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Original Article

Detection of the Level of Interleukin-8 in the Serum of Burn Patients by ELISA Technique

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Abstract

Burn injuries are the most frequent injuries in the world, with a death rate of 2.3-3.6%. Children and people of working age constitute 85-90% of the burn cases. Burn injury results in metabolic problems, a generalized inflammatory response, inefficient energy use, and other physiological alternations that may cause organ and system dysfunction and sepsis. Sepsis is mostly caused by multiple organ failures and has unique characteristics in burn injuries, which make it the most dangerous complication of burn injuries. This study aimed to investigate the correlation between sepsis in burn patients and the level of interleukin 8 (IL-8) in their serum. In total, 60 patients with burn injuries were included in this study. Blood samples were obtained from 60 burn patients and 30 healthy individuals as controls. The BacT/Alert and Vitek2 systems were used to identify the bacteria and determine their susceptibility to these bacteria. Moreover, enzyme-linked immunosorbent assay (ELISA) was used to determine IL-8 serum levels. Based on the results, elevated levels of IL-8 were observed in the serum of burn patients, compared to healthy individuals. Concentration of IL-8 was significantly higher in patients with sepsis, compared to healthy individuals without sepsis.

Keywords: Detection; Interleukin-8; Serum of Burn Patients; ELISA Technique

1. Introduction

Burn is an injury to the skin or other organic tissue primarily caused by heat, radiation, radioactivity, electricity, friction, or contact with chemicals. Burn injuries result in a significant amount of morbidity. Burns that do not lead to death may cause prolonged hospitalization, disfigurement, and disability, often stigma and rejection (1). Burn wounds provide an ideal environment for the growth of both indigenous and external opportunistic organisms. Due to the changed immunity, there is a higher chance of infection, leading to sepsis.

Infection-related complications contribute to 50-70% of the mortalities of burn patients following their first therapy (2). Invasive infection is determined by characteristics related to the patient, such as age, total body surface area, and depth of burn wound, as well as those connected to microbiological organisms, such as kind and number, enzyme/toxin production, and motility of organisms (3).

Sepsis influences the immune system by affecting the lifespan, generation, and function of homeostatic effector cells. The hematopoietic compartment replenishes terminally differentiated innate and adaptive cells, which are essential for proper tissue regeneration and wound healing and are responsible for immune monitoring against offending microorganisms (4).

Some of these biomarkers are cytokines, which are important mediators of these processes by keeping a

precarious balance between resisting infection and minimizing tissue damage (5). The IL-8 is a chemokine produced by macrophages and other cell types, such as epithelial cells, and plays a crucial role in the regulation of cell adhesion, inflammatory response, cellular response to lipopolysaccharide, and regulation of entry of bacterium into host cells (6).

1. Gram-positive bacteria (*Staphylococcus aureus, Streptococcus* spp., and *Enterococcus* spp.)
2. Gram-negative bacteria (*Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae*, and other Enterobacteriaceae.)
3. Anaerobic bacteria (7).

A life-threatening infection with systemic symptoms that can be fatal proceeds to septic shock, defined by hyperdynamic changes in the body. A state of cardiovascular instability can lead to tissue damage in the future. Hypoperfusion causes multi-organ failure, increased hypermetabolic catabolism, poor wound healing, and inflammatory and stress responses that lead to immunological depletion and failure (8).

Inflammation, proliferation, and remodeling are the three stages of wound healing based on each type of burn. Production of histamine, free radicals, and inflammatory cytokines, which cause vasodilation and tissue edema, initiate the reaction. This attracts neutrophils and monocytes to the lesion site, where they release chemotactic signals that attract macrophages (9).

Some investigations have found a link between the extent of the lesion and serum concentrations of cytokines (IL-6, IL-8, and IL-10) or monocyte chemoattractant protein-1 within 24 or 48 h after the burn. It has also been discovered that IL-10 serum concentrations have a predictive value when assessed in hospitalized patients 24-48 h following a burn (10).

The IL-8 (also known as CXCL8) is a chemokine produced mainly by macrophages after an injury. The IL-8 is a protein involved in inflammation and plays a vital role in the immigration of neutrophils and other immune cells to the infection site. It is produced by epithelial cells, airway smooth muscle cells, endothelial cells, and macrophages.

The IL-8 has been considered a survival biomarker. Expression of IL-8 is consistently higher in burn patients who do not survive, compared to those who survive. Although IL-8 expression in burn survivors returned to baseline within 8-10 days of damage, IL-8 concentration in non-survivors remained considerably higher until death.

The temporal rise in IL-8 expression in non-survivors begins roughly 8-10 days after the burn, suggesting the onset of infection or sepsis when measured across the period of hospitalization (11). In this regard, this study aimed to investigate the correlation between sepsis in 60 burn patients and the level of IL-8 in their serum.

2. Materials and Methods

2.1. Sample Collection

The samples were collected from 60 Iraqi patients admitted to the hospitals after burn injuries from the first of November 2021 to the end of February 2022. Regarding gender, 30 patients were male and the rest were female.

The samples were collected from Specialized Burns Hospital, Medical City, Al-Yarmouk Teaching Hospital, and Al Karama Teaching Hospital, Baghdad, Iraq. The patients suffered burns in different degrees but the most frequent one was the third degree.

2.2. Identification of bacterial Isolates Using Analytical Profile Index 20E System

The isolates were identified by sub-culturing representative colonies from MacConkey agar plates on API 20E microtubes systems. This system performed 20 standard biochemical tests on a single colony on a plate medium. Each test in this system was performed within a sterile plastic micro-tube that contained the appropriate substrates and was affixed to an impermeable plastic strip called a gallery, and each gallery contained 20 microtubes (Figure 1).

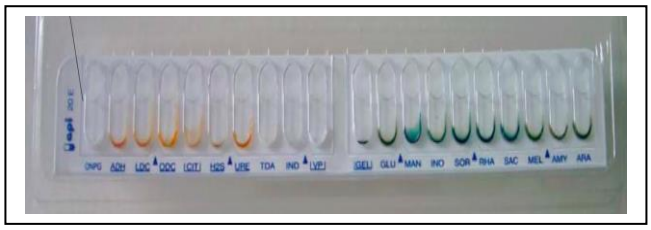

Figure 1. API 20E

2.3. Identification of Bacteria by VITEK-2 System

The VITEK-2 system is an identification system that depends on the biochemical reactions between the bacterial isolates suspended in their solutions and the media in the VITEK-2 Identification Cards to identify the isolates. The bacterial isolates were inoculated onto MacConky agar plates and incubated overnight at 37 °C. A single colony was then taken and suspended in the solution.

Turbidity of the bacterial suspension was adjusted by VITEK Densi-Chek (BioMèrieux) to match the McFarland 0.5 standard in 0.45% sodium chloride. Afterward, the VITEK 2 ID card and the bacterial suspension tubes were manually loaded into the VITEK-2 system. Subsequently, the steps on the software were performed according to the instructions of the manufacturer (BioMèrieux, France) (12).

2.4. Human Interleukin-8 ELISA KIT

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibodies specific to IL-8 had been pre-coated onto a microplate. Standards and samples were pipetted into the wells, and the immobilized antibody bound any present IL-8. After the removal of any unbound substances, a biotin-conjugated antibody specific to IL-8 was added to the wells. After washing, avidin-conjugated horseradish peroxidase was added to the wells. Following a wash to remove any unbound avidin-enzyme reagents, a substrate solution was added to the wells, and color developed in proportion to the amount of IL-8 bound in the initial step. The color development was stopped, and the intensity of the color was measured (Figure 2).

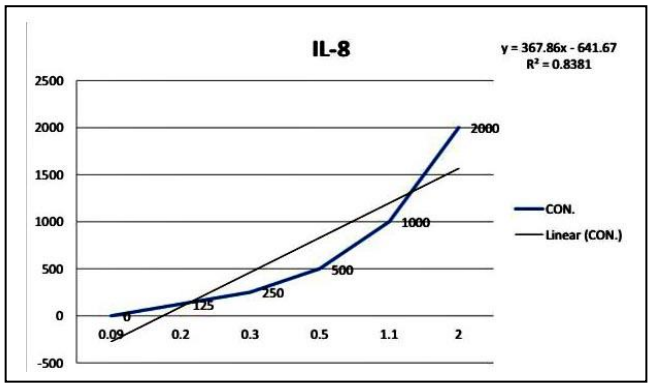

Figure 2. Shows the relation between the IL-8 conc and the OD

3. Results

Similarly, apart from the control and healed-cases groups, as no one had a positive growth of blood culture, the gram-negative cultures of blood were more than the gram-positive cultures among dead-cases groups (96.7% vs. 3.3%). The isolated gram-negative bacteria from the blood culture of the patients were mainly *Acinetobacter baumannii* (47%), followed by *Klebsiella pnuemoniae* and *Pseudomonas aeruginosa* (27% and 23%, respectively). Whereas, *Staphylococcus aureus* was the only gram-positive bacteria that was isolated from blood cultures (3.3%) (Figure 3).

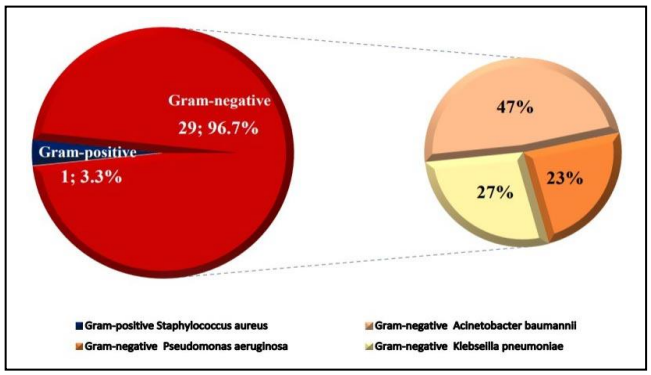

Figure 3. Distribution of isolated bacteria from blood culture among dead-cases groups (n= 30)

A comparison of the studied groups regarding the immunological parameters of IL-8 revealed significant differences among them. Accordingly, the mean value of IL-8 was found to be significantly higher among the dead-cases group

(632.747±132.2323), compared to that of the healed-cases group (452.510±124.5278), which was subsequently higher than that of the controls (188.057±60.3751) (F=122.894, df: 2, 87, and *P*=0.000). It should be mentioned that the dead-cases group and the healed-cases group had significant differences of 820.804 and 364.453 from the control group, respectively (Figure 4 and Table 1).

Among 60 subjects, the optimal cutoff value of IL-8 for the detection of burned patients with a high risk of lethality was 529.650 with a sensitivity of 83.3% and specificity of 73.3%. Moreover, the optimal cutoff value was correctly predicted by the regression model of 76.7% with a good area under the receiver operating characteristic curve of 0.847±0.050 (*P*=0.000) (Figure 5 and Table 2).

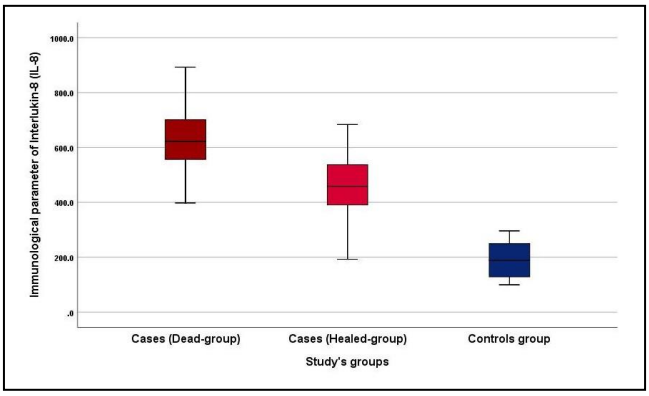

Figure 4. Mean comparison of the immunological parameter of interleukin-8 (IL-8) among study groups (n=90)

Table 1. Mean comparison of the immunological parameter of interleukin-8 (IL-8) among study groups (n=90)

Study Groups (n=90)	Immunological parameter of interlukin-8 (IL-8)		
	(Mean±SD)	Mean difference[a]	Significance[b]
Contorols	188.057±60.3751		F= 122.894, df: 2, 87, *P*= 0.000
Healed-cases	452.510±124.5278	364.453	
Dead-cases	632.747±132.2323	820.804	

[a]: Mean difference from mean value of controls group
[b]: One-Way ANOVA Test

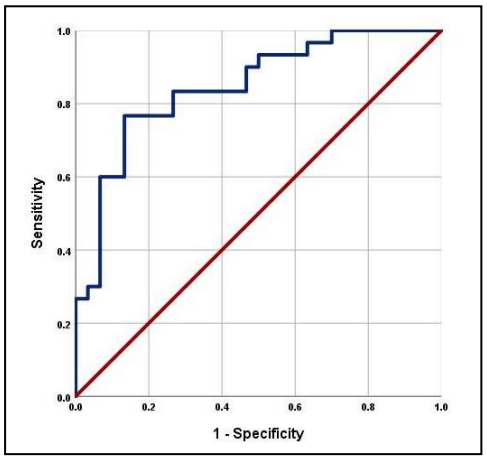

Figure 5. ROC Curve of death risk predicted by the immunological parameter of interleukin-8 (IL-8) among burned cases sample (n=60)

Table 2. Predictive value of interleukin-8 (IL-8) as a marker for lethality risk among burned cases sample (n=60)

Validity of model					
Parameter	**Sensitivity (Sn)**	**Specificity (Sp)**	**Accuracy**	**Area Under the curve (AUC)**	**Significance (*P*-value)**
Interleukin-8 (IL-8)	83.3	73.3	76.7	0.847	0.000

4. Discussion

In this study, the growth in blood culture was only observed in the dead cases; accordingly, the gram-negative and gram-positive values were 96.7% and 3.3%, respectively. The gram-negative bacteria included *A. baumannii* (47%) and *K. pneumoniae* (27%), and *P. aeruginosa* (23%). Furthermore, the gram-positive bacteria was *S. aureus* with (3.3%). Results of a study (13) performed on 41 patients with positive blood cultures in Turkey were similar to those of the present study as the gram-negative and gram-positive bacteria were observed in 40 (97.65%) and 1 patients (2.4%), respectively. The most common gram-negative bacteria were *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae* which were found in 17 (41.5%), 13 (31.7%), and 2 patients (4.9%), respectively.

In addition, in a study carried out in Iraq (14), it was found that 66.6% and 33.3% of the blood culture results were gram-positive and gram-negative bacteria, respectively. However, the results of a study performed by Devrim, Kara (15) in Turkey were inconsistent with those of the present study, which showed that the gram-positive bacteria (66.4%) were the coagulase-negative *Staphylococci* (57.3%), *S. aureus* (2.4%), and *Enterococcus* spp. (6.5%). Moreover, in the above-mentioned research, the gram-negative bacteria (22.1%) included *K. pneumoniae* (6.5%), *A. baumannii* (0.8%), *P. aeruginosa* (7.3%), and *E. coli* (7.3%).

According to the results of the present study, these types of bacteria are the most predominant in bloodstream infections in severely burned patients, and the situation related to medication resistance is severe. Those with severe burns (dead cases) had more complicated types of pathogenic pathogens, and their mortality was higher than those with less severe burns (healed cases).

After a serious burn injury, sepsis and severe infections are two significant consequences that are highly fatal. In order to start the proper treatments and reduce morbidity and mortality, it is crucial to identify infectious complications early. These patients have severely burned hyper-inflammatory due to a physiological response to the trauma. Established pro-inflammatory indicators, such as c-reactive protein or IL-6, may not be helpful as outcome biomarkers. Currently, no recognized biomarkers can identify or track the mediator of the inflammatory response.

The present study investigated the possibility that IL-8 could be used as a predictor and the results were in line with those of a previous study (16). Neutrophils are significantly activated by IL-8, general inflammatory activities, and cell proliferation and angiogenesis, which are tissue repair mechanisms. Findings of this study showed that the value of IL-8 in the dead cases was higher than that in healed cases which were, in turn, higher than that in the controls.

A study conducted in Iraq showed that the value of IL-8 was also elevated due to bacterial infection and the immunological response in patients (17). Another study also carried out in Iraq reached the same results, revealing the elevated value of IL-8 in patients with systemic complications due to sepsis (18). Moreover, in another study performed in the USA, an increase was found in the value of IL-8 in patients with sepsis and organ dysregulations (8). These results confirmed our hypothesis about the critical role of IL-8 in the immunological response in patients with sepsis.

Authors' Contribution

Study concept and design: H. M. F.
Acquisition of data: F. W. A.
Analysis and interpretation of data: F. W. A.
Drafting of the manuscript: F. W. A.
Critical revision of the manuscript for important intellectual content: H. M. F.
Statistical analysis: H. M. F.
Administrative, technical, and material support: H. M. F.

Ethics

This study approved by the ethics committee of the AL-Iraqia University, Baghdad, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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