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Evaluating the Level of IL-22 and Gene Expression of miRNA-146 in Iraqi Patients with Wound and Burn Injury and Bacterial Infection

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Abstract

Impaired and chronic wound healing (WH) is a prevalent and serious medical issue, and it poses a significant challenge to public health since WH is an essential physiological process that maintains skin integrity. This study aimed to evaluate the relationship between serum levels of IL-22 and the gene expression of miRNA-146 in Iraqi patients with wound and burn injuries, to determine their potential as diagnostic biomarkers. In total, 300 blood samples were collected: 200 samples from wound and burn patients, and 100 samples as a control group were collected from healthy individuals. The ELISA technique was used to measure the serum level of IL-22. In addition, real-time quantitative PCR (RT-qPCR) was used to quantify the gene expression level of miRNA-146. Clinical biomarkers showed a significant increase (p-value ≤ 0.05) in all biomarkers that include white blood cell, red blood cell, neutrophil, lymphocyte, platelets, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). Also, the levels of IL22 were significantly increased in the patients' group (p-value < 0.01), which was 6.123 pg/mL compared to the healthy control group, which was 3.802 pg/mL. Molecular evaluation using RT-qPCR demonstrated a marked downregulation in miRNA-146 expression in patients (0.207 ± 0.043) compared to controls (1.0 ± 0.0), indicating approximately an 80% reduction in fold expression.

Keywords: burn and skin injury; microRNA-146; Biomarker; diagnosis; IL22.

تقييم مستوى IL-22 والتعبير الجيني لـ miRNA-146 في المرضى العراقيين المصابين بالحروق والجروح والعدوى البكتيرية

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الخلاصة

يُعدّ ضعف التئام الجروح المزمن (WH) مشكلة طبية شائعة وخطيرة، ويشكل تحديًا كبيرًا للصحة العامة نظرًا لأن WH عملية فسيولوجية أساسية تحافظ على سلامة الجلد. هدفت هذه الدراسة إلى تقييم العلاقة بين مستويات مصل IL-22 والتعبير الجيني لـ miRNA-146 لدى المرضى العراقيين المصابين بإصابات الجروح

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والحروق، لتحديد إمكاناتهم كمؤشرات حيوية تشخيصية. في المجموع، تم جمع 300 عينة دم، 200 عينة من مرضى الجروح والحروق و100 عينة كمجموعة ضابطة من أفراد أصحاء. تم استخدام تقنية ELISA لقياس مستوى مصل IL−22. بالإضافة إلى ذلك، تم استخدام qPCR في الوقت الحقيقي لتحديد مستوى التعبير الجيني لـ miRNA−146. أظهرت المؤشرات الحيوية السريرية وجود زيادة كبيرة (القيمة الاحتمالية ≥ 0.05) في جميع المؤشرات الحيوية التي تشمل (خلايا الدم البيضاء، خلايا الدم الحمراء، العدلات، الخلايا الليمفاوية، الصفائح الدموية، ESR وCRP). كما ارتفعت مستويات IL22 بشكل ملحوظ في مجموعة المرضى (القيمة الاحتمالية < 0.01)، حيث بلغت 6.123 بيكوغرام/مل، مقارنةً بمجموعة الضبط السليمة التي بلغت 3.802 بيكوغرام/مل. أظهر التقييم الجزيئي باستخدام تفاعل البوليميراز المتسلسل الكمي في الوقت الحقيقي (RT−qPCR) انخفاضًا ملحوظًا في التعبير عن miRNA−146 لدى المرضى (0.207 ± 0.043) مقارنةً بمجموعة الضبط (1.0 ± 0.0)، مما يشير إلى انخفاض بنسبة 80% تقريبًا في التعبير عن الطيات.

1. Introduction

Burns and wounds are also the most prevalent forms of traumatic injuries, which cause a high level of morbidity and mortality on the global level. Various causes can produce burns, and the causes of the burns are both thermal effects and chemical substances, electrical causes, and radiation-based agents. The inability to protect against pathogens efficiently and delayed healing of the body are caused by epidermal and tissue damage, which is a consequence of these injuries. Chronic wounds, along with burns, are highly susceptible to bacterial infections, which slow down recovery time and can lead to serious health effects, including sepsis or prolonged hospitalization [1].

The medical facilities in Iraq are under stress due to high rates of burn injuries, together with wound complications, which create a major concern for public health. Public health authorities in Iraq must address burn injuries because the condition affects Iraqis differently depending on their location. Overall, there were 884 cases of burns among the population, which corresponds to 43 instances per 100,000 individuals annually, based on the research. Since these data are now outdated, they require new tabulation; however, the current prevalence numbers should be higher than documented. The path to successful injury management depends on knowledge about immune reactions because they affect both healing processes and infection prevention [2].

The immune response depends on Interleukin-22 (IL-22) as an essential cytokine, which helps defend tissues and promotes healing. Mixed immune cell types produce IL-22 from T-helper 17 cells and innate lymphoid cells, while non-hematopoietic epithelial and endothelial cells receive these effects, which sustain tissue restoration and protect against harmful organisms. The initiation period of wound healing (WH) depends on IL-22 because this factor stimulates proliferative and migratory behavior of keratinocytes, which perform essential roles in skin restoration. The resolution of infection-related inflammation heavily depends on IL-22 because this factor supports equilibrium between immune system responses [3].

MicroRNA-146 (miRNA-146) is a vital non-coding RNA sequence that regulates gene expression during inflammatory states. MiRNA-146 is an anti-inflammatory factor that works by modifying the elements of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) pathway that determines immune reactions towards wounds and infections [4]. This miRNA controls the functions of immune cells to control the degree of inflammation and minimize tissue damage. The miRNA-146 regulation failure acts as a crucial biomarker to investigate burn and wound immune response since it is linked to chronic inflammation and an inability to heal [5].

The IL-22-miRNA-146 interaction can be described as an antagonist-antagonist interaction

and represents a complicated story of a constant antagonist contact that regulates the inflammation and promotes tissue repair. IL-22 enhances tissue healing and the immune response, while miRNA-146 is a mechanism that prevents an excessive immune system response and contributes to further damage. Balance is crucial to the performance of burn and wound infections, in which excessive inflammation and an inadequate immune system response may halt recovery [6]. The study of the interaction between these two molecules may provide insight into more efficient methods for treating infected burns and wounds.
In this study, the researcher intends to determine the expression of IL-22 and miRNA-146 in different tissues of wounds, wounds caused by burns, and wounds caused by surgery, to identify the potential of these molecules as biomarkers of wound healing and as target therapy.

2. Materials and methods

Bioinformatic methods

In this study, several bioinformatics platforms were used to evaluate the primers applied for miRNA-146a detection. These platforms included: miRbase https://www.mirbase.org/, NCBI BLAST,https://blast.ncbi.nlm.nih.gov/Blast.cgi,and,Primer-BLAST https://www.ncbi.nlm.nih.gov/tools/primer-blast/.

Sample collection

This study covered the period from October 1, 2024, to January 15, 2025. The samples were collected from wound and burn patients by taking swabs from the injury area and blood from the same patients who attended Ghazi Al-Hariri hospital and the specialised burns hospital in Baghdad, Iraq. For this study, a total of 200 samples were collected from the patient group, comprising 128 males and 72 females, with a mean age of 35±5 years. In addition, 200 samples were collected for the control group.

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 bacterial infection

All samples (patient and control) were examined for bacterial infection and the presence of Gram-negative and Gram-positive bacteria. The wound and burn samples were collected by taking a swab from the injury or burn area and placed inside transport media, then cultured on blood agar, MacConkey agar, and mannitol salt agar. Next, the bacterial isolates were identified using microscopy, biochemical tests, and VITEK 2 COMPACT to detect which Gram-negative and Gram-positive bacteria were present in the isolates from wound and burn swabs. Patients' blood samples (6 ml) were collected and processed for different purposes. For complete blood count (CBC) and erythrocyte sedimentation rate (ESR), a volume of 2 ml blood samples was added into the ethylene diamine tetra acetic acid (EDTA) tube, for biochemical parameters, C-Reactive Protein test (CRP) and ELISA, a volume of 4 ml blood sample was transferred into gel tube and centrifuged at 4000 rpm for 10 minutes to obtain serum and storage at -20 °C until used. For molecular study, a volume of 400µl of serum is added to 600 µl of TRIzol® and stored at -20 °C until used.

Clinical laboratory analysis

Levels of CBC, CRP, and ESR were measured according to the instruction protocols and manufacturing companies using an automated chemical analyzed, 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 measurement of IL-22 concentration was conducted using an Enzyme-Linked Immunosorbent Assay (ELISA) kit sourced from Sunlong Biotech (China, Cat. No. SL0988Hu). The kit employs a quantitative sandwich ELISA to determine the level of IL-22 in serum samples.

Primers

Primers used in this study were provided by Macrogen® (South Korea), as shown in Table 2.

Table 2: The name, sequence and product size of primers used in this study

NO	Primer name		5`-Sequence-3	Ref
1	*MiRNA-146*	RT primer	GTCGTATCCAGTGCGTGTCGTGGAGTCGG CAATTGCACTGGATACGACAACCCA	[7]
		Forward primer	CAATTGCACTGGATACGACAACCCA	
		Reverse primer	GGGTGAGAACTGAATTCCA	
2	*U6* Housekeeping gene (Reference gene)	Forward primer	CAGTGCGTGTCGTGGAGT	[8]
		Reverse primer	CTCGCTTCGGCAGCACA	

RNA extraction by TRIzol™

A volume of 400µl of serum was added to 600 µl of TRIzol™. The lysate was Pipetted multiple times to ensure homogenization. After that, it was incubated for 5 minutes to completely dissociate the nucleoprotein complex and stored in a freezer (-20°C) until use. The samples were mixed vigorously, then 200 µl of chloroform was added to the TRIzol™ Reagent for lysis. The mixture was vortexed again and incubated for 15 minutes in a freezer. The samples were centrifuged at a speed of 12,000 rpm for 15 minutes. The mixture was separated into a lower layer containing red phenol-chloroform, an intermediate layer, and an upper layer consisting of colorless aqueous solution. The aqueous phase containing RNA was subsequently transferred to a new tube.

RNA was precipitated by the addition of 650 µL isopropanol to the aqueous phase. The mixture was vortexed briefly and then frozen (-20°C) for 25 min. After incubation, the samples were centrifuged at 12,000 rpm for 10 min. This caused the flocculation of a dense white gelatinous RNA pellet, and this was located at the tube's bottom. The above supernatant was taken out using a micropipette. The RNA was precipitated with 600 µl of 75% ethanol. The pellet was dissolved by vortexing and then centrifuged at 7500 × g for 5 minutes. The tube was open for 25-30 minutes to dry, after emptying the supernatant with the micropipette. The pellet was incubated at 60 °C for 15 minutes, then resuspended in 25 µL of RNase-free water using a thermomixer. The collected RNA was kept at -20 °C until processing.

miRNA Quantitation

In case of miRNA quantification, the standard solution (190 µL) was used as the Qubit® working solution along with 10 µL of standard solution to carry out the calibration process, and

the sample tubes had 197 µL of working solution and 3 µL of the sample. All tubes were then incubated at room temperature for 3 minutes after thorough mixing. Qubit was then calibrated with standard tubes, and enzyme concentration in each sample tube was then measured. The cDNA quantification protocol involved the same steps, but it used specific reagents and dyes.

RT-qPCR protocol

RNA reverse transcription (cDNA)

Synthesis of cDNA from miRNA, using a specific primer for miRNA-146 as mentioned in Table 2 and the Protoscript cDNA synthesis kit, was carried out by first transferring 4 µL of the samples containing total RNA into a fresh PCR tube. A Protoscript reaction mix that consisted of (dNTPs, buffer, and other necessary components) was applied at a volume of 10 µL for each sample. The MuLV enzyme was added with a volume of 2 µL for each sample. 1.5 µL of a specific primer was added, then the volume was completed to 20 µL with nuclease-free water. The components of the reaction mixture are listed in Table 3, along with their respective quantities.

Table 3: Components of the cDNA mixture

Component	20 µL Reaction
Enzyme	**2**
Extracted total RNA	**4**
Nuclease-free Water	**1**
Random Primer	**1.5**
Reaction mix	**10**
RT146	**1.5**

The mixture was incubated at 42 ºC for 1 hour in a thermocycler. The enzyme was deactivated at 80 ºC after this. Using Qubit 4.0, the cDNA product was quantified and then kept until the second part (Relative quantitative PCR).

miRNA Detection by RT-qPCR

The cDNA sample was chosen from both the patient and control groups concurrently. Each sample requires the use of two PCR tubes: one for the target miRNA (miRNA-146) and another tube for U6 snRNA, which serves as a housekeeping gene in this investigation. The quantification was done by assessing the fluorescence intensity of SYBR Green. The components of the reaction mixture are indicated in Table 4, along with their corresponding amounts:

Table 4: Components of 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**

The PCR tubes were centrifuged at 2000 g for 1 minute to exclude air bubbles, and the supernatant was collected. Subsequently, the thermocycling parameters listed in Table 5 were set on the RT-PCR software.

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 the Livak formula (18) as follows:
ΔCt patient = Ct patient – Ct U6
ΔCt control = Ct control – Ct U6
ΔΔCt = ΔCt patient – ΔCt control
Folding expression = $2^{-(\Delta\Delta CT)}$

Statistical analysis

The SPSS (IBM Corp, 2016) program was employed to observe the impact of variation in study parameters. Student's *t*-test was employed to conduct a significant comparison between means. In this investigation, the p-values between 0.05 and 0.01 were projected in all statistical analyses. For bacterial statistics, only descriptive analysis was conducted which used percentages.

3. Results and Discussion

Characteristics of patients

In this study, 200 samples were collected, which include 120 (60%) samples from burn cases and 80 (40%) samples from wound cases from multiple healthcare centers with in-hospital follow-up, from Ghazi Al-Hariri hospital and the specialized burns hospital in Baghdad, Iraq.

The age ranges from 35 to 50 years, including 72 females and 128 males. The injury type (wounds) includes surgical and accidental cases from mild to severe injury, while the burn cases also include accidental burns with fires and stage II. All cases of wounds and burns were diagnosed, and the specialist doctor determined the extent of damage at the hospital.

Isolation and identification of pathogenic bacteria

All wounds and burn swab samples were investigated for the presence of pathogenic bacteria. All samples were cultured and underwent morphological and microscopical examination to determine 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.
Approximately 160 samples in this study were placed on MacConkey agar, and it was found that approximately 96 were pink (lactose fermenters) and 64 were non-pink (colorless) (non-lactose fermenters). MacConkey agar is a selective and differentiating medium that is mainly applied in isolating gram-negative bacteria according to their capability to ferment lactose.

Lactose-fermenting bacteria secrete acidic products that reduce the pH, which causes the neutral red dye to change to pink or red, thus producing round, flat, and moist colonies. Fermenters that do not ferment lactose form colorless or pale colonies, which means that they produce no acid and do not ferment. Bile salts and crystal violet are also present in the medium and prevent the growth of Gram-positive organisms and selectively promote the growth of

Gram-negative bacteria.

In this study, 180 samples were grown on blood agar, of which 50 formed alpha-hemolytic colonies, 80 formed beta-hemolytic colonies, and 50 formed gamma-hemolytic colonies. Blood agar is an enriched medium used to support the growth of a wide variety of bacteria, particularly fastidious organisms. While it is not selective, it differentiates bacteria based on their ability to hemolyze red blood cells. The medium contains sheep blood, which allows for differentiation through hemolytic activity. Alpha-hemolytic organisms produce greenish discoloration due to partial hemolysis of blood, while beta-hemolytic organisms produce clear zones around their colonies due to complete hemolysis of blood.
Gamma-hemolytic organisms show no hemolytic activity, leaving the medium unchanged. 40 samples that can grow in mannitol salt agar and are suspected to be *Staphylococcus aureus*, which fermented mannitol and turned the medium yellow.

Mannitol Salt Agar (MSA) is a selective medium-differential media that is mainly employed to isolate *Staphylococcus* species. The salt concentration is so high (7.5 percent) as to inhibit the growth of most types of bacteria, except *Staphylococci*. MSA distinguishes bacteria according to their fermenting capacity of mannitol, which reduces the PH level and results in a change of phenol red indicator (red to yellow). Mannitol fermenters, such as *S.aureus*, form yellow colonies, whereas non-fermenters, such as *Staphylococcus epidermidis*, do not change color and turn pink or red. The selectivity of the medium towards the salt-tolerant organisms and the capability to distinguish based on mannitol fermentation make it specifically helpful in isolating and identifying *Staphylococci* spp.

Biochemical tests were performed for 180 isolates with their morphological characteristics. The biochemical tests included in this study were (indole, methyl-red, Voges-Proskauer, citrate, oxidase, and catalase).
The biochemical results for different isolates of Gram-negative bacteria and Gram-positive bacteria showed the presence of 4 suspected isolates, including *Pseudomonas spp*, *Acinetobacter spp*, *Klebsiella spp,* and *S. aureus*, as shown in Table 6.

Table 6: Results of biochemical tests for bacteria isolated from wounds and burns

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, *S. aureus* indicated negative results for (indole, methyl-red, Voges-Proskauer, citrate, and oxidase) and positive results for catalase.

Additional confirmatory identification of the isolates to the species level was carried out using VITEK2 dense automated system tests that included several biochemical tests. The results showed the presence of 4 diagnosed species, including *Pseudomonas* (30%), *Acinetobacter* (27.50%), *Klebsiella* (22.50%), and *S. aureus* (20%), as shown in Table 7.

Table 7: Classification and occurrence of microorganisms identified in cultures

Bacterium	Numbers
Pseudomonas	*30%*
Acinetobacter	*27.50%*
Klebsiella	*22.50%*
Staphylococcus auras	*20%*

A study by Al-Ani and Fahad found that patients with burns and wounds have the Gram-negative bacteria included *A. baumannii* (47%), *K. pneumoniae* (27%), *and P. aeruginosa* (23%), and Gram-positive bacteria *S. aureus* (3.3%), and that 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 *S. aureus* (12%). Conversely, among the Gram-negative isolates the highest isolation rate was reported for *P. aeruginosa* (36%), followed by *Enterobacter spp.* (33%) and *Klebsiella spp*. (17.1%) [10].

Also, another study by Al-Azzawi and Alkalifawi found different percentages of bacterial isolates, *S. aureus* (25%), *Acinetobacter baumannii* (20%), *P. aeruginosa* (16.7%), *K. pneumoniae* (13.3%), *E. coli* (11.7%), *Proteus mirabilis* (10%), and *Burkholder cepacian* (3.3%) burn and wound infections [11].

The most common species were Gram-negative bacteria, which constituted 83%, and *Pseudomonas spp*., and Gram-positive bacteria, especially *Streptococcus* and *S. aureus*, are in line with this study [12].

hematology analysis

Clinical biomarkers showed a significant increase (p-value ≤ 0.05) in all biomarkers, including white blood cell, red blood cell, neutrophil, lymphocyte, platelets, ESR, and CRP. The hematological test results of all patients with wound and burn infections are presented 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 15.61 $\times 10^3/\mu L$, alongside a significantly reduced lymphocyte (LYM) count of 0.1612 $\times 10^3/\mu L$ and a high neutrophil (NET) count of 14.68 $\times 10^3/\mu L$. Platelet (PLT) levels were also elevated, reaching 403.6 $\times 10^3/\mu L$.

Markers of inflammation, including ESR and CRP, were significantly increased, measuring 57.66 mm/hr and 86.42 mg/L, respectively. Additionally, the red blood cell (RBC) count was 5.004 $\times 10^6/\mu L$. 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 $10^3/\mu L$	15.61	7.221	3.60-10.20	<0.0001**

LYM 10^3/µL	2.605	2.021	1.00-3.20	0.0010**
NET 10^3/µL	14.68	4.890	1.85-5.94	<0.0001**
PLT 10^3/µL	403.6	193.5	152.4-3547.9	<0.0001**
ESR mm/hr	57.66	10.98	0.0 - 20.0	<0.0001**
CRP mg/L	86.42	27.70	0.0 - 10.0	<0.0001**
RBC× 10^6/µL	5.004	3.999	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, as shown in Figure 1.

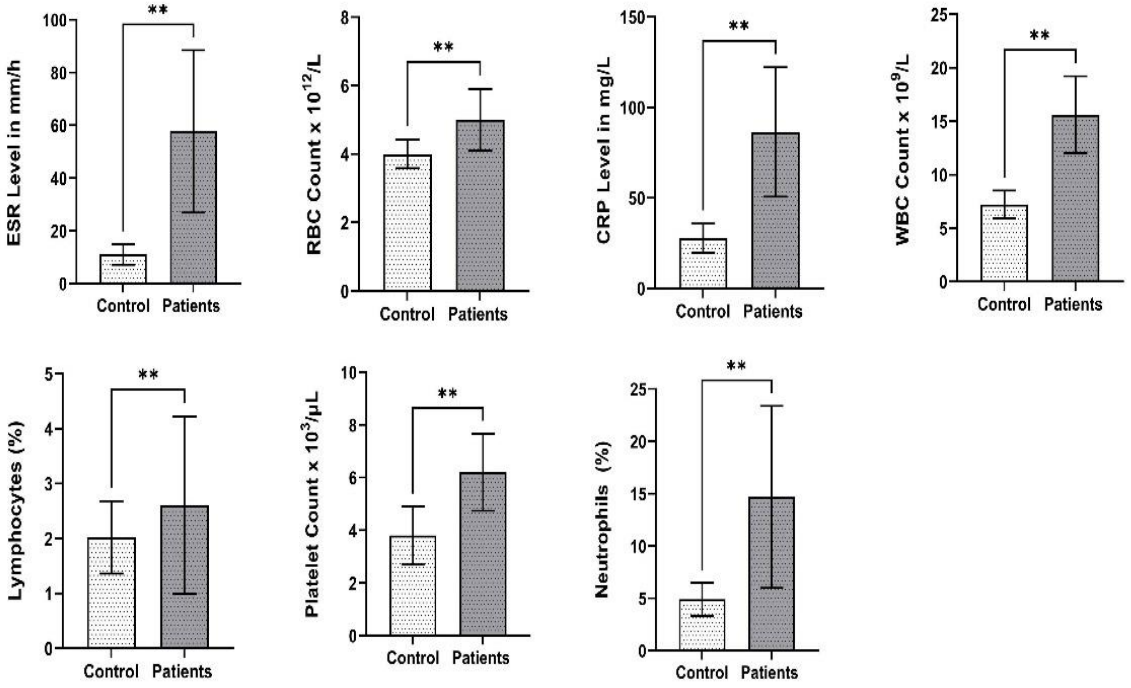

Figure 1: Elevated inflammatory and hematological parameters in patients compared to healthy controls

Complete Blood Count (CBC): This is an exam that determines the amount of different blood cell types, such as white blood cells (WBCs), which play a vital role in the detection of infections and the response to injury in the body. An increase in the WBC levels may suggest an active inflammatory process or infection, whereas lower levels may indicate an active resolving or non-infectious process. The results showed significant increases in its value.
With respect to WBC, like ESR, both markers were observed to be markedly elevated in burn patients compared to healthy controls; this finding is consistent with an earlier study.

The height of WBC is an indication of the immune response of the body to the tissue destruction brought about by burns, whereas ESR is a nonspecific marker of inflammation that indicates that there are inflammatory processes taking place, which is consistent with the other research study [13].
Lymphocytes and neutrophils are key components of the immune system that play a central role in responding to infections and tissue damage, such as wounds and burns. Neutrophils first respond to the area of injury or infection. They are attracted by cues like cytokines and

chemokines that have been emitted by injured tissues. Neutrophils carry out phagocytosis which involves the ingestion and digestion of pathogens, debris, and dead cells. Also, they secrete antimicrobial molecules, including reactive oxygen species (ROS) and enzymes, to counteract pathogens. Neutrophils play a crucial role in preventing secondary infections and facilitating tissue repair in burn injuries by clearing tissue damage. The results showed significant increases in its value.

T-cells and B-cells become more pronounced in the late stages of wound healing and burn healing. T-cells are used in the regulation of the immune system and have the capability of attacking infected or damaged cells directly and destroying them. They also release cytokines in order to enlist other immune cells and assist in the repair of the tissues. B-cells make antibodies, and they aid in neutralizing the pathogens and in inhibiting further infection. Lymphocytes play a critical role in burn injuries in preventing systemic infections, long-term immune memory, and tissue regeneration. Neutrophils and lymphocytes work together to provide the first line of defense and provide continued healing of tissue after injury.
Platelets play a vital hemostatic role, such as preventing bleeding at the site of vascular injury. It is a process that entails platelets adhesion, aggregation, and the development of a procoagulant surface, which helps in the generation of thrombin and the production of fibrin. In addition, platelets also play a role in tissue repair by means of expressing and releasing bioactive substances that regulate angiogenesis, inflammatory responses, and immune activity. These cellular components have large amounts of secretable bioactive proteins and also secrete newly generated active metabolites into the microenvironment. The present findings revealed that platelet values increased significantly. It is observed that at the onset of the infection, the platelet count increased and then decreased after a few days.

A study found that white blood cell and neutrophil counts were strongly increased in burn cases, with cell counts rising in the first days after injury, while platelet counts in burn cases were lower than those in healthy controls during the first week after injury [14]. A significant increase was observed in platelet counts 3 weeks after trauma. These data suggest a delayed immune “second hit” and progression of burn injury even weeks after the initial injury. Our results demonstrated a significant increase in lymphocyte counts.
Erythrocyte Sedimentation Rate (ESR): ESR measures the rate at which red blood cells settle in a test tube over a specified period. An increased ESR indicates the presence of inflammation, which can be associated with infection or tissue damage. Monitoring ESR levels over time helps assess the progression of inflammation and the effectiveness of treatment interventions [15].

However, as noted by Zhang *et al.,* ESR’s utility in diagnosing wound infections is limited due to its lack of specificity. Both ESR and CRP are frequently used in clinical settings to assess inflammation, but their ability to specifically identify infections in burn patients is constrained [16].

One of the studies has examined the contribution of cytokines in systemic response to burns. It is established that burn injuries result in high amounts of inflammatory responses, such as discharge of numerous inflammatory cytokines. CRP is produced by stimulation by these cytokines especially those of the acute-phase response. The figures depicted that its value increased substantially [17].
C-Reactive Protein (CRP): CRP: This is an acute-phase reactant produced in the liver as a reaction to inflammation. High levels of CRP indicate acute inflammation, infection or tissue damages. When it comes to wound and burn healing, CRP can be monitored to track the

inflammation and identify the possible complications, including infections [18]. The findings indicated its values to have increased significantly.

CRP is however extensively utilized to measure inflammation; it is not totally reliable. As an example, Yigit and Demir Yigit stated that CRP levels, as well as WBC counts, tend to stay constantly high during the hospitalization of burn patients in intensive care units, which is why it is not a narrow indicator of infection. The observation has indicated that though CRP is raised in response to burns, the levels of CRP must be taken with caution during infection diagnosis [19].

CRP is a helpful biomarker in conjunction with other instruments and measurements of cytokines. Palmieri and Heard suggest that CRP is produced by the hepatocytes in reaction to a high level of cytokines, including IL-22, during the inflammatory reactions, particularly with burns. Consequently, CRP is prone to rise immediately after a burn, and slowly falls as the patient heals [20].
Kolls and Linden pointed out that a high CRP, which is caused by pro-inflammatory cytokines like IL-22, is a typical attribute among patients who are in burning conditions [21]. Its value was highly increased in the present study.

Wound and burn healing requires the presence of Red Blood Cells (RBCs) because they are essential in the transportation of oxygen, which is essential in repairing and regenerating tissues. They play an active role in the inflammatory process, regulating the extracellular matrix and influencing the healing process. Studies show that RBCs can influence the functioning of the dermal fibroblasts, which are vital in wound healing, by activating matrix metalloproteinases and fibronectin, as well as suppressing type-I collagen. Extracellular regulated protein kinase 1/2 (ERK 1/2) is the mediator of this modulation. The complexity of RBCs in wound healing can be used to design new therapies for fibrotic diseases , such as hypertrophic scars and keloids [22]. In our current study, there was a great increase in the number of RBCs in burn patients when compared to controls.

Enzyme-linked immunosorbent assay (ELISA)

The results of the analysis of IL22 serum level in (wound and burn patients) and (the control group) showed an increase in IL22 serum level in patients (6.123 pg/mL) compared to the control group (3.802 pg/mL) with a significant difference ($p \leq 0.01$), as shown in Table 9.

Table 9: Comparison of serum IL-22 levels between patient and control Groups

Groups	Mean ± SD conc. pg/mL
Control	3.802 ± 1.162
Sample	6.123 ± 1.498*
p-value	≤ 0.01

A study indicated that IL-22 is involved in wound and burn patients; spatially, it was elevated in all patients. The activation of NF-κB promotes the expression of pro-inflammatory cytokines, including IL-22, which are secreted by different immune cells, including T and B lymphocytes, macrophages, microglia, and non-immune cells, such as endothelial cells and neurons [23].

A previous report by Liu *et al.,* found significant differences in IL-22 serum levels between patients and control groups. In an experiment involving wound infection with *P. aeruginosa*, it was found that IL-22, IL-17A, and IL-17F were strongly expressed [24]. Also, Zaharie *et al.,*

found increased levels of IL-22[25]. This is in line with the current findings, which show a significant increase ($p \leq 0.01$) in the level of IL22.

One of the studies has demonstrated that IL-22 is a cytokine that has a key role in communication between the immune system and the tissue barriers. It plays a vital role in skin homeostasis and the control of inflammation. It has been associated with a variety of inflammatory skin diseases where the IL-22 levels are elevated [26].

Wound and burn healing is incomprehensible without IL-22 because it induces proliferation, migration, and differentiation of cells engaged in tissue-repairing. IL-22 stimulates the regeneration of epithelial cells, promotes the synthesis of the extracellular matrix, and is involved in wound contraction and tissue remodeling. There are, however, pathological scarring that may occur in case of IL-22 overexpression, including keloid scarring or peritoneal adhesions [27].
Thus, IL-22 is crucial to the successful healing process, though the level of this cytokine should be balanced to avoid negative results. It is expressed in the inflammatory stage after an acute skin injury and induces fibroblast proliferation and migration of not only epithelial cells but also of dermal fibroblasts, which play a role in the formation of the extracellular matrix [3].

The IL-22 is very much involved in the control of the fibroblasts, particularly in the area of tissue repair and fibrosis. Immune cells, such as T helper cells and innate lymphoid cells, produce IL-22, which stimulates fibroblasts to synthesize extracellular matrix components, thereby aiding in the process of fibrosis following tissue injury. Although this reaction is vital in wound healing, over or incompetent IL-22 reaction may cause pathological fibrosis and long-term inflammation. The IL-22 also favors the secretion of pro-inflammatory cytokines in fibroblasts, thus increasing the inflammatory response, which can be advantageous during the initial phases of repair but destructive when chronic [28].
IL-22 is also important in the healing process of corneal wounds, particularly in the resident stromal cells of the cornea, known as keratocytes. IL- 22 is a cytokine that stimulates the increase and migration of keratocytes to regenerate damaged tissue. It also enhances the synthesis of extracellular matrix components that aid in healing by restoring the corneal structure. Nevertheless, the long-term or excessive stimulation of IL-22 can cause corneal scarring that affects vision and clarity of the cornea. Therefore, although IL-22 plays a crucial role in tissue repair and regeneration in fibroblasts and gene keratocytes, its imbalance can lead to disease-related tissue developments, including fibrosis and scarring [29].

Gene expression of miRNA-146

The real-time PCR amplification program was conducted using melting curve analysis for all evaluated gene expressions, following the manufacturer's stimulation programs, using relative gene expression. Total RNA extracted from blood samples was converted to cDNA for gene expression level analysis using real-time PCR (qPCR) for *miRNA-146* gene. Gene expression was standardized to the level of the housekeeping gene (U6) and the Ct value, as shown in Figure 2. Each colour in the curve displayed in these images refers to a certain Ct value.

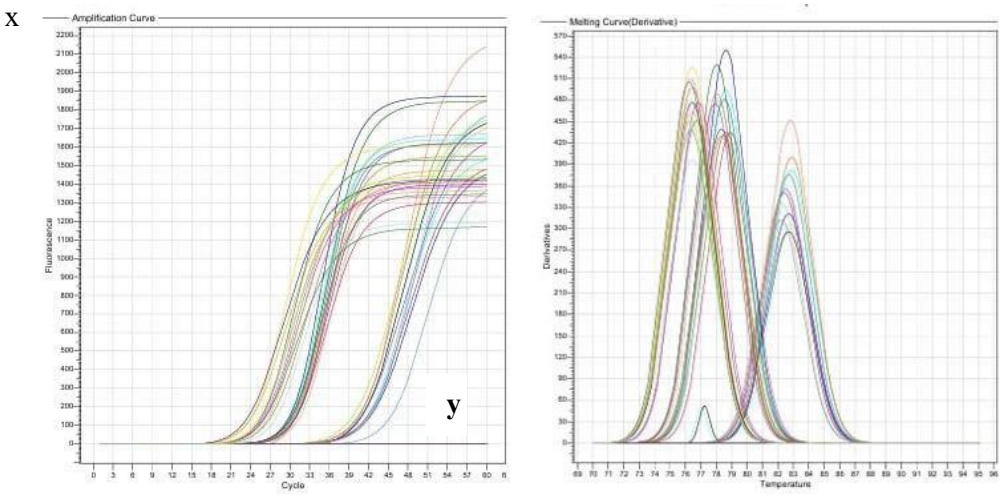

Figure 2: Amplification plots (left) and melting curves (right) for *miRNA-146* and *U6* obtained by RT-qPCR. The X-axis in the amplification plot represents the cycle number, and the Y-axis shows the relative fluorescence units (RFU). In the melting curve, the X-axis represents the temperature (°C). Different colors represent different sample groups (patients, controls, and *U6* reference).

MiRNA-146 gene expression was quantified using a 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 10: 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-146	**Patients**	**41.29**	**25.93**	**16.40**	**2.27**	**0.207 ± 0.043**	**<0.0001****
	control	**15.16**	**26.90**	**14.13**		**1.0 ± 0.0**	**----------**
Non-significant, ** p < 0.01							

The results of miRNA-146 expression in burn and wound patients significantly reduced ($p < 0.0001$) with a value of 0.207 ± 0.043 in patients' group and 1.0 ± 0.0 in the control group, the folding change about 80% reduction, as shown in Figure 3. miRNA-146 is a well-known regulator of inflammation, particularly in controlling excessive immune responses through the NF-κB signaling pathway [23]. A reduction in miRNA-146 expression in burn and wound patients suggests an impaired ability to regulate inflammatory responses, potentially leading to excessive inflammation, delayed healing, or increased risk of infection. Also, downregulation of *miRNA-146* has been linked to chronic wounds, where prolonged inflammation prevents proper tissue repair [5].

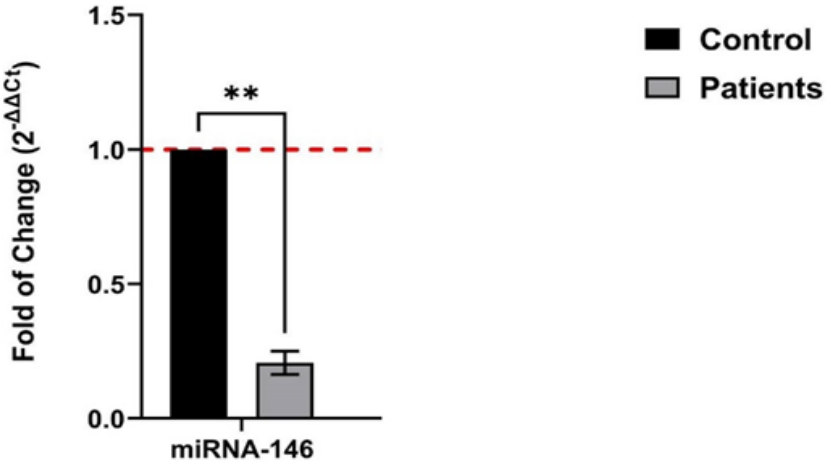

Figure 3: Relative expression of *miRNA-146* in patients compared to controls, showing significant downregulation ($p < 0.01$).

Burn and wound patients exhibit a significant decrease in *miRNA-146* (~80% reduction) compared to controls.

A study by Herrnreiter *et al.,* showed that downregulation of miRNA 146 following burn injury, which is in line with the current study, a decrease in miR-146a may lead to an increase in pro-inflammatory target genes, negatively impacting the healing process [30].
Sen discussed the broader relationship between microRNAs and wound healing mechanisms and the evidence for changes in microRNA expression after burns, during wound healing, and scar formation, with a focus on the role of miR-146a in these processes [31].
In addition, the role of microRNAs in cutaneous wound healing, focusing on how changes in miR-146a expression affect inflammation and healing [32]. The role of miR-146a in the proliferative phase of wound healing, noting its effect on promoting angiogenesis.
Biological implications of burn and wound repair, excessive inflammatory response, SO miRNA-146 assists in the regulation of inflammation. Their downregulation implies uncontrolled inflammation that is a significant problem among the burn and chronic wounds patient population. Chronic inflammation hinders normal wound healing by destroying tissue structures and increasing the risk of acquiring new infections. The miRNA-146 structural repair functions facilitate cellular proliferation of fibroblasts alongside the movement of keratinocytes to facilitate healing of wounds. The wound healing process is delayed due to the insufficient quantity of miRNA-146, which hinders the rate of tissue remodeling and skin wound healing [33].

The miR-146a small RNA molecule regulates important biological processes during inflammatory and immune response processes. The miR-146 intervenes by modulating significant signal pathways as an inhibitory feedback loop whose principal outcomes are on NF- KB and MAPK pathways. MiR-146a, as a significant therapeutic target, is unique in that it regulates the immune system through inflammatory pathways; thus, it offers a potential treatment approach for managing the inflammatory process and promoting tissue healing. This molecule is the one that acts essentially in physiological and pathological events. This miRNA plays a regulatory role in controlling the synthesis of inflammatory cytokines, thereby preventing unnecessary tissue destruction and promoting tissue repair [6].

The mechanisms of action of MiR-146a involve inhibiting the activation of NF-κB, thereby preventing inflammation that can lead to tissue destruction and the development of

autoimmunity. The miR-146a regulatory mechanism goes further to p38 MAPK (mitogen-activated protein kinase) pathway activation, as well as NF- kb inhibition. Both stress factors and cytokines trigger the p38 MAPK pathway. Nevertheless, miR-146a plays a mitigating role in response to pro-inflammatory cytokines because it targets specific molecules in the MAPK pathway, which facilitates tissue repair processes [23]. StrtokiR-146a controls pathways of fundamental signaling, regulating the interaction between inflammation and immune tolerance processes to facilitate injury healing [34].

The above regulatory roles of miR-146a in various inflammatory signaling pathways have rendered it a major area of investigation in studies of inflammatory regulation. The NF- kB signaling pathway is one of the most critical pathways that miR -146a regulates in immunologic and inflammatory events [23].

It has been shown that miR-146a, an angiogenesis-controlling expression molecule, selectively interacts with P21-Activated Kinase 1 (PAK1) in the process of regulating the expression of vascular endothelial growth factor (VEGF) during the development of new blood vessels to repair damaged tissue areas. VEGF is a vital element in wound healing as it is necessary to develop new blood vessels to supply the wound with oxygen through a crucial process regulated by miR-146a. This process hastens the healing process and forms an oxygen and nutrient supply to the injured tissue [32].

Experiments have shown that miR-146a helps reduce fibrosis, which hinders healing processes during chronic inflammation. Wound scarring, together with impaired tissue function, results when fibrosis develops due to high levels of collagen deposition in the wound site. Through its effect on inflammatory pathways, miR-146a minimizes fibrotic tissue production, which results in maintaining the normal trajectory of wound healing [4].

Exercise, together with other external stimuli, regulates the expression levels of miR-146a. Multiple research studies have demonstrated that exercise serves as a modulator of miRNA expression. A 2023 review further showed how exercise affects miR-146a levels in burn wound tissue. The therapeutic potential of exercise-induced miRNA regulation includes anti-inflammatory response management alongside tissue healing improvement and vessel development enhancement, which supports exercise as a supplemental treatment for burn recovery [30].

Research demonstrates that miR-146a participates in the management of intestinal inflammation aside from its familiar function in wound healing and burn injuries. Systemic inflammation with a disrupted intestinal barrier becomes a risk factor for sepsis when patients suffer severe burns [30].

The expression of miR-146a regulates vital processes in inflammation, immune responses, and tissue repair, significantly impacting burn wound healing and other inflammatory conditions. Because miR-146a controls important signaling pathways, including NF-κB and p38 MAPK, as well as Signal Transducer and Activator of Transcription 3 (STAT3), it demonstrates utility as a possible recovery outcome enhancer in burn patient treatment [35].

The microRNA miR-146a negatively regulates IL-22 signaling through its response to IL-22 by downregulating the proteins TRAF6 and IRAK1, responsible for NF- κB and MAPK inflammatory pathway activation. The feedback loop uses these regulatory proteins to prevent

unwanted inflammation that would otherwise result in endogenous tissue damage. IL-22 needs to maintain a proper balance because it helps skin and gut tissue maintain barrier protection and aids in repair processes [8]. The data from this study show that IL22 levels increase remarkably while miR-146a expression decreases substantially.

Studies on miR-146a demonstrate its complexity because it acts as both an anti-inflammatory molecule and a cancer-related factor [8]. Future investigations into miR-146a are likely to identify its potential value in treating inflammatory diseases, wound healing, and cancer treatment.
Inhibition of the IL-22/miR-146 axis can promote more rapid wound and burn healing [34]. IL-22, is an epithelial cell mitogen and barrier protector. Concurrently, miR-146 defends against excessive inflammation by inhibiting IRAK1 and TRAF6, which are the major signaling molecules in the NF-kB signaling pathway. These inducers may have a synergistic effect systemically, allowing tissue regeneration to occur in the presence of a well-regulated healing environment that does not induce chronic inflammatory tissue and scar tissue development. Recently, miR-146a was found to promote angiogenesis by targeting PAK1, thereby stimulating VEGF expression and facilitating tissue repair [32].

Conclusions

The signaling mechanism of IL-22 becomes essential for both immune regulation and tissue repair; however, IL-22-induced immune activity prevents both chronic inflammation and tissue damage. MiR-146 and IL-22 play a role in controlling inflammation and promoting wound healing. MiR-146 is also considered one of the main regulators of the inflammatory process, whereas IL-22 facilitates tissue repair and antimicrobial activities. It is linked to poor wound healing among burn and wound patients. The analysis revealed that the amount of IL-22 was significantly increased, and miRNA-146 was reduced in the study participants.

Conflict of Interest

The authors declare no conflict of interest.

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