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Evaluation of miRNA-155 expression in burn and wound injuries with bacterial infection in Iraqi patients

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Abstract

Maintaining skin integrity and controlling infection depend on effective wound healing; nonetheless, poor healing still presents a major clinical and public health issue. The purpose of this research was to find the correlation between the expression levels of microRNAs-155 (miRNAs) and the prevalence of burn and wound injuries aggravated by bacterial infection in Iraqi patients. Three hundred blood samples in all—one hundred from healthy controls and two hundred from burn and wound injured patients. Patients had notably higher clinical parameters than controls ($p < 0.05$: white blood cell count, red blood cell count, neutrophils, lymphocytes, platelets, ESR, C-reactive protein). Reverse transcription quantitative polymerase chains reaction (RT-qPCR) was used to examine miRNA-155 expression. The findings showed a significant downregulation of miRNA-155 in the sick group (0.62 ± 0.027) relative to the control group (1.0 ± 0.0), therefore approximating a 38% drop. These results suggest that miRNA-155 could be a useful molecular biomarketer for burn and wound injuries, especially those aggravated by bacterial infection. Its lower expression emphasizes its possible function in the inflammatory response and tissue damage, therefore stressing its importance for further diagnostic and therapeutic uses.

Keywords: miRNA-155, wound healing, burn injury, gene expression, RT-QPCR.

Introduction

Burn and wound injuries represent some of the most severe and complex forms of trauma, often accompanied by microbial infections that significantly impair the healing process. These injuries trigger strong local and systemic inflammatory responses, which are essential for initial wound defense but can become harmful if dysregulated. Understanding the molecular mechanisms that govern wound and burn healing is therefore critical for improving patient outcomes, particularly in resource-limited settings, such as certain regions in Iraq.

MicroRNAs (miRNAs) have emerged as key post-transcriptional regulators of gene expression, influencing a variety of cellular processes including inflammation, cell proliferation, differentiation, and

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immune response modulation. Among them, microRNA-155 (miR-155) has gained considerable attention due to its central role in both innate and adaptive immunity[1]. It plays a critical role in regulating inflammatory mediators and modulating immune cell communication, making it a strong candidate for studies focused on wound healing, especially in infections aggravated by bacterial pathogens[2].

Recent studies have demonstrated that tissue injury and microbial infection can significantly alter the expression of miR-155. Its levels may increase or decrease depending on the type of microbial invasion and the stage of the healing process[3]. A downregulation of miR-155 may impair immune surveillance and tissue regeneration, whereas its upregulation may exacerbate inflammation through the overproduction of pro-inflammatory cytokines[4]. This dual functionality highlights the complexity of miR-155 regulation in wound environments.

MicroRNA-155 (miR-155) is a key regulator in the wound healing process, particularly during the inflammatory phase. It modulates immune responses by influencing macrophage activity, cytokine production, and T-cell function—essential for clearing pathogens and initiating repair. miR-155 also affects keratinocyte and fibroblast behavior, which are critical for skin regeneration and extracellular matrix remodeling. Its expression is tightly regulated; reduced levels may impair immune defense and tissue repair, while overexpression can lead to excessive inflammation. This dual role highlights miR-155's importance in maintaining the balance needed for effective wound healing, especially in cases complicated by bacterial infection. In the context of Iraqi patients, factors such as high infection rates, delayed hospital visits, and the emergence of multidrug-resistant bacteria pose additional challenges to wound management and recovery. Evaluating miR-155 expression in this population could provide valuable insights into the molecular mechanisms contributing to poor healing outcomes. Furthermore, such data could help identify miR-155 as a potential biomarker for infection severity and wound prognosis, ultimately supporting the development of locally relevant, targeted therapeutic strategies.

Therefore, this study aims to assess the expression level of miR-155 in Iraqi patients with burn and wound injuries, particularly in those with concurrent bacterial infections. The study seeks to identify expression patterns and correlate them with clinical outcomes to better understand the role of miR-155 in wound pathophysiology and to explore its potential utility in guiding future therapeutic interventions.

Materials and Methods

Sample Collection

The search included the period from October to January 2025. Samples were obtained from wound and burn patients by swabs from the injury sites and blood draws from the same individuals at Ghazi Al-Hariri Hospital and the Specialized Burns Hospital in Baghdad, Iraq. A total of 200 samples were obtained for the patient group, including individuals with a mean age of 35±5 years, including 128 men and 72 females. Furthermore, 200 samples were gathered for the control group.

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Ethical Approval

The project was approved by the College of Biotechnology, Department of Molecular and Medical Biotechnology Institute's Ethics Committee (Date: 15/1/2025).

Inclusion criteria for burns and wounds include bacterial infection

All samples, both from patients and controls, were assessed for the presence of bacterial infection. Swabs from wounds and burns were immediately transferred to transport media and cultured on blood agar, MacConkey agar, and mannitol salt agar to identify both gram-negative and gram-positive bacteria. Identification was confirmed using microscopy and biochemical tests via the VITEK® 2 COMPACT system or the API system. Blood samples (6 mL) were also collected from the same patients and divided for multiple analyses: 2 mL were placed in EDTA tubes for complete blood count (CBC) and erythrocyte sedimentation rate (ESR) analysis; 4 mL were placed in gel tubes, centrifuged at 4000 rpm for 10 minutes to separate serum, and stored at –20°C until used for biochemical tests including C-reactive protein (CRP) and IL-22 analysis using ELISA; and 400 µL of serum was mixed with 600 µL of TRIzol® and preserved at –20°C for subsequent RNA extraction.

Clinical laboratory analysis

The complete blood count (CBC), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were quantified following the specified methods and manufacturer guidelines with an automated chemical analyzer, as shown in Table 1.

Table (1).The tests were done for all samples (patients and controls)

Test	Company	Origin
CBC	Genex	USA
ESR	Biobase	China
CRP	Agappe	Switzerland

The IL-22 concentration was measured using an Enzyme-Linked Immunosorbent Assay (ELISA) kit obtained from Sunlong Biotech (China, Cat. No SL0988Hu). The kit utilizes a quantitative sandwich ELISA to measure the concentration of IL-22 in serum samples.

Table (2). Primers used in this study were provided from Macrogen® (South Korea)

NO	Primer Name		5`-Sequence-3	R
1	**MiRNA-155**	**RT primer**	(GCGAGGCGGTGGCAGTGGAAGCGTG ATTTATTCACCGCCTCGCACCCCTAT)	5
		Forward primer	(CTCAGACTCGGTTAATGCTAATCGTGATAGG)	
		Reverse primer	(GCTGTGGCAGTGGAAGCGTGATTTATT)	

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2	**U6 Housekeeping gene (Reference gene)**	**Forward primer**	(CTCGCTTCGGCAGCACA)	6
		Reverse primer	(AACGCTTCACGAATTTGCGT)	

RNA extraction by TRIzol™

RNA was extracted from serum using TRIzol™ reagent. A volume of 400 µL serum was combined with 600 µL TRIzol™, thoroughly mixed, and incubated at room temperature for 5 minutes. Subsequently, 200 µL of chloroform was added, and the mixture was vortexed and incubated at –20°C for 15 minutes. After centrifugation at 12,000 rpm for 15 minutes, the upper aqueous phase containing RNA was transferred to a new tube and mixed with 650 µL of isopropanol. Following another incubation and centrifugation step, the RNA pellet was washed with 75% ethanol, dried at room temperature, and resuspended in 25 µL of RNase-free water. The RNA was stored at –20°C until further use.

miRNA Quantitation

For mRNA quantification, 190 µL of Qubit® working solution was mixed with 10 µL of standard solution for calibration, while sample tubes contained 197 µL of working solution and 3 µL of sample. Following comprehensive mixing, all tubes were incubated at ambient temperature for 3 minutes. The Qubit was calibrated with standard tubes prior to detecting miRNA levels in each sample tube. The cDNA quantification methodology adhered to the same procedures using specified reagents and dyes.

RT-qPCR protocol

RNA Reverse Transcription (CDNA)

The synthesis of complementary DNA (cDNA) from total RNA was performed using the Protoscript cDNA synthesis kit and specific primers for miRNA-146, as detailed in Table 2. For each reaction, a total of 4 µL of RNA was transferred into a PCR tube. To this, 10 µL of the Protoscript reaction mixture—containing dNTPs, buffer, and other essential reagents—was added. Subsequently, 2 µL of the MuLV reverse transcriptase enzyme and 1.5 µL of the specific primer were included in the reaction. Finally, 2 µL of nuclease-free water was added to bring the total reaction volume to 20 µL. The complete composition of the cDNA reaction mixture is provided in Table 3. The reaction tubes were incubated at 42°C for 60 minutes to allow reverse transcription, followed by enzyme inactivation at 80°C. The resulting cDNA was stored at –20°C until use in qPCR analysis.

Table (3) component of the cDNA mixture

Component	20 µL Reaction
Enzyme	2
Extracted total RNA	4
Nuclease-free Water	1
Random Primer	1.5

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Reaction mix	10
RT146	1.5

miRNA Detection by RT-qPCR

The cDNA sample was selected simultaneously from both the patients and control groups. Each sample necessitates two PCR tubes: one for the target miRNA (miRNA-146) and another for U6 snRNA, which functions as a housekeeping gene in this study. The quantification was conducted by evaluating the fluorescence intensity of SYBR Green. The reaction mixture comprises the components specified in Table 4, together with their respective quantities:

Table (4). component of the reaction mix

Component	20 µL Reaction
Forward primer (10 µM)	1
Luna Universal qPCR Master Mix	10
Nuclease-free Water	3
Reverse primer (10 µM)	1
Template DNA	5

Execute a high-speed centrifugation of the PCR tubes for 1 minute at 2000g to eliminate air bubbles and consolidate the liquid. Subsequently, modify the Real-Time PCR software in accordance with the chosen thermocycling protocol outlined in Table 5.

Table (5) RT PCR setup

Cycle Step	Temperature	Time	Cycles
Initial Denaturation	**95°C**	**8 minutes**	**1**
Denaturation Extension	**95°C-60°C**	**15 seconds30 seconds (+plate read)**	**50**
Melt Curve	**60-95°C**	**20 minutes**	**1**

The result was collected and analyzed by Livak formula (18) as follows:

ΔCt patient = Ct patient – Ct U6

ΔCt control = Ct control – Ct U6

ΔΔCt = ΔCt patient – ΔCt control Folding expression = 2-(ΔΔCT)

Statistical analysis

The SPSS (2016) (IBM Corp., 2016) software was used to analyze the effects of changes in research parameters. The student's t-test was used to make a meaningful comparison of means. In this inquiry, p-values between 0.05 and 0.01 were used in all statistical analyses.

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Results and Discussion

Characteristics of patients

This research gathered around 200 samples, including 120 (60%) from burn patients and 80 (40%) from wound cases, sourced from Ghazi Al-Hariri Hospital and the Specialized Burns Hospital in Baghdad, Iraq. The age range is from 35 to 50 years, including 72 females and 128 males. The category of injuries (wounds) includes both surgical and accidental incidents, ranging from mild to severe, while burn cases also include accidental burns from fires, classified as stage II. All instances of wounds and burns have been assessed, and the degree of damage has been evaluated by the expert physician at the hospital.

Isolation and identification of pathogenic bacteria

This research examined all 200 wounds and burn swab samples for harmful microorganisms. All samples were grown and subjected to morphological and microscopic analysis to identify those infected with Gram-negative and Gram-positive bacteria. Morphological exams rely on traits such as form, size, arrangement, color, and surface aspects to identify and categorize bacterial species. All specimens were cultivated on blood agar, MacConkey agar, and mannitol salt agar.

In this investigation, about 160 samples were cultivated on MacConkey agar, resulting in approximately 96 pink colonies (lactose fermenters) and 64 colorless colonies (non-lactose fermenters). MacConkey agar is a selective and differential medium used largely for the isolation of gram-negative bacteria, contingent upon their capacity to ferment lactose[7]. This investigation included the cultivation of 180 samples on blood agar, resulting in 50 alpha-hemolytic colonies, 80 beta-hemolytic colonies, and 50 gamma-hemolytic colonies. Blood agar is an enriched medium used to facilitate the development of several bacterial species, especially fastidious ones. Although it is not selective, it distinguishes bacteria based on their capacity to hemolyze red blood cells[8].

This investigation included forty samples capable of growth on mannitol salt agar, presumed to be Staphylococcus aureus, which ferments mannitol, resulting in a yellow coloration of the medium. Mannitol Salt Agar (MSA) is a selective and differential medium used largely for the isolation of the *Staphylococcus* species. Biochemical studies were conducted on around 180 isolates, characterized by their physical traits. This research comprised the following biochemical tests: indole, methyl red, Voges-Proskauer, citrate, oxidase, and catalase. The biochemical analysis of several isolates of gram-negative and gram-positive bacteria revealed the presence of four suspected isolates: *Pseudomonas spp*., *Acinetobacter spp*., *Klebsiella spp*., and *Staphylococcus aureus*, as shown in Table [6].

In this study, all 200 wounds and burn swab samples were investigated for the presence of pathogenic bacteria. All samples were cultured and undergo morphological and microscopical examination for detection which samples were infected with Gram- negative bacteria and Gram-positive bacteria. Morphological examinations depend on morphological characteristics like shape, size, arrangement, color, and surface features to identify and classify bacterial species. All samples were cultured on blood agar, MacConkey agar and mannitol salt agar.

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Table (6): Results of biochemical tests for bacteria isolated from wound and burn

Biochemical Test	Indole	Methyl red	Voges-Proskauer	Citrate	Oxidase	Catalase
Pseudomonas spp	-	-	-	+	+	+
Acinetobacter spp	-	-	-	-	-	+
Klebsiella spp	-	+	-	+	-	+
Staphylococcus auras	-	-	-	-	-	+

The results showed that *Pseudomonas* indicated negative results for (indole, methyl-red, and Voges-Proskauer) and positive results for (catalase, citrate, and oxidase). *Acinetobacter* indicated negative results for (indole, methyl red, Voges-Proskauer, citrate), and positive results for catalase tests. Also, *Klebsiella* indicated negative results for (indole, methyl-red, and oxidase) and positive results for (Voges Proskauer, citrate, and catalase). Finally, *Staphylococcus aureus* indicated negative results for (indole, methyl-red, Voges-Proskauer, citrate, and oxidase) and positive results for catalase.

Additional confirmatory identification of the isolates gave information to the species level was carried out using VITEK2 dense automated system tests that included several biochemical tests. *Escherichia coli* isolates were subjected to further identification. After that, to confirm the results, the tests were performed using Vitek2 20 tests. The results showed that the presence of 4 diagnosed isolates included *Pseudomonas* (29.5%), *Acinetobacter* (27.20%), *Klebsiella* (23 %), and *Staphylococcus aureus* (19.45%).

Table (7): Classification and occurrence of microorganisms identified in cultures

Bacterium	Numbers
Pseudomonas spp.	**29.5%**
Acinetobacter spp.	**27.20%**
Klebsiella spp.	**23%**
Staphylococcus auras	**19.45%**

A study by Al-Ani and Fahad found that patients with burns and wound patients were observed the gram-negative bacteria, including *A. baumannii* (47%) and *K. pneumoniae* (27%), and *P. aeruginosa* (23%). Furthermore, the gram-positive bacteria were *S. aureus* with (3.3%), which is in line with the current study[9].

Also, another study by Kadhim et al found that among the Gram-positive isolates, the highest isolation rate was for *Bacillus spp*. (55%) followed by *Staphylococcus albus* (27%) and *Staphylococcus aureus* (12%). Conversely, among the Gram-negative isolates identified in the present study, the highest isolation rate was for *Pseudomonas aeruginosa* (36%) followed by *Enterobacter spp*. (33%) and *Klebsiella spp*. (17.1%)[10]. According to a study by Hateet, *Pseudomonas aeruginosa* is the most common pathogen (20%), followed by *Staphylococcus aureus* (17.14%), *Enterobacter spp* (16.19%), *Proteus vulgaris* (13.33%), *Proteus mirabilis* (10.47%), *Escherichia coli* (7.6%), *Klebsiella pneumoniae* (6.6%), and finally *Staphylococcus lentus* and *Aeromonas sobria*, which have the same proportion (4.7%) in wound and burn patients[11]. Also, another study by Al-Azzawi and Alkalifawi found different percentages of (25%) *Staphylococcus aureus*, (20%) *Acinetobacter baumannii*, (16.7%) *Pseudomonas aeruginosa*, (13.3%)

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Klebsiella pneumoniae, (11.7%) *Escherichia coli*, (10%) *Proteus mirabilis*, and (3.3%) *Burkholder cepacian* from the same patients[12]. The most common species were Gram-negative bacteria constituted 83%, and *Pseudomonas spp*. Gram-positive Bacteria, especially *Streptococcus* and *Staphylococcus aureus*, and that is in line with this study.

Hematology analysis

Clinical biomarkers showed that there was significant increase (p-value ≤ 0.05) in all biomarkers that include (white blood cell, red blood cell, neutrophil, lymphocyte, platelets, ESR and CRP). The hematological test of all Patients who had wound and burn infection was shown in (table 8).

Clinical parameters were evaluated in patients with wounds and burns and compared to those of a control group. The results demonstrated statistically significant differences ($p < 0.01$) across several key markers. The white blood cell (WBC) count was markedly elevated in the patient group, with a value of 15.30 $\times 10^3/\mu L$, alongside a significantly reduced lymphocyte (LYM) count of 0.1618 $\times 10^3/\mu L$ and a high neutrophil (NET) count of 14.40 $\times 10^3/\mu L$. Platelet (PLT) levels were also elevated, reaching 398.0 $\times 10^3/\mu L$. Markers of inflammation, including erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), were significantly increased, measuring 56.80 mm/hr and 86.30 mg/L, respectively. Additionally, the red blood cell (RBC) count was 4.980 $\times 10^6/\mu L$. These findings highlight the pronounced inflammatory and hematological responses observed in patients with wounds and burns

Table (8): Clinical parameters for patients with wound and burn and (control group)

Group Parameter	Patient group	Control group	Normal Range for Healthy Cases	p-Value
WBC 103/µL	15.30	7.00	3.60-10.20	<0.0001**
LYM 103/µL	0.1618	1.580	1.00-3.20	0.0010**
NET 103/µL	14.40	4.75	1.85-5.94	<0.0001**
PLT 103/µL	398.0	192.0	152.4-3547.9	<0.0001**
ESR mm/hr	56.80	10.70	0.0 - 20.0	<0.0001**
CRP mg/L	86.30	27.00	0.0 - 10.0	<0.0001**
RBC× 10^6/µL	4.980	3.950	4.70 - 6.10	<0.0001**
significant, ** p < 0.01				

The findings of this study demonstrate that the inflammatory markers, particularly CRP, WBC, and ESR, are significantly elevated in burn patients compared to healthy individuals. This is shown in Figure 1.

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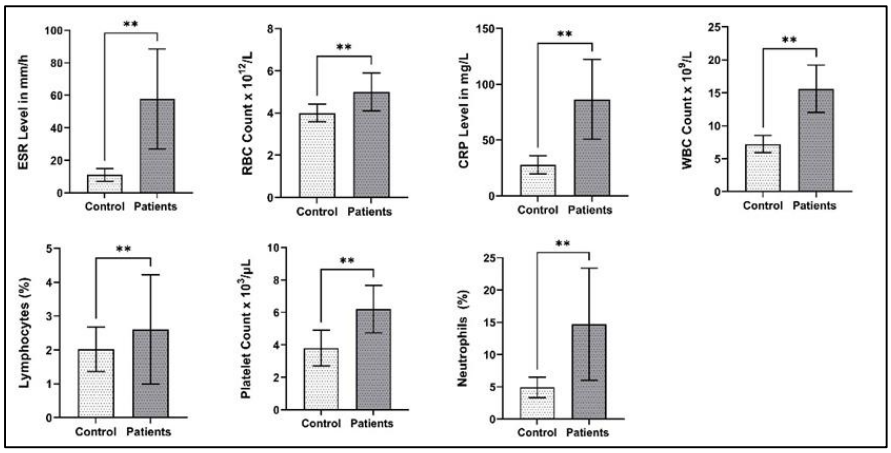

Figure (1) Elevated Inflammatory and Hematological Parameters in Patients Compared to Healthy Controls

The Complete Blood Count (CBC) test measures the amounts of blood cells, including white blood cells (WBCs), which are very vital for tracking the body's reaction to injury and infection detection. Elevated WBC counts might point to a continuing inflammatory response or infection. It's worth exhibiting a notable rise in the outcomes. In keeping with previous research[13], both WBC and ESR were observed to be considerably raised in burn patients compared to healthy controls.

While ESR, a nonspecific indicator of inflammation, confirms the presence of inflammatory activity as reported in this study [14], the concurrent rise in WBC count reflects the body's immunological response to tissue damage caused by burns. Within the differential count, both neutrophils and lymphocytes demonstrated notable increases, indicating the activation of both innate and adaptive immune responses during the inflammatory phase of wound and burn healing.

Lymphocytes are very essential in burn injuries for avoiding systemic infections, boosting long-term immunological memory, and promoting tissue regeneration. Neutrophils and lymphocytes taken together guarantee the first defense and continuous tissue regeneration after damage[15].

By stopping bleeding at areas of vascular damage, platelets play a vital hemostatic role. Johnson et al.'s research shows that a rise in platelets was seen at the start of the infection, then a decline in number a few days later; this is consistent with the persistence of a modest but considerable platelet hyperreactivity after burn damage. The results revealed notable rise in its worth[16].

We observed a notable rise in platelet counts three weeks following trauma. White blood cell and neutrophil counts were found to be significantly higher in burn cases, with cell counts rising in the first days following injury, while platelet counts in burn cases were lower than those in healthy controls during the first week after trauma. These results point to a delayed immunological "second hit" with burn damage progressing even weeks after the original insult. Absolute lymphocyte counts revealed no difference between controls and burn victims and that in keeping with current studies[17] and course of action. The findings revealed a notable rise in its worth. An elevated ESR suggests inflammation, which may be related

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to tissue injury or an infection. Tracking ESR over time enables one to evaluate the course of inflammation and the success of treatment programs. Zhang et al. do point out that ESR's lack of specificity limits its usefulness in identifying wound infections. Although both ESR and CRP are routinely used in clinical settings to evaluate inflammation, their capacity to particularly detect infections in burn victims is limited[18].

The function of cytokines in the systemic reaction to burns has been investigated. Burn injuries clearly cause major inflammatory reactions, including the production of many inflammatory cytokines. Especially those engaged in the acute-phase response, these cytokines boost the generation of CRP[19]. Its value exhibited a notable rise among the findings. Made in reaction to inflammation by the liver, C-reactive protein (CRP) is an acute-phase reactant. Raised CRP levels point to either acute inflammation, infection, or tissue damage. In the framework of wound and burn healing, CRP levels may be tracked by the inflammatory response and identify possible problems such infections[20]. The results revealed a notable rise in its worth.

Burn patients with raised CRP levels on days three and seven were more likely to acquire wound infections, according to a research by Song et al. Although its diagnostic specificity has limitations, this supports the theory that CRP may be a marker for infection in burn patients[21]. It also rises in this research. Yiğit and Demir Yiğit noted that, throughout the hospital stay of burn patients in critical care units, CRP levels and WBC counts typically remain continuously high, therefore providing a less precise indication of infection. This finding implies that while CRP increases in response to burns, its levels should be cautiously considered in relation to infection diagnosis[22]. Together with other techniques including cytokine analyses, CRP is still a valuable biomarker. Palmieri and Heard claim that hepatocytes synthesis CRP in response to increased cytokine levels, including IL-22, during inflammatory responses—particularly in burn injuries. Consequently, after a burn, CRP levels usually rise; they then progressively drop as the patient heals[23]. Because of their function in oxygen transport—which is crucial for tissue repair and regeneration—red blood cells (RBCs) are absolutely necessary for healing of burns and wounds. By upregulating matrix metalloproteinases and fibronectin and downregulating type-I collagen, research shows RBCs may influence the activity of dermal fibroblasts, cells vital for wound healing. Extracellular regulated protein kinase 1/2 (ERK 1/2) pathway meditates this regulation. Knowing the many functions of RBCs in wound healing helps one to design new therapies for fibrotic diseases like keloids and hypertensive scars[24]. The findings revealed a notable rise in its worth.

Iraqi journal of Bioscience and Biomedical
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Gene expression of miRNA-155

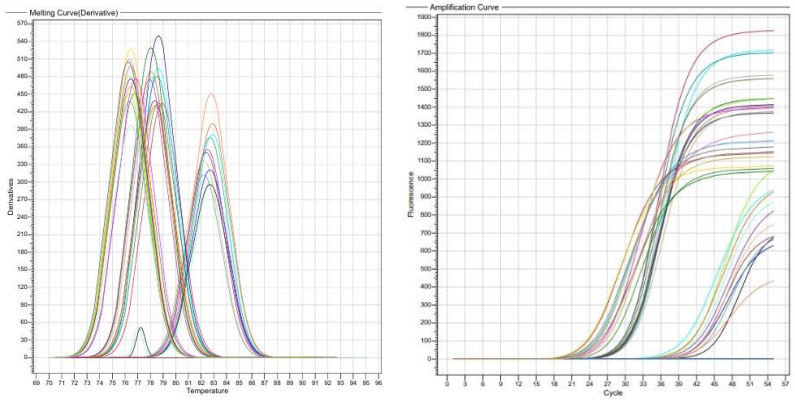

Figure (2) amplification plots for miRNA 146 and U6 obtained by RT-qPCR, melting curve analyzed for miRNA-146 and U6 expression.

The real time PCR amplification program was conducted using melting curve analysis for all evaluated gene expressions, following the manufacture stimulation programs, using relative gene expression. Total RNA extracted from blood samples were converted to cDNA for gene expression level analysis using Real time PCR (qPCR) for miRNA-155 genes. The gene expression was standardized to the level of the housekeeping gene (U6) and measured using the Ct value, as depicted in Figure 2. Each colour in the curve displayed in these images refers to a certain Ct value. MiRNA-155 gene expression was quantified using reference gene U6 and compared between patient and healthy control groups in different stages (Ct, ΔCt, ΔΔCt, and fold change) using qRT-PCR, as shown in Table 10.

Table (9): comparison between the studied groups (patients and healthy control) in different stages of gene expression level Ct, ΔCt, ΔΔCt and fold.

Parameters		Mean CT	CT U6	ΔCt	ΔΔCt	Mean ± SD Fold	p Value
MiRNA-155	Patients	41.29	25.93	5.40	0.70	0.62±0.027	<0.0001**
	Control	15.16	26.90	4.70	----------	1.0 ± 0.0	----------
Non-significant, ** p < 0.01							

The expression levels of miRNA-155 were significantly reduced in burn and wound patients compared to healthy controls ($p < 0.0001$). As shown in Figure 3, the mean relative expression in the patient group was 0.62 ± 0.027, while it was 1.0 ± 0.0 in the control group, indicating a 38%-fold reduction in miRNA-155 expression. This downregulation was calculated based on ΔCt values, where patients exhibited a mean ΔCt of 5.40 compared to 4.70 in controls, and a resulting ΔΔCt of 0.70. A decrease in miRNA-155 expression in burn and wound patients suggests a possible impairment in the regulation of immune activation, potentially leading to weakened inflammatory responses, delayed tissue repair, or increased

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susceptibility to infection. Furthermore, reduced miRNA-155 levels have been associated with chronic wounds, where inadequate immune signaling hampers normal healing processes.

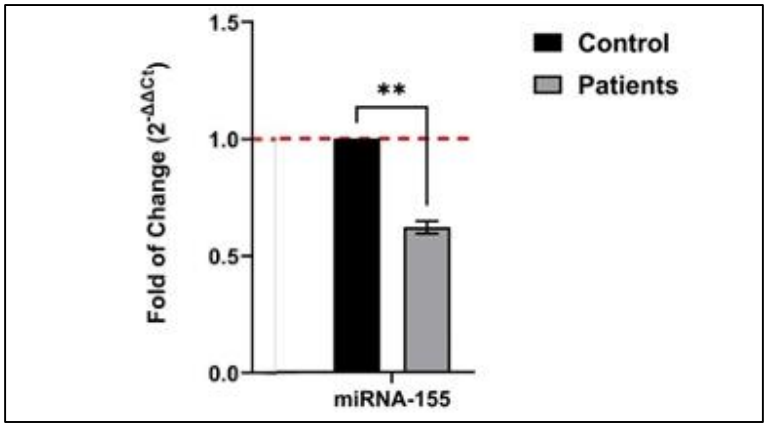

Figure (3) Relative expression of miRNA-155 in patients compared to controls, showing significant downregulation ($p < 0.01$).

miRNA-155 plays a key regulatory role in immune cell activation, particularly in macrophages, which are essential for effective wound healing. A moderate reduction in miRNA-155 expression may indicate an altered immune response, potentially leading to impaired defense mechanisms at the wound site. Given its role in balancing pro- and anti-inflammatory cytokines, decreased miRNA-155 levels have been associated with delayed healing and an increased risk of infection [25]. Recent studies have highlighted the critical involvement of miR-155 during the inflammatory phase of wound repair. It is notably upregulated in fibroblasts and macrophages, where it contributes to extracellular matrix (ECM) deposition and fibrosis. This microRNA modulates key signaling cascades, such as the TGF-β1 pathway, which promotes collagen synthesis, an essential process for tissue regeneration.

However, excessive miR-155 expression can be detrimental, as it may lead to pathological fibrosis and abnormal tissue remodeling. In fibrotic wound models, miR-155 suppression was shown to downregulate collagen production, suggesting its potential as a therapeutic target for preventing fibrosis and enhancing healing in chronic wounds [26]. Overexpression of miR-155 has also been linked to reduced levels of fibroblast growth factor 7 (FGF7), a crucial mediator of keratinocyte proliferation and re-epithelialization. Notably, topical inhibition of miR-155 in diabetic wound models restored FGF7 expression, reduced inflammation, and accelerated wound closure. These findings support the therapeutic benefit of miR-155 modulation in improving wound outcomes.

Additionally, miR-155 upregulates matrix metalloproteinase-2 (MMP-2), facilitating keratinocyte migration and re-epithelialization—key processes for wound closure. Nonetheless, dysregulation of miR-155 can disturb this balance, resulting in impaired healing [27]. A study demonstrated that acute downregulation of miR-155 led to decreased collagen production by limiting macrophage-derived inflammatory signals via SHIP1, further confirming its role in coordinating collagen synthesis and immune responses during healing [28]. A more recent investigation in 2023 using miR-155 knockout mice reported

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enhanced wound healing, increased collagen deposition, and greater macrophage infiltration, suggesting a negative regulatory role of miR-155 in cutaneous wound repair [29].

Eissa and Artlett also investigated the role of miR-155 in fibrotic processes during wound healing and found that it influences major fibrosis-related pathways [30]. This supports its potential as a target in anti-fibrotic therapies for chronic wounds. Huang et al. (2025) explored miR-155 expression in diabetic foot ulcers (DFUs) treated with negative pressure wound therapy (NPWT) and observed significant downregulation of miR-155. This was associated with enhanced fibroblast proliferation, migration, reduced cell death, and increased FGF7 expression, all contributing to improved healing outcomes. Similarly, Shahin et al. (2023) reported that miR-155 regulates adipose-derived mesenchymal stem cell (AD-MSC) activity, influencing the expression of key genes involved in tissue regeneration [31]. These genes are essential for repair processes, reinforcing miR-155's central role in modulating AD-MSC regenerative potential. In a systematic review, Smith et al. (2023) examined how physical activity influences miRNA profiles in burn wound healing, further emphasizing the therapeutic value of regulating miR-155 expression [32].

Moreover, the interplay between miR-155 and miR-146 is critical in modulating inflammation in burn and chronic wound settings. Elevated levels of these miRNAs are associated with poor healing outcomes, while their downregulation can signal uncontrolled inflammation—a known contributor to chronic wound pathology [33]. Prolonged or excessive inflammation may lead to delayed healing, tissue breakdown, and increased vulnerability to secondary infections [34,35]. miR-146, for example, is essential for fibroblast proliferation and keratinocyte migration, both critical for wound closure. Its reduction can impair epithelialization and remodeling. Likewise, miR-155 is vital for macrophage activation and antimicrobial function. A decrease in its expression may compromise the host immune defense, increasing the risk of infection. This study focused only on gene expression and did not include protein level analysis, which may provide more insight into the biological effects. In addition, the study did not track changes in miRNA-155 over time, so the dynamic role of this marker during different stages of wound healing remains unclear.

Conclusions

The present investigation highlights the significant role of miRNA-155 as a molecular biomarketer connected with wound and burn injuries worsened by bacterial infections in Iraqi patients. Apart from more clinical inflammatory markers, the discovered downregulation of miRNA-155 expression in affected individuals highlights its likely relevance in the management of the immune response and inadequate healing processes. The rather low drop in miRNA-155 levels indicates a diagnostic significance enabling early detection and evaluation of wound degree. These findings support the inclusion of miRNA profiling—especially miRNA-155—into clinical diagnostic processes and provide directions for future therapeutic therapies targeted at molecular routes implicated in immune control and tissue repair.

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Author's Declaration

- We hereby confirm that all the Figures and Tables in the manuscript are original and have been created by us.
- We have obtained ethical clearance for our study from the local ethical committee at [Al-Nahrain University/College of Biotechnology]. This approval underscores our commitment to ethical research practices and the well-being of our participants.
- Ethical Clearance: The project was approved by the local ethical committee at [Al-Nahrain University/College of Biotechnology], ensuring adherence to ethical standards and the protection of participants' rights and welfare.

Author's Contribution Statement

Zahraa H. Khdair: Contributed to the conception and design of the study, conducted some experiments, data rearrangement, drafted the initial manuscript and facilitated processes to ensure the project ran smoothly.

Sahar M. Hussein: Conducted some experiments, collected a part of literature review and conducted some characteristics of the products.

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