to melanocyte death, especially in vitiligo, where persistent inflammation intensifies autoimmune.

The association between miR-9 and HLA-Class I expression demonstrates intriguing and intricate dynamics. Statistical analysis revealed minor relationships, with Spearman and Pearson coefficients demonstrating minimal associations. In the vitiligo cohort, Spearman ($\rho = 0.27$, $p = 0.40$) and Pearson ($r = 0.16$, $p = 0.69$) analyses demonstrated a marginal positive tendency, whereas, in burn patients, Spearman ($\rho = -0.35$, $p = 0.30$) and Pearson ($r = -0.25$, $p = 0.72$) revealed a weak negative correlation. Linear regression demonstrates negligible effects, with R-squared values of 1.6% (vitiligo) and 1.5% (burn), signifying that miR-9 expression accounts for quite a minor portion of HLA-Class I variability. The Statistical results indicate that the downregulation of miR-9 is slightly correlated with the overexpression of HLA-Class I, reinforcing the idea that miR-9 may regulate HLA-Class I expression.

The observed lack of association between miRNA-9 and HLA-Class I gene expression may be ascribed to many variables, as demonstrated by Spearman's ρ and Pearson's r values. Gene expression regulation is a complex process affected by numerous transcriptional and post-transcriptional processes. MiRNA-9 may not independently regulate HLA-Class I expression but instead operates within a more extensive network that includes other microRNAs and transcription factors. Moreover, external factors, including inflammatory signaling, immune cell activity, and patient-specific variances (e.g., genetic background, immunological status, or environmental exposures) may influence alterations in HLA-Class I expression independently of miRNA-9 (Figures 6 and 7).

Future studies could conduct a more comprehensive examination of regulatory molecules influencing HLA-Class I expression to investigate this association better. This may entail high-throughput transcriptome profiling, such as RNA sequencing, to find additional differentially expressed microRNAs and transcription factors implicated in HLA-Class I regulation. Furthermore, functional validation tests, such as luciferase reporter assays, may be utilized to evaluate the direct binding of miRNA-9 to HLA-Class I mRNA. Experiments involving miRNA overexpression and knockdown through small interfering RNAs (siRNA) or miRNA mimics/inhibitors in cell culture models may yield additional mechanistic insights. Moreover, examining cytokine profiles with enzyme-linked immunosorbent assays (ELISA) or multiplex cytokine assays may clarify the impact of inflammatory mediators on HLA-Class I expression during bacterial infections or immunological activation. Increasing the sample size and using systems biology methodologies, such as pathway enrichment analysis, may elucidate the extensive regulatory network that governs HLA-Class I expression.

The influence of bacterial colonization in burn wounds offers further context to these findings. The significant incidence of *S. aureus* and *P. aeruginosa* in patients with burns highlights their propensity to exacerbate inflammatory responses. *Staphylococcus spp.* and *Pseudomonas spp.* are more common in burn patients owing to their capacity to exploit impaired skin barriers and their frequent occurrence in hospital settings. These bacteria represent a substantial percentage of burn infections, with *S. aureus* and *P. aeruginosa* responsible for around 19.7% and 20.25% of cases, respectively [34].

Their pathogenicity is amplified by biofilm formation, which increases resistance to antibiotics and complicates therapeutic interventions. *P. aeruginosa* also synthesizes many virulence factors that enhance its pathogenicity in burn wounds. The release of damage-associated molecular patterns (DAMPs) from diseased and injured tissues might stimulate the immune system, thereby facilitating the development of autoantibodies against melanocytes.

The activation of the immune system, combined with ongoing infections, may foster a pro-inflammatory milieu that exacerbates autoimmune reactions associated with vitiligo [35].
Statistical analysis indicated no significant correlation between the presence of specific bacterial species and the expression levels of the analyzed genes. An intriguing tendency was noted: HLA-class I expression was elevated in 95% of burn patients, in contrast to 65% in vitiligo patients. This indicates that burn injuries may foster an environment that enhances immunological activation and may be affected by bacterial infections.

Burn injuries elicit a pronounced inflammatory response characterized by generating damage-associated molecular patterns (DAMPs), which activate the immune system and augment HLA-class I molecule expression. This response constitutes a component of the body's innate defensive mechanism, wherein the production of DAMPs stimulates antigen-presenting cells, hence enhancing HLA-class I expression. Bacterial infections in burn wounds may serve as an extra stressor, exacerbating the immunological response. The absence of statistical association between bacterial species and HLA-class I or miRNA-9 expression indicates that microbial influences may not directly influence these gene expressions. The observed changes may come from a complicated interaction of tissue damage, immune system activation, and systemic inflammatory responses.

Bacterial colonization is common in burn wounds; nevertheless, its lack of association with gene expression in this study suggests that microbial interactions may be indirect rather than direct. Bacterial infections likely lead to sustained inflammation, which may subsequently affect immunological signaling pathways.
Future research should examine whether bacterial infections affect immune modulation via indirect mechanisms, such as changes in cytokine profiles, variations in antigen presentation efficiency, or effects on the stability of miRNA components pertinent to immune regulation. Additional experimental validation, such as measuring cytokine levels in infected and non-infected burn patients, would be crucial in establishing a direct correlation between bacterial infections and the start of vitiligo. Enhancing research in this domain may yield profound insights into the processes via which bacterial infections induce immune dysregulation and autoimmunity, hence advancing our comprehension of post-burn vitiligo progression.

Subsequent studies utilizing bigger sample sizes and sophisticated molecular methodologies such as transcriptome analysis or functional assays may enhance understanding of the mechanisms regulating HLA-class I and miRNA-9 expression in burn injuries. Comprehending these pathways may provide significant insights into the pathophysiology of autoimmune illnesses like vitiligo, especially in post-burn scenarios, when immune dysregulation could lead to melanocyte death and disease advancement.

Conclusion

The research reveals notable immunological dysregulation in individuals with vitiligo and burns, marked by the overexpression of HLA-class I and the downregulation of miR-9, indicating increased immune activation and a regulatory function of miR-9. Bacterial infections, especially with *S. aureus* and *P. aeruginosa*, were common in burn patients; however, no direct association was found between particular bacterial species and gene expression. Increased HLA-class I expression was noted in 95% of burn patients, in contrast to 65% of vitiligo patients, suggesting that inflammation, possibly driven by bacterial colonization, may contribute to immune dysregulation and the onset of vitiligo. These findings underscore the intricate relationship among trauma, infection, and autoimmune.

Ethical Approval

This study was approved by the local Ethical Committee, the Department of Biology (CSEC/1023/0078) on 29/10/2023, the College of Science, the University of Baghdad, Baghdad, Iraq, and the Iraqi Ministry of Health all granted their sanction for this study.

Conflict of interest statement

The authors declare they have no conflict of interest.

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