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Study The Relation between the IL-6 Fold Increase in Expression and the Type of Bacteria Causing Sepsis in Burn Patients in Baghdad Governorate

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Abstract: Interleukin 6 (IL-6) is a well-established indicator of inflammation and is one of the first immune responses to occur in cases of sepsis. It is possible to assess serum levels quickly, typically within a few hours. The precise clinical importance of IL-6 in the initial phase of sepsis in burn individuals remains unverified. This study aimed to investigate the prognostic significance of IL-6 in burn patients with suspected sepsis, specifically in relation to bacterial infection. 44 patients with age (1.5–75 years), and 19 healthy controls with the same age range, were involved in this study during their attendance to Specialized Burns Hospital in the Medical City, Al-Kindi Teaching Hospital in Baghdad. The study was conducted from 1st December 2023 to 1st April 2024 and approved by ethical committees of Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad. Blood samples and data were collected to evaluate the level of IL-6 associated with bacterial infection for burn patients by RT-qPCR System. Results appeared that mean IL-6 percentages were significantly ($P \leq 0.01$) increased in burn patients as compared with control. The current study is consistent with other research as it shows no significant differences in IL-6 levels among patients with gram-positive, gram-negative, and mixed growth.

Keywords: *Sepsis; Interleukin-6 gene expression; bacterial infection.*

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Introduction

IL-6 is a multifunctional cytokine that stimulates the growth and specialization of B lymphocytes and also enhances the synthesis of platelets. Additionally, it stimulates the secretion of inflammatory chemicals such as C-reactive protein (CRP) and fibrinogen from the hepatocytes (1). According to reports, macrophages exhibit heightened activity and an enhanced ability to produce proinflammatory mediators following a burn. The research findings confirmed the presence of increased IL-6 levels in burn sufferers (2). The plasma concentration of IL-6 in patients with burns reached its highest level during six hrs following the burn injury. Increased concentrations of IL-6 are directly Persistent elevation of IL-6 after a burn injury may serve as an indicator of both the severity of the burn and the probability of fatality, which is associated with the severity of the burn(3). IL-6, an inflammatory mediator, is often raised in burn patients. Higher levels of IL-6 on the first day after the injury raise the likelihood of developing sepsis (4).

Sepsis is characterized by a dysregulated immune response to an invading pathogen, resulting in

persistent inflammation and compromised immune function(5). Elevated cytokine production after burn damage seems to play a vital part in the development of sepsis and septic shock, both in people and in animal models(6). Early after a burn, monocytes, endothelial cells, and fibroblasts release pro-inflammatory interleukin (IL-6), which may activate B and T lymphocytes and cause fever. Studies have shown that IL-6 is one of the most significant components that may be connected to the risk and outcome of sepsis and may also be essential in the inflammatory response to microbial invasion (7). confirmed comparable results in both trauma individuals and experimental sepsis models, showing that detecting high levels of IL-6 early on is a sign of developing multiple organ malfunction syndrome and an increased likelihood of death (8).

Sepsis is defined by an imbalance in the immune system caused by an infection. This leads to both an excessive production of cytokines that are pro-inflammatory and a suppression of the immune response in the early stages of the illness (9). If the host's immune system can eliminate the pathogen rapidly, the body will regain equilibrium with the use of medications and infection management methods. Nevertheless, several hosts exhibited persistent immunosuppression, rendering their immune system incapable of promptly eliminating pathogens. This condition may progress to secondary infections, ultimately resulting in multiple organ dysfunction syndrome (MODS) and death (10).

The severity of a burn is determined by the specific location, temperature, and length of the patient's exposure. Additionally, there is a direct correlation between temperature and exposure time(11). *Pseudomonas aeruginosa* and *Staphylococcus aureus* are the predominant bacteria implicated in producing serious infections in burn wound patients, which might possibly compromise the efficacy of burn wound therapy (12).

Material and methods

This research was carried out in the immunological and Bacteriology laboratory of Specialized Burns Hospital in the Medical City/ Baghdad. This research used a Case-Control design, using 63 samples of burn infection patients of varying age and sex. Additionally, a control group consisting of healthy persons was included in the investigation. from both male and female in several hospitals in Baghdad, during the time between 1st December 2023 to 1st April 2024. These patients age range (1.5–75 years).

Physicians confirmed the patients' clinical diagnosis. Patients were classified into three main groups based on burn severity by measuring burn severity: mild group, moderate group, and severe group. The research was conducted with ethical approval from all participants as well as the hospital.

Control group

2 mL of blood samples were taken from (19) healthy individuals as control group who are free from any sign and symptoms for any illness. RNA was extracted from these samples in order to be studied later.

Gene expression of IL-6: RT-PCR

After collection of blood samples from patients and healthy individuals, the total RNA was extracted by using Tri RNA Pure Kit (TransGen Biotech company, China). The real-time quantitative polymerase chain reaction (qPCR) procedures were conducted using specific primers targeting the reference gene GAPDH and the target

gene IL-6. The final amount of the reaction mixture was 20 µL. Conversion the total RNA to cDNA and amplification of DNA was done according to instructions provided by GoTaqR 1-Step RT-qPCR System (Promega, USA). Using BRYT GreenR dye, where RT-qPCR primers and conditions were summarized in Table 1,2. Relative expression fold was calculated (2−(ΔΔCt)) (13).

Statistical analysis

The statistical study was conducted using SPSS version 26, developed by IBM Company in Chicago, Illinois. The molecular result was exploration by using Chi-square ($\chi 2$) test. *P* values less than (0.05) is considered. Data were expressed as mean ± SD.

Table (1): Primers sequences used for genes amplification.

Primer		Sequence (5'-3')	Length (bp)	product	Ref.
Gene IL 6	F	GGCACTGGCAGAAAACAACC	20	85	(14)
	R	GCAAGTCTCCTCATTGAATCC	21	85	
GAPDH	F	GTCTCCTCTGACTTCAACAGCG	22	131	(15)
	R	ACCACCCTGTTGCTGTAGCCAA	22	131	

Table (2): Program for qPCR amplification

Steps	Temperature	Time	Cycles
Reverse transcription	37℃	15 MIN.	1
Reverse transcriptase inactivation and GoTaq® DNA Polymerase activation	95 ℃	10 MIN.	1
Denaturation	95 ℃	10 SEC.	40
Annealing and data collection	60 ℃	30 SEC.	

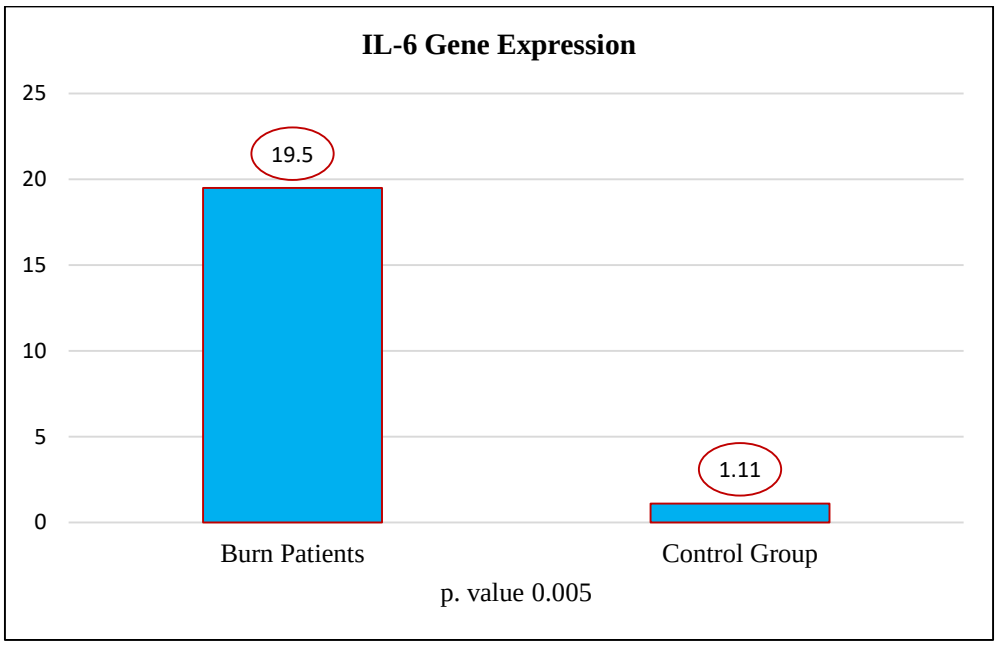
Result and discussion

Gene expression IL-6 level using real time PCR

A total of 44 blood samples were collected. RNA was extracted to revision the gene expressing of IL-6 by means of RT-PCR (ΔΔCt). The results found that the level of expression to IL-6 gene in the patients' samples as well as in control samples, normalized by means of house-keeping gene GAPDH, represents high statistical significance at $P \leq 0.05$, as shown in Figures (1), Tables (3).

Table (3): Expression of IL 6-fold gene between control and burn patients versus the reference gene (GAPDH)

Groups	No.	CT (IL-6)	Ct (GAPDH)	ΔCT	ΔΔCT	Folding	*P* value
Controls	19	30.05	21.86	8.18	0.00	1.11	*< 0.001
Patients	44	29.69	25.76	3.92	-4.25	19.59	
* Represent a significant difference at P ≤ 0.05							



Figure(1): IL 6-fold gene expression between control and burn patients versus the reference gene (GAPDH).

A wide range of stimuli (bacterial infections, inflammation, tissue injury response, and other cytokines and growth factors), IL-6 gene expression are easily elevated in cells. The promoter IL-6 is turned on at the molecular level via a number of signaling mechanisms(16). The study examined the use of IL-6 as a marker for bacterial infection in burn patients. It supported previous research that also identified IL-6 as a marker for bacterial infection during acute inflammatory reactions in burn infections. The study focused on the cellular and molecular processes and interactions that effectively reduce the risk of harm or infection caused by invading microorganisms(17). IL-6 has a vital function in the first phases of inflammation by activating different cells to generate and release acute-phase proteins. Throughout the course of an infection, it promotes the production and stimulation of neutrophils, the proliferation and differentiation of lymphocytes such as B cells and T lymphocytes, and the synthesis of immunoglobulins (18). The concentration of IL-6 in individuals without any health issues is often quite little, usually not surpassing 7 pg/mL. However, in patients diagnosed with sepsis, the amount of IL-6 in their bloodstream rises significantly within 2 hours of the beginning of infection. Elevated levels of IL-6 are linked to the diagnosis of infection(19).

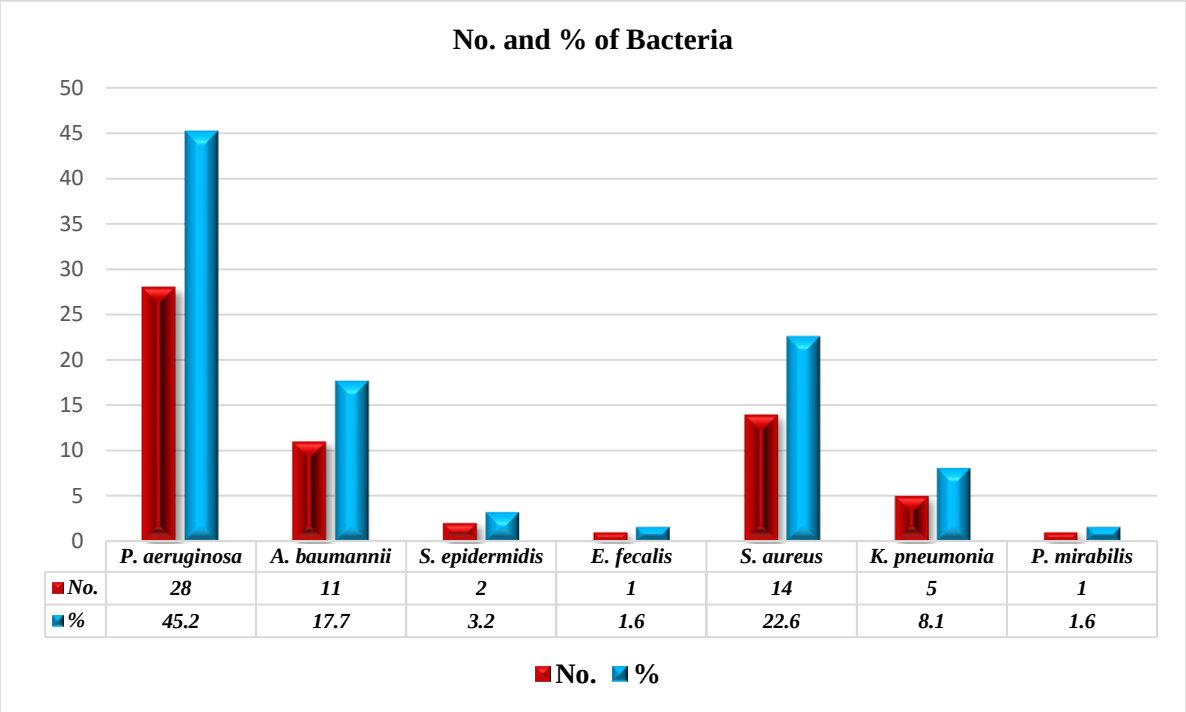
A notable fatality rate is linked to sepsis, a bacterial infection-induced SIRS. Sepsis patients often experience rapid deterioration and serious harm (20). The expansion of burn circumference in burn patients results in a reduction of their fundamental immune response. Additionally, patients with a substantial surface area affected by grade III burns have an even higher likelihood of developing sepsis after the burn injury(21). Currently, the underlying mechanism of sepsis after burns has not been comprehensively investigated, although it is potentially associated with the generation of bacterial endotoxins, the release of inflammatory mediators,

and immunological dysfunction (17).

Despite notable advancements in clinical identification and treatment of sepsis, its morbidity and death rates continue to be elevated in recent years. The 2016 "SCCM" and "ESICM" have revised their definitions, according to the modified definitions, sepsis is now described as a condition when the body's reaction to infection becomes uncontrolled, leading to life-threatening organ failure (22). The discovery of biomarkers for prompt diagnosis of sepsis is crucial due to the limitations of diagnostic criteria and the lack of clinically meaningful data for many patients.

Identification of bacterial isolates from burn patients causing sepsis

Many bacterial species may be found in burn sample, bacterial isolates were diagnosed by using the bacterial cultural, biochemical tests and API 20E system for the bacterial isolates were investigated in the laboratory, the result revealed 7 bacterial species the most frequent was *P. aeruginosa* 28 (45%), *Staph. aureus* 14 (22.6 %), *Acinetobacter baumannii* 11 (17.7%), *Klebsiella pneumoniae* 5 (8.1%) *Staph. epidermidis* 2 (3.2%), *Proteus mirabilis* 1 (1.6%) and *Enterococcus faecalis* 1 (1.6 %), as shown in Figures (2).



Figure(2): Showed all the types of bacteria isolated from burn patients.

It is crucial to emphasise the significance of determining the frequency of bacterial infections and antibiotic resistance in patients with severe burns. Two autopsy conducted on burn patients have shown that the primary reason for death, accounting for up to 65% of cases, is multi-organ failure resulting from sepsis(23). Sepsis frequently occurs in the intensive care unit of burn centres and is influenced by various factors, including the duration of hospitalisation, advanced age, the extent and severity of burns, and the underlying

causes of burns, as well as related conditions that compromise the immune system, such as HIV infection or autoimmune diseases. The primary objective of this research, which aimed to identify the disease-causing bacteria responsible for infections in patients admitted to burn units, was successfully accomplished in a cohort of 44 patients between December 1, 2023, and April 1, 2024. In our country, Pseudomonas aeruginosa, Staphylococcus aureus, and Acinetobacter baumannii are the most often encountered bacterial pathogens in burn victims. These results align with previous worldwide research; however, the distribution of different bacteria varies across burn centres. A Bulgarian research conducted over three decades ago found Staphylococcus aureus as the primary causative agent in burn wound infections, in contrast to studies conducted in South-East Europe (24, 23).

The relation between the IL-6 fold increase in expression and the type of bacteria causing sepsis

In gram-positive bacterial, the parameters of IL6 were 20.081 ± 4.124 (max: 25.244, min: 12.192). In gram-negative bacterial, the parameters of IL6 were 19.261± 4.404 (max: 28.798, min: 12.448). In mixed bacterial, the parameters of IL6 were 19.726± 4.404 (max: 28.205, min: 14.103). The study's results revealed that there was no statistically significant difference (P> 0.05) in both of gram positive, gram negative, and mixed growth. as show in figure (3).

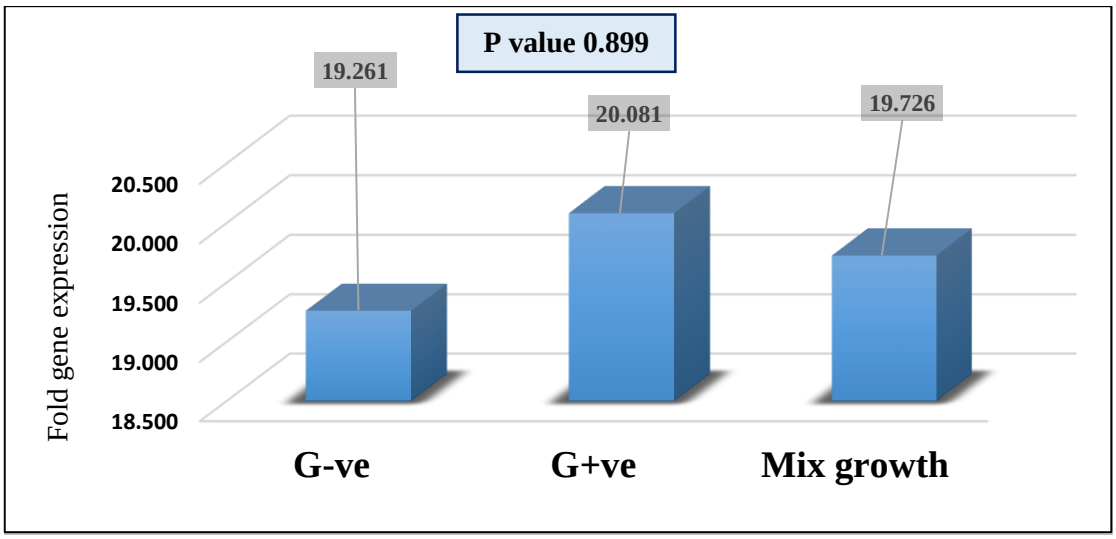


Figure (3): The relation between the IL6 fold increase in expression and the type of bacteria causing sepsis.

The primary discovery of this research is that IL-6 has the potential to forecast bacterial infection in people who are clinically suspected of having sepsis. The findings align with previous studies in burn victims, indicating a strong association between increased IL-6 levels and bacterial infections(25). Currently, there is a lack of pertinent data pertaining to burn victims. We discovered a robust predictive capacity of IL-6 levels for bloodstream infections produced by gram-positive, gram-negative, and mixed growth pathogens. Our recent research found that there were no significant differences in IL-6 levels between patients with Gram-positive, Gram-negative, and mixed

growth. This outcome is consistent with earlier research conducted by(16).

Conclusion

This research demonstrates that interleukin 6 levels are up in individuals with burn infections caused by various kinds of bacteria. This rise is attributed to the activation of T1 cells, which significantly contribute to the immune system's inflammatory response. The study's conclusive findings revealed that there were no significant differences in IL-6 levels among patients with Gram-positive, Gram-negative, and mixed growth.

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