Evaluation of IL8 in burn injuries and its correlation with bacterial infections and burn severity

Haider Naama Ouda Al-Jubouri, Israa Saeed Abbas, Ali Hmood Al-Saadi

1- College of Applied Medical Sciences, University of Karbala
2- College of Science, University of Babylon

E.mail: hayder_jubouri@yahoo.com

Abstracts

Burn injuries are a major public health concern in many nations across the world, since the risk of these injuries is determined by a variety of factors such as the length and depth of the burn, numerous host characteristics, and virulence factors for bacterial colonies associated with burns (1). Burn lesion is concern in high risk of nosocomial infections. Denatured and dead, moist tissue makes the burn wound sensitive to P. aeruginosa infection, breakdown of skin barriers, reduced immunity, and prolonged hospital stays significant factors leading to burn wound infected with these opportunistic pathogens. A total of 170 samples were obtained from burn victims and categorized into the following categories: All 140 samples from swabs and blood samples were taken from patients admitted to burn units and centers at the following hospitals: 100 swabs from the burn area and 40 blood samples from burn patients (Burns Unit at Imam Al-Sadiq Hospital (peace be upon him) / Babel and Burn Center at Al-Hussein Teaching Hospital / Karbala and Burn Center in Sadr Medical City / Najaf Al-Ashraf), in addition of ( 30 blood samples collected from healthy people from outside the hospital used as control for IL8 detection). Culturing swabs from burn sites 72 hours following patients' admission to hospital burn units to discover the most common microbes isolated from burn injuries, and then determining the IL8 association with bacterial infection and burn severity.

The most frequent bacterial Isolates obtained from burned patients was P. aeruginosa bacteria, followed by Klebsiella, E. coli, Proteus, Acinetobacter and
**Citrobacter** IL8 concentration in burn patients group were have significant (increase) in serum level of IL-8 between Patient and control at $P \leq 0.001$ and the highly level in sever burn injuries, additionally significant difference (increase) in IL8 level among burned patients whom have bacterial infection and burned patients with no growth of bacteria, $P \leq 0.05$.

**Keywords:** burn, burn injuries, bacterial infection in burn, IL8.

### 1. Introduction

Burn injuries are a major public health concern in many nations across the world, since the risk of these injuries is determined by a variety of factors such as the length and depth of the burn, numerous host characteristics, and virulence factors for bacterial colonies associated with burns (1). Burn lesion is concern in high risk of nosocomial infections. Denatured and dead, moist tissue makes the burn wound sensitive to *P. aeruginosa* infection, breakdown of skin barriers, reduced immunity, and prolonged hospital stays significant factors leading to burn wound infected with these opportunistic pathogens particularly the "*P. aeruginosa*" (MDR). The multi-drug-resistant *P. aeruginosa*" induces 4-60% nosocomial inflammation of different countries as the cause of death and morbidity in burning-unit patients (2).

*Pseudomonas* is a gram-negative, aerobic, rod-shaped bacterium with unipolar pinions (3). *Pseudomonas* forms a positive oxidase reaction with certain members capable of generating colors (4). *P. aeruginosa* is also a non-fermented gram-negative bacillus with a high inherent antibiotic resistance, together with its rapid ability to acquire new antimicrobial resistance, this pathogen is an increasing problem for the pathology of infectious diseases, particularly if the infection is nosocomial. No medical trials exist to investigate the potential survival factors of hospitalized patients with *P. aeruginosa* urinary tract infections (5).
**Pseudomonas aeruginosa** possess a highly capacity to form biofilms that are cell communities enclosed in an extracellular self-produced matrix protects cells from antibiotics and host immune responses, Biofilm can increase *P. aeruginosa* infection in comparison with planktonic bacterial cells and increase the degree of antibiotic resistance such as (β-lactam and carbapenem antibiotic group) and its capacity to develop more resistance to several groups of antibiotics including aminoglycosides and fluoroquinolones (6).

Although burn damage induces considerable activation of inflammation and cytokine release, the acute and subacute inflammatory response time resolution has yet to be fully defined. So CXCL8 (IL-8) is a substance secreted by macrophages at the start of the post-injury process. IL-8 is an essential inflammatory molecule that recruits neutrophils and other immune cells at the site of infection; IL-8 is also secreted by epithelial cells, smooth airway muscle cells, and endothelial cells, in addition to macrophages. This chemokine has been demonstrated to engage in a variety of cell processes, including cell proliferation, tissue remodeling, and angiogenesis (7).

Interleukin-8 is a chemokine that belongs to the Cysteine X Cysteine (CXC) family and has the amino acid sequence Glu-Leu-Arg (ELR) before the first conserved cysteine amino acid residue in its main structure. It operates as a chemical signal that draws neutrophils to the site of inflammation. IL-8 is implicated in the course of severe sepsis, and it has been observed that serum and plasma IL-8 levels are elevated in sepsis patients (8,9).

Furthermore, Interleukin-8 is a neutrophil chemoattractant cytokine that has been linked to numerous organ failure. IL-8, also known as CXCL-8, is a key chemokine for leukocyte recruitment that is produced by a variety of cells including macrophages/monocytes, endothelial cells, fibroblasts, epithelial cells, and neutrophils (10,38-53).
Interleukin-8 activates quickly, implying that early monitoring of this chemokine may offer early infection information. Based on the role of IL-8 as an inflammatory and multiproliferating mediator, we hypothesized that systemic levels of IL-8 may be utilized to predict infections and sepsis in severely burned patients (11).

Interleukin-8 is a key mediator of inflammation. When exposed to inflammatory agents such as tumor necrosis factor (TNF) or interferon (IFN), it is created in a range of organs. When it is released, it attracts lymphocytes and stimulates neutrophil granulocytes, which can then create IL-8, reinforcing the inflammatory signal. IL-8 degrades rather slowly compared to other cytokines and can survive in its active state for many days in the immediate vicinity of the cells from whence it was produced, making it simpler to assess in studies than other interleukins. When triggered by cytokines or other infectious stimuli, a range of cells inside the CNS, including astrocytes, endothelial cells, and microglial cells, can generate IL-8 (12).

Elevated sTNFR1 and IL8 plasma levels reliably identified sepsis patients at increased risk of death and may be used as predictive enrichment variables in future studies by boosting trial efficiency and power and lowering the number of survivors, and thus in summary, he discovered that IL-8 is a sensitive and specific biomarker for burn size, and that at greater levels, the degree of plasma IL-8 corresponds substantially with the occurrence of septic episodes. Another research concluded that IL-8 might be a valuable biomarker for predicting infections and septic episodes in burn patients (13).

2. Materials and Methods

2.1. Study design

170 samples were gathered from burn victims during a 5-month period, from December 2020 to April 2021, and were divided as follows: All 140 samples from
swabs and blood samples were collected from patients who were admitted to burn units and centers in the following hospitals (Burns Unit at Imam Al-Sadiq Hospital (peace be upon him) / Babel and Burn Center at Al-Hussein Teaching Hospital / Karbala and Burn Center in Sadr Medical City / Najaf Al-Ashraf), in addition of (30 blood samples collected from healthy people from outside the hospital used as control for IL8 detection).

2.2. Patients

Patients ages ranged from (1 year to 80 years), males were 46 patient (14 adult, 32 children) and 54 patient was females (40 adult ,14 children). they were suffered from second to third degree burns by means of (flame, hot fluids, gasoline, oil, electricity The burn rate ranged between (5-100%) of the total body surface (TBSA). All patients were diagnosed by a consultant physician specializing in the aforementioned hospitals.

2.3. Cultivation

A total of 100 swab samples were collected from burn patients after three and six days from the pus of the burnt region in the morning before washing the afflicted area (before hydrotherapy) using transport and collecting swabs (sterile swab stick, sterile transport medium swabs). Each swab was put in a sterile container until it arrived at the laboratory to be inoculated on culture media (Blood agar, MacConkey agar) and cultured aerobically for (24-48) hours at 37°C. (14).

*P. aeruginosa* identification was confirmed using VITEK2-Compact, an advanced colorimetric technology for bacterial identification, ID gram negative (GN) card was used for this purpose for P. aeruginosa identification, and gram-negative susceptibility card (AST-GN76) was used for susceptibility testing against specified antimicrobials (15).

2.4. Interleukin-8 (IL-8)
A 40 Blood samples were taken from burn patients: 5 ml after (3-6) days from burn injury in addition to 5 ml blood sample were taken from 30 healthy people as a control group used to determine the level of IL8 and placed in a sterile gel tube. The samples are centrifuged at 1500 cycles for 5 minutes and the serum is stored at -20 °C until use in analysis (16).

3. Results and discussion

All samples were taken from burned patients with age from 1-80 years for both sexes male and female (table 1).

Table (1). Distribution of burn patients according to the age.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>No. of patient (%)</th>
<th>χ²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10</td>
<td>42 (42%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-20</td>
<td>27 (27%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21-30</td>
<td>15 (15%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31-40</td>
<td>11 (11%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41-50</td>
<td>3 (3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51-60</td>
<td>1 (1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Above 61</td>
<td>1(1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (1) showed the distribution of burn patients according to the age the most prevalent age group (1-10 years), followed by (11-20 years), (21-30 years), (31-40 years), (41-50 years), (51-60 years) and the least prevalence age group is those over (61 years) it represented by the following percentages (42%, 27%, 15%, 11%, 3%, 1%, 1%) respectively of the collected burned patients included in this study. There were high significant differences (P ≤ 0.0001) this increase in child (1-10 years) linked to several reasons like lots of movement of children a lack of awareness and lack of knowledge of the nature of fire- causing materials and how to deal.

This result was in agreement with of study that conducted by (17), which reported that scalds were seen commonly in children, and with the study from
Palestine published by (18) which showed that burn injuries in children (72%) were much more than burn injuries in adult (28%). This might be because children have more movement within dwellings and have a lower feeling and knowledge of threats. Furthermore, this study was consistent with (19), which discovered that the highest rate of burn injuries was in the children's group in the age group between (0 - 9) years, at a rate of 58.54.2 percent, where deaths caused by burns occur at a rate ten times higher in developing countries than in the developed world.

The prevalence of bacterial growth in collected swab sample was 74% while growth negative was 26%, (figure 1).

![Figure 1](image)

**Figure (1). Distribution of growth positive bacteria in collected samples.**

Severe burns are one of the most dangerous types of traumas, resulting in the loss of the skin barrier and tissue degradation. Tissue damage at burn sites does, in fact, result in the generation of biological fluids known as burn wound exudates (BWEs) (20,21). The immunosuppressive condition and burn tissue microenvironment are favorable factors for burn wound pathogen colonization and proliferation, resulting in the spread and multiplication of various types of bacteria in the burn region depending on the degree of body burning (21).
Table (2) showed that the most frequent bacterial isolates were obtained from burned patients. The results showed the numbers and percentages that were obtained from clinical samples of burn patients of both sexes, males and females, which showed high levels of *P. aeruginosa* bacteria, followed by Klebsiella, E. coli, Proteus, Acinetobacter and Citrobacter they represent the following percentages 50 (67.6%), 9(12.2%), 8(10.8%), 3(4%), 2(2.7%) and 2(2.7%) respectively as shown in the figure (2) which shows the distribution of these percentages.

**Table (2). Gram negative bacterial isolates obtained from burned patients explain adult and children.**

<table>
<thead>
<tr>
<th>Isolated bacteria</th>
<th>Male Adult</th>
<th>Male Children</th>
<th>Female Adult</th>
<th>Female Children</th>
<th>Total Number</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>4(8%)</td>
<td>19(38%)</td>
<td>21(42%)</td>
<td>6(12%)</td>
<td>50(67.6%)</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>1(11.1%)</td>
<td>2(22.2%)</td>
<td>5(55.6%)</td>
<td>1(11.1%)</td>
<td>9(12.2%)</td>
</tr>
<tr>
<td>E. coli</td>
<td>2(25%)</td>
<td>2(25%)</td>
<td>2(25%)</td>
<td>2(25%)</td>
<td>8(10.8%)</td>
</tr>
<tr>
<td>Proteus</td>
<td>1(33.3%)</td>
<td>0</td>
<td>2(66.7%)</td>
<td>0</td>
<td>3(4%)</td>
</tr>
<tr>
<td>Acinetobacter</td>
<td>0</td>
<td>1(50%)</td>
<td>1(50%)</td>
<td>0</td>
<td>2(2.7%)</td>
</tr>
<tr>
<td>Citrobacter</td>
<td>1(50%)</td>
<td>0</td>
<td>1(50%)</td>
<td>0</td>
<td>2(2.7%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>33(45%)</td>
<td>41(55%)</td>
<td></td>
<td></td>
<td>74(100%)</td>
</tr>
</tbody>
</table>

\(\chi^2\) 22.561

p-value 0.026*

This result was in agreement agrees with the other result, obtained by (22), they found that the *P. aeruginosa* was the most common source of burn wound infection (23). The high frequency rate of *P. aeruginosa* observed in burn units may be attributed to the longer hospital stay and aggressive usage of antibiotics, according to the study. Pseudomonas aeruginosa [35.84 percent], Klebsiella species [27.30 percent], Acinetobacter species [20.13 percent], Escherichia coli [2.38 percent], and Staphylococcus aurous [8.87 percent] were the most common organisms isolated from burn wounds, according to the research (24) and (25). (26)
showed that the highest isolate rate was for *P. aeruginosa* bacteria with an isolate rate of 38 (32.47%), followed by *K. pneumonia* bacteria with an isolated percentage of 25(21.36) and the lowest percentage of infection with *S.epidermis* bacteria With an isolate rate of 2(1.7), while the percentages of the following bacterial species *A.baumenii, E.coli, E.cloacae, S.auraus, B.cepesa, P.miribilles, P.agglomer* were 15 (12.82%), 13(11.1%), 6(%5.12), 6(5.12%), 5(4.27%), 3(2.56%) respectively. These bacteria are considered opportunistic pathogens and rarely cause disease in healthy people, but they are highly virulence in patients with weak defensive mechanisms causes bacteremia, and therefore the contamination in hospitals with these pathogens have a pathological effect to deteriorate the condition of those sleeping there (27). (28) found that the isolates of *P. aeruginosa* bacteria were with an rate of 43 (41.3%).

![Figure (2). Distribution percentage of gram-negative bacterial isolates.](image)

The current study revealed that the concentration of IL8 in burn patients’ group were have significant (increase) in serum level of IL-8 between patient and control at P ≤ 0.001. There is significant (increase) in serum level of IL-8 between degree 2 burn patients and control and there is significant difference (increase) in
serum level of IL-8 between degree 3 burn patients and control, additionally the concentration of IL8 showed a highly elevation in degree 3 burn patients, (table 3).

Table (3). Mean of IL-8 concentration (pg/ml) of burned patient (degree2 and degree 3) and control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SD</th>
<th>Sig. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=28)</td>
<td>33.308±25.790</td>
<td></td>
</tr>
<tr>
<td>Degree 2 burn (n=18)</td>
<td>80.051±58.373</td>
<td>0.000276*</td>
</tr>
<tr>
<td>Degree 3 burn (n=18)</td>
<td>116.594±108.769</td>
<td>0.000159*</td>
</tr>
<tr>
<td>Patient (n=36)</td>
<td>98.322±88.004</td>
<td>0.000178*</td>
</tr>
</tbody>
</table>

*Sig P ≤ (0.05)

The current study accords with past studies that approved the difference in serum IL8 levels between the burnt and control groups; IL-8 expression has been proven to be higher in burn patients (29,30,31,32). More recent research found that IL-8 concentrations that reached or above a threshold value of 234 pg/mL in 468 pediatric burn patients were related with a greater incidence of MOF (multiple organ failure), sepsis, and death (11). In comparison to another study, the levels of IL-6, IL-8, IL10, TNF-, and G-CSF in non-burn controls were considerably greater in burn patients over the observed period following burn damage (P ≤ 0.05) (33).

In another research of 322 badly burnt children, a panel of biomarkers, including burn size, was discovered (34). One possible reason for these disparities is that, while an increase in cytokine concentration is associated with burn size generally, other variables such as fever, bacterial infection, and WBC may generate a fast rise in cytokine concentration (33).

Our study explained that there is significant difference (increase) in IL8 level among burned patients (degree 2 and degree 3) whom have bacterial infection and burned patients (degree 2 and degree 3) with no growth of bacteria, (P ≤ 0.05) (table 4).
Table (4). Correlation of IL8 with growth of bacteria and burn degree.

<table>
<thead>
<tr>
<th>Degree of burn</th>
<th>Growth</th>
<th>IL-8 concentration (pg/ml)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>No</td>
<td>89.06±4.69</td>
<td>0.023*</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>167.58±15.63</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>117.09±7.31</td>
<td>0.011*</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>255.70±14.62</td>
<td></td>
</tr>
</tbody>
</table>

*Sig P ≤ (0.05)

The present findings concurred with another study that found IL8 to be higher in bacterial infection (13). Elevated plasma levels of sTNFR1 and IL8 reliably identified sepsis patients at increased risk of death and may be used as prognostic enrichment variables in future studies by boosting trial efficiency and power and lowering the number of survivors, and hence to summarize his findings, he discovered that IL-8 is a sensitive and specific biomarker for burn size below a threshold of 234 pg/ml, and that at greater levels, the degree of plasma IL-8 corresponds substantially with the risk of septic episodes. Another research concluded that IL-8 might be a valuable biomarker for predicting infections and septic episodes in burn patients (13).

Other previous research built on that, ILs such as IL-6, which is, like IL-8, clinically employed as a biomarker during sepsis (35), and other prior studies underlined the importance of IL-8-induced alterations during systemic inflammation (35,36). ILs such as IL-6 and IL-8, on the other hand, are commonly employed as diagnostic and prognostic markers for infectious (e.g., septic) and other inflammatory (e.g., traumatic) disorders (11,37).
4. Conclusions

The current study concludes the following:

The prevalence of burns in the category of children and women is more than in men and less in the elderly. The most common causes of burns are liquid burns. The second-degree burns were prevalent at all ages, it was observed that *P. aeruginosa* was the dominant and most prevalent type in burn victims compared to other Gram-negative bacterial species and Pseudomonas chromogenic agar medium was a very good optional medium for the growth of this bacteria, and can be used instead of vitek system, and increased in the level of (IL8) among burn patients with bacterial infection compared to burn patients without bacterial infection.

References


www.turkjphysiotherrehabil.org


48. NGAFWAN, N., RASYID, H., ABOOD, E. S., ABDELBASSET, W. K., AL-
fluorescent carbon nanomaterials in food analysis. *Food Science and
Technology*. [https://doi.org/10.1590/fst.37821](https://doi.org/10.1590/fst.37821)

49. Marofi, F., Abdul-Rasheed, O. F., Rahman, H. S., Budi, H. S., Jalil, A. T.,
immunotherapy; A promising frontier. *Cancer Science, 112*(9), 3427.
[https://doi.org/10.1111/cas.14993](https://doi.org/10.1111/cas.14993)

Abdullah, M. M., ... & Almashhadani, H. A. (2021). Role of vitamin C in the
protection of the gum and implants in the human body: theoretical and
experimental studies. *International Journal of Corrosion and Scale
Inhibition, 10*(3), 1213-1229. [https://dx.doi.org/10.17675/2305-6894-2021-
10-3-22](https://dx.doi.org/10.17675/2305-6894-2021-10-3-22)

51. Jumintono, J., Alkubaisy, S., Yánez Silva, D., Singh, K., Turki Jalil, A., Mutia
Syarifah, S., ... & Derkho, M. (2021). Effect of Cystamine on Sperm and
Antioxidant Parameters of Ram Semen Stored at 4° C for 50 Hours. *Archives

52. Raya, I., Chupradit, S., Kadhim, M. M., Mahmoud, M. Z., Jalil, A. T.,
Surendar, A., ... & Bochvar, A. N. (2021). Role of Compositional Changes on
Thermal, Magnetic and Mechanical Properties of Fe-PC-Based Amorphous

53. Chupradit, S., Jalil, A. T., Enina, Y., Neganov, D. A., Alhassan, M. S.,
Nanoparticles on the Turbulator Installment in a Shell Tube Heat Exchanger:
A CFD-Based Simulation Approach by Using Nanofluids. *Journal of
Nanomaterials*. [https://doi.org/10.1155/2021/3250058](https://doi.org/10.1155/2021/3250058)