Microbial Index and TNF-α, IL-4, CCL17 Level Among Burn Wound Infections in Hilla City, Iraq

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ABSTRACT

Burn wound infections is one of the most common nosocomial infections and it is mainly due to bacterial infection. A total of 30 blood and burn swab samples were taken from patients with burn attending Al-Hilla, General Teaching Hospital, in Babylon, Iraq. Twenty blood samples were taken from healthy individual as a control. Conventional bacterial culture were performed to recover bacteria and measurement of TNF-α, IL-4 and CCL17 were performed by ELISA. The results revealed that Pseudomomas aeruginosa were the common agent and compile (76.66%) followed by Escherichia coli and Klebsiella pneumoniae (6.66%) for each and (3.33%) for each of Staphylococcus aureus, Streptococcus pyogenes and Acinetobacter baumannii. Concern the serum levels of TNF-α, IL-4 and CCL17, the results revealed significant decreases of TNF-α level (51.00±16.98 pg/ml) for burn patients in comparison with healthy control (150.70±34.60 pg/ml) p<0.005. For IL-4 and CCL17 the results displayed non-significant differences between burn patients and healthy control. This study conclude that the Pseudomonas aeruginosa still the main pathogen among burn wound infections and the levels of some cytokines depend upon time of collection and may revealed the worseness degree of burns.

Keywords: Pseudomonas aeruginosa, TNF-a, IL-4, CCL17, Burn, Infections.

INTRODUCTION

The skin is one of the principal organs in the body that achieves vital functions, including fluid homeostasis, thermoregulation, immunologic functions, neurosensory functions, and metabolic functions (eg, vitamin D synthesis). The most important role is the primary protection against infection by acting as a physical barrier. When this barrier is damaged, pathogens can directly infiltrate the body, resulting in infection (Church et al., 2006). In Contrast to other types of injury, burn wounds induce metabolic and inflammatory changes that predispose the patient to various complications. Infection is the most common cause of morbidity and mortality in this population, with almost 61% of deaths being caused by infection (Gomez et al., 2009).

Burn is one of the most common and devastating forms of trauma. It is an injury to the skin that damages or destroys skin cells and tissue. It is generally caused when skin makes contact with flames, chemical electricity, or radiation. Thermal burns are caused by intense external sources of heat, such as flames, scalding liquids, or steam. Burns resulting from an impaired driving crash are most likely thermal burns. The pathogens that infect the wound are primarily gram-positive bacteria such as methicillin-resistant Staphylococcus aureus (MRSA) and gram-negative...
bacteria such as Acinetobacter baumannii, Pseudomonas aeruginosa, and Klebsiella species (Keen et al., 2010).

The surface of the burn wound is free of microorganisms immediately following a burn injury. Thereafter a complex and changing microbial ecology rapidly develops. There is rapid colonization by predominantly Gram-positive bacteria, which are harbored in the deep unburnt cutaneous structures. Over the next 5-7 days, other microbes, including gram-negative and gram-positive bacteria, colonize the wound. These potential pathogens typically come from the patients’ gastrointestinal tract, upper respiratory tract, or the hospital environment, transferred through contact with healthcare workers (Armour et al., 2007).

The immunological changes among burnt patients includes increasing of T regulatory cells; decreasing in T helper cells, inflammatory cytokines, complement, chemotactic, phagocytic activity, and bactericidal activity of neutrophils (Church et al., 2006). Major burn injury induces an inflammatory response, which is accompanied by the release of various cytokines. During this response, pro-inflammatory cytokines such as interleukin (IL)-1α, IL-6, IL-8, tumor necrosis factor (TNF)-α, or interferon (IFN)-α, or anti-inflammatory cytokines such as IL-4, IL-10, and granulocyte-colony stimulating factor (G-CSF) are released. Inflammation is controlled by the balance between these proand anti-inflammatory mediators; simultaneous production of anti-inflammatory cytokines may counteract the effects of proinflammatory cytokines and modify the intensity of the inflammatory response (Finnerty et al., 2006).

This study was conducted to investigate bacterial profile and TNF-α, IL-4, CCL17 level among burn patients in Hilla city-Iraq.

**MATERIALS AND METHODS**

**Sample collection**

A total of 30 blood and burn swab samples were taken from patients with burn attending Al-Hilla, General Teaching Hospital, in Babylon, Iraq. Twenty blood samples were taken from healthy individual as a control. All blood samples were prepared to separate serum which preserved at -20 °C until using while the swabs were cultured on blood agar (for primary bacterial isolation) for 48 h at 37 °C. The culture plates were processed using standard microbiological procedures (Olayinka et al., 2009).

**Identification of Bacteria**

Characterization and identification of bacteria species were carried out by using a combination of colonial morphology, Gram stain characteristics, motility test, pigmentation, oxidation fermentation tests, catalase and oxidase activity tests, and pyocyanin production for primary identification and then confirmed by Viteck 2 compact system (Paranjothi and Dheepa, 2010).

**Cytokine Concentration Measurement**

Five ml of blood were collected from healthy donor and patients. The blood specimen was centrifuged at 3000 rpm for 5 min after allowing the blood to volt at room temperature. Serum separated and transferred in to test tube, and stored at -20 °C until being used. Serum level of cytokines TNF-α, IL-4 and CCL17 (Elabscience /China) were measured using Enzyme linked Immunosorbent assay (ELISA) kits according the instructions of manufacturer company Elabscience/China).

**RESULTS**

Concern bacterial isolates recovered from burn wound swabs, the results revealed that 23(76.66%) give positive results for only Pseudomonas aeruginosa, 2(6.66%) for each of Escherichia coli and klebsiella pneumoniae,

<table>
<thead>
<tr>
<th>Bacterial Isolates</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>23(76.66%)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>2(6.66%)</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>2(6.66%)</td>
</tr>
<tr>
<td>Staphylococcus anreus</td>
<td>1(3.33%)</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>1(3.33%)</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>1(3.33%)</td>
</tr>
</tbody>
</table>

Table 1: Distribution of bacterial isolates among burned patients
Concern the serum levels of TNF-α, IL-4 and CCL17 measured by ELISA, the results revealed significant decreases of TNF-α level (51.00±16.98 pg/ml) for burn patients in comparison with healthy control (150.70±34.60 pg/ml) p<0.005. For IL-4 and CCL17 the results displayed non-significant differences between burn patients and healthy control (Table 2).

Regarding the correlation among TNF-α, IL-4 and CCL17, the results showed no correlation among patient (Table 3) and among control (Table 4).

### DISCUSSION

Burn wounds are a suitable site for multiplication of bacteria and are more persistent richer sources of infection than surgical wounds, mainly because of the larger area involved and longer duration of patient stay in the hospital (Church et al., 2006). Initially the burn wound is sterile and the source of Gram positive bacteria like S. aureus and S. pyogenes may be hair follicles and sweat glands, which may survive thermal injury, colonize the wound within 48 hours of injury.

### Table 2: Serum level of IL-4, CCL17 and TNF-alpha

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean±SD of Concentration (pg/ml)</th>
<th>Patienit</th>
<th>Control</th>
<th>sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>485.01±270.61</td>
<td>480.49±210.89</td>
<td>0.385</td>
<td></td>
</tr>
<tr>
<td>CCL17</td>
<td>368.97±183.67</td>
<td>241.30±360.80</td>
<td>0.312</td>
<td></td>
</tr>
<tr>
<td>TNF-alpha</td>
<td>51.00±16.98</td>
<td>150.70±34.60</td>
<td>0.002*</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: Correlation among IL-4, CCL17 and TNF-alpha levels for patients.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>IL4</th>
<th>CCL17</th>
<th>TNF-alpha</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pearson Correlation</td>
<td>.995</td>
<td>.598</td>
</tr>
<tr>
<td>N</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>CCL17</td>
<td>Pearson Correlation</td>
<td>.995</td>
<td>.825</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.001-</td>
<td>1</td>
<td>.053</td>
</tr>
<tr>
<td>N</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>TNF</td>
<td>Pearson Correlation</td>
<td>.598</td>
<td>.825</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td>.126-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>N</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

Azzopardi et al.,(2014) stated that P. aeruginosa, K. pneumoniae, E. coli, Enterobacter spp. and Proteus spp. were identified as the commonest Gram-negative pathogens to be isolated from clinically infected burn wounds. Occurrence of Acinetobacter baumannii among burn patients were reported in many studies (Simor et al., 2002 ; Wong et al., 2002 ; Bayat et al., 2003 ; Trottier et al., 2007).
Microorganisms are still transmitted to the burn wound surfaces of recently admitted patients by the hands of personnel, by fomites, and to some extent by hydrotherapy. The common pathogens isolated from burn wounds are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and various coilform bacilli (Lari and Alaghehbandan, 2000). The results not report fungi or yeast in this study and this may be due to the effect of silver sulfadiazine which was used for the wound management as it is effective against both fungi and yeast (Jan et al., 2015).

Burn injury induces an inflammatory state characterized by the release of both proinflammatory and antimflammatory proteins. Proinflammatory cytokines induce not only alocal inflammation at the site of injury, but also systemic responses (Abston et al., 2000). Increased levels of proinflammatory cytokines are also associated with a higher incidence of postoperative infections (Church et al., 2006; Porth, 2007; Brunicardi et al., 2009). The plasma TNF-alpha levels at the time of admission were very low and did not correlate with the extent of the burn or the prognosis (Endo et al., 1993). The increased level of TNF-alpha have significant role in the pathophysiology of sepsis in burned patients (Yeh et al., 1999). The serum level of IL-4 in both burned and healthy control were same and no significant differences (Struzyna et al., 1995).

This study conclude that the *Pseudomonas aeruginosa* still the main pathogen among burn wound infections and the levels of some cytokines depend upon time of collection and may revealed the worseness degree of burns.

### REFERENCES


