Impact of Bacterial Infection and Cytokines Imbalance on Pathophysiology Burns Infections

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Abstract
Burns create traumatic injuries that are unpredictable and deadly which are linked to high morbidity and mortality among affected patients. So, this study aimed to measure dynamic shifts in the levels of IL-23 and IL-17 and association to a bacterial infection in the pathophysiology of burn infection. A case-control study for 60 patients suffering from burn during March-July 2021 entered the specialized burn center in middle Euphrates hospital in the Iraqi province of Al Najaf. Swabs from central burn cultured on Chromogenic medium and cetramid for 24h at 37 ℃. Two ml blood sample were taken from all patients and from 30 apparently healthy subjects as control group to evaluate IL-17 and IL-23 levels. Our results found that 28 (70%) of patients with injury > 50%, 20 isolates related to P. aerogenosa, 12 isolates for S. aureus as most common bacterial agents. Immunological study explains that IL-17 and IL-23 concentration in burn P. aeroginosae infected patients were (129.22±32.47, 114.09±11.56 pg/ml, respectively), in burn sterile patients (98.13±13.71, 192.64±28.70 pg/ml, respectively) in comparison to healthy group (18.32±4.18, 16.50±2.34 pg/ml) with significantly differences at p≤ 0.0. In conclusion: burn infection with antibiotic-resistant bacteria and persistent elevation a pro-inflammatory cytokines lead to the development of pathophysiological burn and suggest that using anti-IL-23 or IL-17 may help improve burn treatment, prevent complications, and reduce the length of hospital stay.
KEY WORD: IL-23, IL-17, *Pseudomonas aeruginosa*, Burn infections.

1. Introduction

Burn injuries cause a distinct and dynamic stress response that may contribute to progressive and mortality depended on degree of burn and respective causes that include thermal, radiation, chemicals, electricity which cause destroys the physical skin barrier then lead to increased susceptibility to infection and subsequent increased mortality (Laggner *et al.*,2022).

According to estimates, between 7 and 12 million people (up to 33,000 every day) suffer burn injuries every year that necessitate medical attention, cause extended absences from work or school, or even result in death (American Burn Association, 2019). Sepsis and the accompanying invasive infection, which are brought on by pathogens penetrating the injured skin that is devoid of its protective function, are the leading causes of death after the first 24 hours (Boldeanu *et al.*, 2020).

Burn infection can cause a skewed inflammatory response state that can last and eventually cause host tissue damage and organ malfunction. Early immune responses are controlled by a number of variables, including the size of the infected body surface area, the depth and kind of burns, inhalation injuries, patient age, and chronic medical conditions (Markiewicz-Gospodarek *et al.*, 2022).

Burn and cell damage release intracellular molecules (DAMPs) that recognized by host body proteins called PRRs which activate the innate immune cells to produce pro-inflammatory cytokine to activate microbicidal functions of immune cells that control or eliminate infections after primary injury. Production of cytokines during severe burns can be excessive, which can cause an overabundance of inflammation. burns and cells destroy release intracellular molecules (DAMPs) that are identified by proteins in the host body called PRRs, activating innate immune cells to create pro-inflammatory cytokines, which activate the microbicidal capabilities of immune cells to control or eradicate infections after primary injury. With severe burns, cytokine production may be overly high, leading to an excess of inflammation.

Pseudomonas aeruginosa is a motile aerobic Gram-negative bacilli, with great diversity and adaptability in a wide range of environments, including non-clinical and clinical settings (Azam and Khan, 2019). As an opportunistic pathogen, it belongs to the multi-drug resistant (MDR) ESKAPE pathogens. In 2017, MDR *P. aeruginosa* caused 32,600 infections among hospitalized patients and 2700 estimated deaths in the United States (CDC, 2020).

2. Patients and Clinical Specimens

During the study period from May-December 2022, a case–control study conducted on 60 specimens were collected from burns patients to each sex: male (34 swabs) and female (31 swabs) with age group from 15-70 years from specialized burn center in middle Euphrates hospital in AL Najaf /Iraq. Thirty healthy subjects were enrolled in this study as control group with age and sex compatible with patients age range (18 -68 years and male to female 18:12). Swab and two ml of blood sample were taken from all patients and only blood specimens from 30 apparently healthy subjects as

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control group to evaluate IL-17 and IL-23 levels by ELISA system.

Bacterial Isolates

The collected swab specimens were inoculated on three types of culture media which included blood agar, Mannitol salt agar and MacConkey agar, incubated at 37˚C for 24 h. Following, single pure isolated from all positive bacterial growth were subculture on brain heart infusion agar for maintenance and morphological identification by Gram staining, biochemical assays (Collee *et al.*, 1996) and Vitek -2 compact system that confirmed the identification of isolates (Nakasone *et al.*, 2007). Two milliliter of blood were collected from each subjects placed in gel tubes to separate sera to measure IL-17 and IL-23 concentration.

Included and Excluded criteria

Patients who suffering from any type of burn were included. While Patients who were receiving antibiotic therapy at the time the sample was collected, patients who did not provide all required information, and patients who provided incorrect samples were all eliminated from the study.

Antimicrobial Susceptibility Tests

Antimicrobial susceptibility test was performed on the isolates by disk diffusion method based on the Clinical and Laboratory Standards Institute (CLSI) guideline. Briefly, the turbidity of a suspension of each *P. aeruginosa* isolates were adjusted to that of 0.5 McFarland standard and were inoculated on Muller-Hinton agar plate for 24 hours at 37 °C. The tested antibiotic disks (MastGroup Ltd., UK.) for *P. aeruginosa* isolates were Piperacillin (PRL, 100 µg), Erythromycin (E, 60 µg), Levofloxacin (LEV, 5 µg), Chloramphinecol (C, 30 µg), Imipenem (IPM, 10 µg), Meropenem (MEM, 10 µg), Gentamicin (GN, 30 µg), Tetracyclin (TE, 30 µg), Ceftazidim (CAZ, 30 µg), ciprofloxacin (CIP, 5 µg), Rifampin (RA, 5 µg), Doxycyclin (DO, 30 µg), Trimethoprim-sulfamethoxazole (SXT, 25 µg), and Fusidic acid (FC, 10 µg). *P. aeruginosa* ATCC 27853 was used as the reference strain for antibiotic susceptibility testing (CLSI, 2020).

Ethical Considerations

The Declaration of Helsinki, the World Medical Association's code of ethics for research involving human subjects, was followed in this study. The Institutional Ethics Committee, which was approved by the Ministry of Health in Iraq, also obtained consent from all subjects before collecting data.

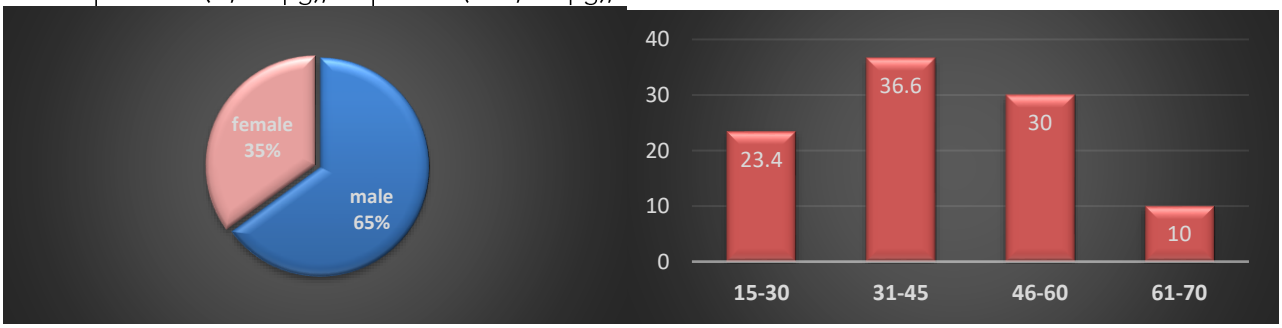
Statistical analysis

The result of IL-17,IL-23 mathematical mean ±SE was used to represent concentrations. T-test analysis was used to compare patients and controls.

3. Results and Discussion

Demographical Distribution of Burn Patients

According to a patients-control research of burn center patients in the province of Al-Najaf, there were 39 (65%) male patients and 21 (35%) female patients. A statistical analysis found substantial disparities between the sexes, as shown in Figure (1-A). When the patients were divided into age groups, it was discovered that the age group 15 to 30 had the highest frequency of patients (36.6%), followed by the age groups 31 to 45 (30%), 46 to 60 (23.4%), and 61 to 70 (10%), as shown in Figure (1-B).



A B

Figure (1): Distribution of burn patients according to A- sex B- age

In local study, Al-Hasnawi and Motaweq, (2021) founded that the percent of burn males 57% in Al-Najaf province. Also, Hassan and Darweesh, (2021) revealed that male were outnumber 53.7% and the age between 16-30 were high frequency 36.6% followed by 30% for patients with age between 31-45 also, observed that 63.3% of patients with a 50% burn injury.

This result in line with Chaganti *et al.*, (2019) founded that he highest rates of burn occur between 16–35 years of age. Ahmadi, (2007) confirmed that age rang for burn patients were 14-50 with median age 28.6 years. Aldemir *et al.*, (2005) reported that in adult more burns are caused by the fire, during working (the kitchen, the backyard and the cooking were identified as the places where the majority of accidents) or the suicides. Also Thombs and Bresnick, (2008) illustrated that people under the age of 25 have burns the most frequently.

Distribution the Patients According Body surface area (BSA) of Burn

The patients classified according to size of burn 38 (63%) with injury greater than 50% BSA and 22 (37%) BSA the injury fewer than 50% as show in figure (2).

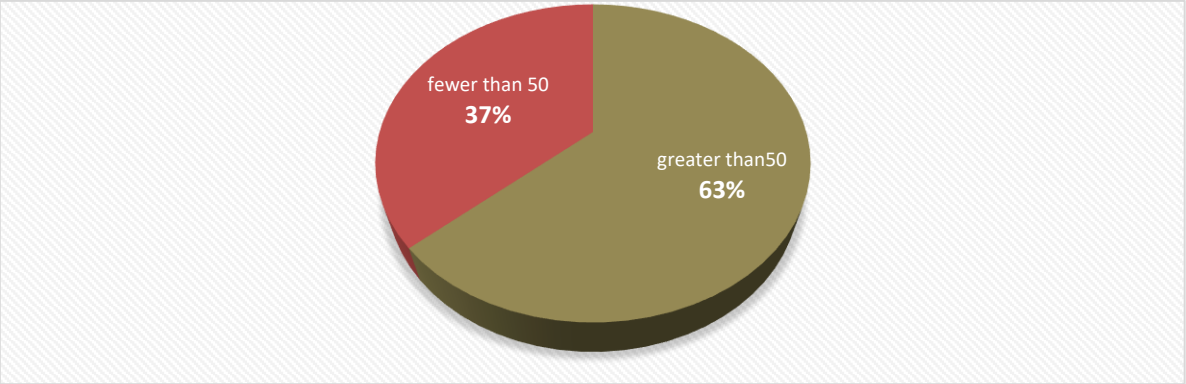


Figure (2) Distribution of Burn Patients by Size of Body Surface Injury

The results appear that the surface area and depth of burn wounds, host immunity, and the kinds and quantity of microbial flora colonizing the burn wounds all affect the risk of invasive burn wound infection. and indicated that an occurrence of septic is much influenced by burn size . This agrees with Li *et al.*, (2020) found that burn person with burn size greater than 40% BSA have more chance to dead related to septic and the incidence of sepsis in burn reach to 30% for burns with more than 20% TBSA. Besides, Aldemir *et al.*, (2005) in Turkey observed a strong association between the burn extended with death and confirmed that dead frequency elevated as the burn extended increased. The size of burn was an important predictor of patient survival, destruction large area of skin lead to dysfunction of skin ultimately colonization of microbes to burn wound increases then invasion into underlying tissue results in multiple organ failure and death (Wood *et al.*, 2016).

Isolation of Pathogenic Bacteria

The results showed that 60 burn swab from which 10 swabs appears No bacterial growth while 50 swabs illustrated that 12 (24%) isolates belong to *S. aureus,* 6(12%) isolates has been belong to coagulase negative staphylococci and 23 (46%) of isolates were *P. aerogenosa* then *E. coli* and *E. coloca* record 5(10%) and 4(8%) respectively.

Latifi and Karimi, (2017) found that *Staphylococcus* spp. (55.1%) are the most abundant bacteria found in burn wound cultures, followed by *P. aeruginosa* (14.29%), *Enterococcus* sp. (12.24%), *Escherichia coli* (4%), *Klebsiella* sp. and *Proteus* sp. (both 2%). Norbury *et al.*, (2016) founded that *S. aureus* major cause of early burn wound infection that plays an important role in invasive burn wound infection and sepsis. In burn centers, the introduction and spread of methicillin-resistant *S. aureus* (MRSA) leads to poor outcomes as prolonged hospitalization, bacteremia or sepsis, and even mortality (Issler-Fisher *et al.*, 2015).

P. aeruginosa is one of the most important pathogens causing different infections such as bacteremia and burn infections (Maraolo *et al.*, 2018). This pathogen has adapted successfully to hospital surroundings due to the production of biofilms, which provide the pathogen with long-term survival advantages and efficiently prevent eradication by the host immune system or antimicrobial drug treatment (Groenewold *et al.*, 2018). *P. aeruginosa* has become responsible for more than 70% of mortality in burn patients (Roham *et al.*, 2017). The levels of IL-17 and IL-6 proportional to the size of the burn, and persistently high levels of IL-6 post burn injury may be indicative of both the severity of the burn and likelihood of mortality.

Similar to *S. aureus*, *P. aeruginosa* also has quite a lot of virulence factors, including adhesins, lipopolysaccharides, elastases, exoenzyme S, exotoxin A, leukocidins and proteases. These make *P. aeruginosa* a major cause of bloodstream invasion, sepsis and poor prognosis in severely burned patients (Chevalier *et al.*, 2017).

Antibiotic Susceptibility

This test was conducted to all isolates by Kirby-Bauer disk diffusion method was used to study routinely used antibacterial drugs. All *P. aeurogenosa* isolates were tested with 14 antibiotics disc which included; carbapenem class (imipenem and meropenem); cephalosporins (ceftazidime); aminoglycoside (gentamycin); floroquniolone (ciproflaxcin, levofloxacin); tetracyclins (tetracyclin, doxycycline); ansamycin (rifampin); phenicols (chloramphenicol, pipracillin); folate pathway antagonists (trimethoprim sulfamethoxazole); macrolides (fusidic acid, erythromycin). The results were interpreted based on the inhibition zone diameter and compared to standard inhibition zones determined by CLSI, (2020).

The outcomes of this experiment revealed that *P. aeurogenosa* have great resistance to most commonly antibiotics used in hospitals, *P. aeurogenosa* showed susceptibility to the antibiotics utilized in this study differed. Fusidic acid, ceftazidim and rifampin exhibits the highest rate of resistance in *P. aeurginosa* 100%, Ciproflaxacin and Levofloxacin 90%, chloramphenicol 85%, trimethoprime sulfamethoxazole 80%, erythromycin 80%, while gentamycin, tetracyclin, doxycycline and pipracillin with presentage 65%. This study show increase rate of resistance for meropenem proximally 60%, and imipenem 20% as show in figure (3)

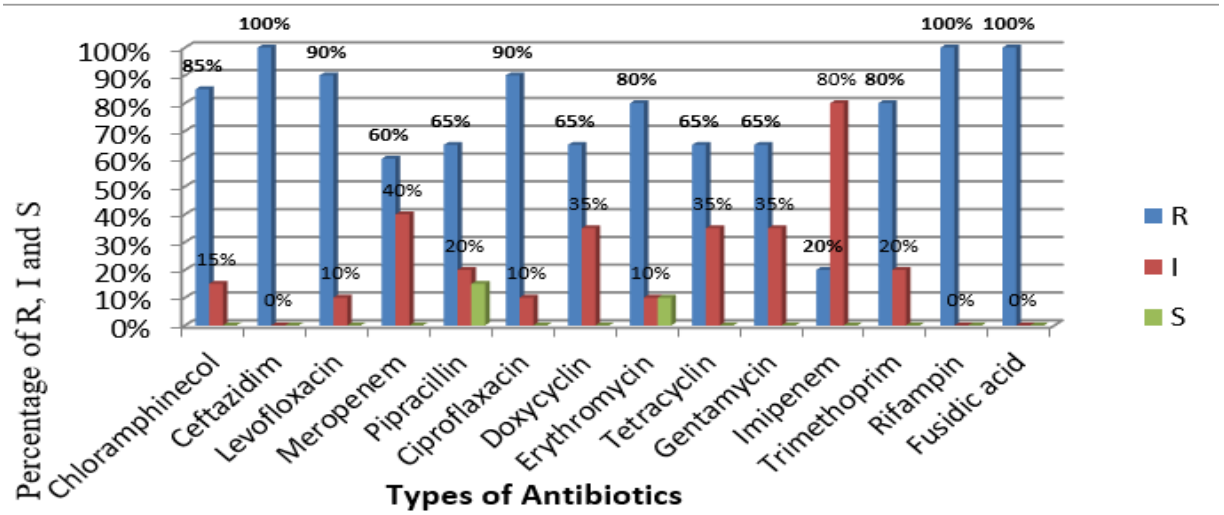


Figure (3): Antimicrobials Profile of P. aeurginosa Isolates from Burns Infections

P. aeruginosa showed resistance to antibiotics under study (fusidic acid, chloramphenicol, ceftazidime, meropenem, imipenem, piperacllin, gentamycin, ciprofloxacine, tetracycllin, doxycycllin, rifampin, trimethoprim sulfamethoxazole, erythromycin and levofloxacine) approximately with the same percentages which may be due to that bacterial isolates utilized similar mechanisms to resist these antibiotics, where *P. aeruginosa* has intrinsic resistance and is one of the most antimicrobial-resistant organisms encountered. Therefore, it needs to be correctly identified of *P. aeruginosa* antimicrobial drugs such as aminoglycosides, first and second generation cephalosporins, antipseudomonal penicillins, and polymyxins have inherent differences. As a result, the correct identification of *P. aeruginosa* is extremely important (Omar *et al.*, 2015). A study done *P. aeruginosa* isolates were found to be resistant to imipenem (13.4%) by Bashir *et al.*, (2011).

P. aeruginosa strains showed 100% resistance to Ampicillin and Norfloxacin, 83.3% resistance to piperacillin, ticarcillin, and tetracycline, 66.6% resistance to ceftazidime, imipenem, gentamicin, amikacin, tobramycin, and cotrimoxazole, and 50% resistance to cefoperazone, Cefotaxime was effective against 83.3% of *P. aeruginosa* strains, a study done according to Sivanmaliappan and Sevanan, (2011).

In addition, extended-spectrum β-lactamase (ESBL)-producing bacteria are considered as a potent pathogens due to it their resistance to a wide range of antimicrobials like, cefotaxime, ceftriaxone and ceftazidime, that lead to difficulty in the treatment of most infections such as burn infection and urinary tract infection (Bennett *et al.*, 2010 ; Walker *et al.*, 2018).

Burn infection is characterized by difficult healing due to administration of unsuitable treatment, long stays in hospital and the contaminates of hospital environments lead to the emergence of new multi-drug resistant bacterial isolates causing dangerous complications such as, bacteremia, septicemia and death (Glik *et al.*, 2012; Lachiewicz *et al.*, 2017).

Determination Level of IL-17 and 23 in Burn Patients and Healthy Controls

The results appear that the IL-17 and IL-23 concentration in burn *P. aeroginosae* infected patients were (129.22±32.47, 114.09±11.56 pg/ml, respectively), in burn sterile patients (98.13± 13.71, 192.64± 28.70 pg/ml, respectively) in compare to healthy group (18.32 ± 4.18, 16.50 ± 2.34pg/ml) with significantly differences at p≤ 0.05 as show in figure (4).

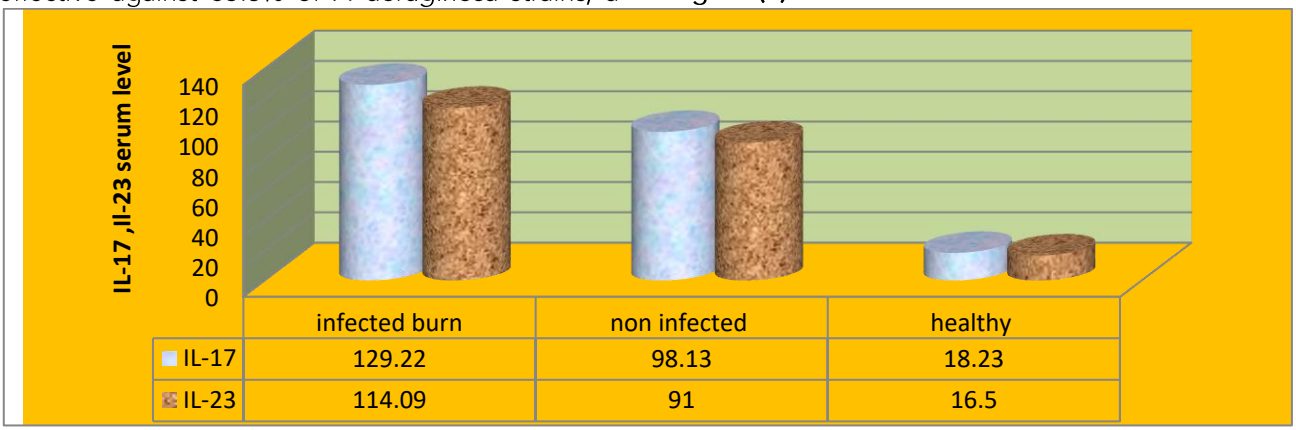


Figure (4): Determination Level of IL-17 and IL-23 in Burn and Healthy subjects

The overall burn surface area appears to be correlated with the severity of the inflammatory reaction. Teisserenc *et al.*, (2021) observed high serum levels of IL-17 in burn compare to healthy

and observed that septic shock developed bacterial infected burn. Finnerty *et al.*, (2007) reported IL-17, IL-6, and IL-8 concentration greater in septic burn patients and appear that sepsis-related deaths from burn injuries were found to have dramatically different inflammatory profiles from their non-septic counterparts. Singer *et al.*, (2016) observed that persistence elevated inflammatory processes contributors to organ damage and malfunction in critically-ill patients, especially in sepsis patients.

IL-17A production is increased after a skin injury, and the administration of IL-17A neutralizing antibodies on wound beds significantly improved wound healing (Li *et al.*, 2018). Li *et al.*, (2022) observed that IL-23 induction of IL-22 and IL-17 production in neutrophils play a critical role in protection against bacterial infection following burn injury. Kim *et al.*, (2017) found that injury burn patients revealed elevations in levels of IL-17 contribute to promoting sepsis. In another hand, Flierl *et al.* (2007) explain that neutralization of elevated IL-17 burn sepsis murine model correlated with decreased plasma levels of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6, decreased bacteremia, and increased survival. A previous study found significant burn injury triggers inflammatory immunological reactions in the peripheral blood and affected tissues with produces the acute-phase protein, especially in infection burn (Nielson *et al.*, 2017).

The immune response can persist for up to months and can lead to additional health problems, including systemic inflammatory response syndrome (SIRS), hypermetabolic state, and damage to surrounding tissues and even distant organs (Bergquist *et al.*, 2019). The immune system may still be in a pro-inflammatory state rather than moving to a state of resolution based on the chronically elevated levels of pro-inflammatory cytokines (IL-17, IL-23). According to this study, anti-IL-23 may be used to lower pro-inflammatory levels and resolve inflammation at an early stage in order to enhance burn care, avoid complications, and shorten hospital stays.

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