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RESEARCH ARTICLE

Microbial drug resistance and potential immune response by IL7 and IL10 in burn patients with septic and death cases

Ahmed Abdul-Ameer Mohammed, Mahdi Hussain Muheel and Fatima Abdul-Hussain Mujbel*
Department of Biology, College of Science, University of Kufa, AN Najaf-54001, Iraq

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Corresponding Author

Fatima Abdul-Hussain Mujbel

Abstract

Burn injury is one of the critical health problem caused by direct or indirect action of heat. Subsequently, burn injury provides novel sites for bacterial colonization, infection and clinical sepsis. In burn patients serum cytokine levels alter in first 7 days after admission which can be used as predictive markers for identifying patients have high risk of sepsis as well as mortality. A total of 45 blood specimens were collected from patients with burn injury for blood culture. The bacterial pathogen were isolated from blood and tested for antibiotic sensitivity. Also, the sera were used for ELISA to determine the level of cytokines. This study showed that septic and mortality increases with the increase of burn size. It is observed that ciprofloxacin, norfloxacin and amikacin are the most influential on Gram negative bacterial isolates and chloramphenicol, vangomycin and nitrofurantion on Gram positive bacterial isolates. In burn patients, increase in the concentration of IL-7 and IL-10 was observed. Gram negative bacteria found to be more predominant as causative agent for bloodstream infections than the gram positive bacteria in burn injury patient. P. aeruginosa is the most frequent species among bacterial isolates. The presence of endotoxin in septic burn patients might induce IL-7 and IL-10 cytokines production. This study conducted to investigate the role of bacterial infection and their Antibiograms as well as immunological aspects among burn patients

INTRUDUCTION:

Burn injury is one of the critical health problem caused by direct or indirect action of heat. Burn injury destroys the physical skin barrier which is considered as the most important tool against invasion of microorganisms. Subsequently, this injury provides novel sites for bacterial colonization, infection and clinical sepsis (Vale et al, 2005). The microorganisms can originate from the patient's own skin (hair follicles and sweat glands), gut and respiratory flora (endogenous), as well as from contact with health care personnel and environment (exogenous) (Church et al, 2006). Invasion of microorganisms into the tissue layers below the dermis lead to bacteremia, sepsis and multiple organ dysfunctions. Bacteremia and infection can release toxins into the blood that leads to sepsis and cause systemic inflammatory response (Forner et al, 2006). Bloodstream infection and the subsequent development of sepsis are among the most common infection complications occurring in burn patients in the intensive care unit (Santucci et al, 2003). A burn patient is extremely susceptible to hospital infections that contribute to excess morbidity and mortality (Murray et al, 2003).

In burn patients early alterations in serum levels of IL-7 within the first 7 days after admission may constitute useful predictive markers for identifying patients those who have high mortality. Patients with decreased IL-7 serum levels had a significantly greater risk for mortality, and in contrast the survival patients had high levels of IL-7. This cytokine plays an important role in supporting cell survival; it drives the survival and proliferation of human T-cells after lymphodepletion (Gauglitz et al, 2008). IL-7 produced in response to lymphopenia stimulates proliferation of both native and memory human T-cells but also has a direct stimulating effect on thymic activity. These make IL-7 a master modulator of T-cell-mediated immune responses, in addition to its role as master regulator of peripheral T-cell homeostasis (Ponchel et al, 2005).The elevation of anti-inflammatory cytokines such as IL-10 alters immune

function, which can lead to decreased resistance to infection in severely burned patients. IL-10 levels usually higher in septic and non-survivor than in non-septic and survivor burn patients (Pileri et al, 2008). IL-10 can inhibit protective immune response to infections. Prolonged IL-10 expression increases the risk for infectious complications. The main target of IL-10 is macrophages, and these cells play a central role in infections, as a target for pathogens and in the activation of both specific and innate immune response. In the human system, IL-10 seems to inhibit both the Th1-type and the Th2-type responses, although the effect on Th1 cells appears to be stronger (Asadullah et al, 1998; Moore et al, 2001).

MATERIALS & METHODS:

Patients:

A total of forty five (45) burned patients whose ages range between (1-75) years and with burn size (10-90%) were included in this study which lasted from November (2012) to May (2013). Those patients were admitted to the burn unit at Al Sadder Teaching Hospital in AN Najaf and Al-Hassian General Hospital in Karballa. Their burn percentage was ranging from 10-90% from the total body surface area (TBSA) and twelve apparently healthy subjects as controls group. Their ages range between (3-57) years.

Blood Collection:

Blood were taken from patients, (1-4) ml from infants and children and (8-10) ml from adult after (5-10) days of burn injury for blood culture. The blood samples were incubated aerobically at 37 ^{0}C in BacT/ALERT 3D for 2-7 days. Then cultured on different types of culture media (Blood agar, MacConkey agar and Mantol salt agar) and incubated aerobically for 24- 48 hours at 37 ^{0}C. Also, two ml of blood were taken from patients after (4-7) day of burn injury use for determine level of cytokines, put in sterilized plane tube to clot at 37^{0}C for (30-45) minutes, and then sera were separated by centrifugation for 15 minutes at 3000 rpm. The sera were used for ELISA to determine the level of cytokines.

Bacterial Diagnosis:

Isolation and identification of bacteria were carried out by morphological and cultural characteristics, biochemical tests and identification by Vitec 2 Compact (Baron et al, 1995; Collee et al, 1996; MacFaddin, 2000) .The identification by Vitec 2 Compact is performed according to the manufacturer's instructions.

Antibiotic Sensitivity Test:

The antimicrobial susceptibility test was carried out according to Kirby-Bauer method. Zone size was compared to standard zones depending on clinical and laboratory standard institute (CLSI), 2012.

Cytokine Measurements:

The assay was performed in accordance with the manufacturer's instructions by Enzyme-linked Immunosorbant Assay (ELISA). Briefly, serum samples were thawed, centrifuged at 3000 rpm for 5 minutes, and incubated with micro beads for 2 hours. After a wash step, the beads were incubated with the detection antibody for 2 hours. After an additional wash step, the beads were incubated with Streptavidin-HRP conjugate for 30 minutes. After wash the beads were incubated with substrate for 11minutes. After adding stop solution the samples were placed in the array reader at absorbance was measured at 450 nm for determination of the respective cytokine concentration.

Statistical Analysis:

Data were analyzed using the software packages Graphpad prism for Windows (5.04, Graphpad software Inc. USA). Data are presented as the mean ± standard error (SE). The comparison between the patients and healthy groups were analyzed by one-way T-test [p-value 0.01 was consider significant].

RESULTS:

The results showed that the number of septic cases and death increase with increasing percentage of burn. The number of septic cases and patient death is increased when burn percentage is more than 30% of (TSBA), and this percentage reach to maximum rate when burn percentage is more than 60%. This indicates that an occurrence of septic and death are very influenced with burn percentage as shown in (Table I).

Burn Percentage (%)	No. of Cases and (death)	No. of non-Septic and (death)	No. of Septic and (death)
11 –20	10 (0)	9 (0)	1 (0)
21 –30	8 (1)	6 (0)	2 (1)
31 – 40	8 (1)	6 (1)	2 (0)
41 – 50	5 (2)	3 (1)	2 (1)
51 – 60	3 (1)	1 (0)	2 (1)
61 –70	1 (1)	0 (0)	1 (1)
71 – 80	0 (0)	0 (0)	0 (0)
81 – 90	5 (5)	0 (0)	5 (5
Total	15 (9)	30 (2)	45 (11)

Table I: Correlation between Burn Percentage and Number of Septic Cases and Death

The results revealed that 15 samples (33.33%) gave positive bacterial culture whereas 30 samples (66.66%) showed no bacterial growth (negative bacterial culture). It was found that P. aeruginosa occupied large part of bacterial isolates as shown in (Table II).

Bacterial Isolates	Number (%)
Pseudomonas aeruginosa	9 (31.03 %)
Klebsiella pneumoniae	7 (24.13 %)
Staphylococcus aureus	5 (17.24 %)
Staphylococcus epidermidis	4 (13.79 %)
Enterobacter cloacae	3 (10.34 %)
Acinetobacter baumannii	1 (3.44%)
Total	29 (100%)

Table II: Number and percentage of bacterial isolates

Gram negative bacteria are more frequent (68.96%) than Gram positive (31.04%). The antibiotic sensitivity of gram negative and gram positive bacteria was summarized in table III and IV which showed varying resistance antibiotics.

Type of bacteria	Type of antibiotics													
	AK	AMP	CAZ	CN	CIP	CTX	F	FEP	IPM	MEM	NOR	PRL	SXT	TOB
Pseudomonas aeruginosa (9)	6 (66.6)	9 (100)	9 (100)	9 (100)	7 (77.7)	9 (100)	9 (100)	9 (100)	7 (77.7)	7 (77.7)	7 (77.7)	9 (100)	9 (100)	8 (88.8)
Klebsiella pneumonia (7)	2 (28.5)	7 (100)	7 (100)	6 (85.7)	1 (14.2)	7 (100)	5 (17.4)	7 (100)	5 (71.4)	6 (85.7)	3 (42.8)	7 (100)	7 (100)	7 (100)
Enterobacter cloacae.(3)	3 (100)	3 (100)	3 (100)	3 (100)	0 (0)	3 (100)	3 (100)	3 (100)	3 (100)	3 (100)	0 (0)	3 (100)	3 (100)	3 (100)
Acinetobacter baumannii (1)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Total	12 (60)	20 (100)	20 (100)	19 (95)	9 (45)	20 (100)	18 (90)	20 (100)	16 (80)	17 (85)	11 (55)	20 (100)	20 (100)	19 (95)

Table III: Antibiotic Resistance of Gram Negative Bacterial Isolates [Number (%)]

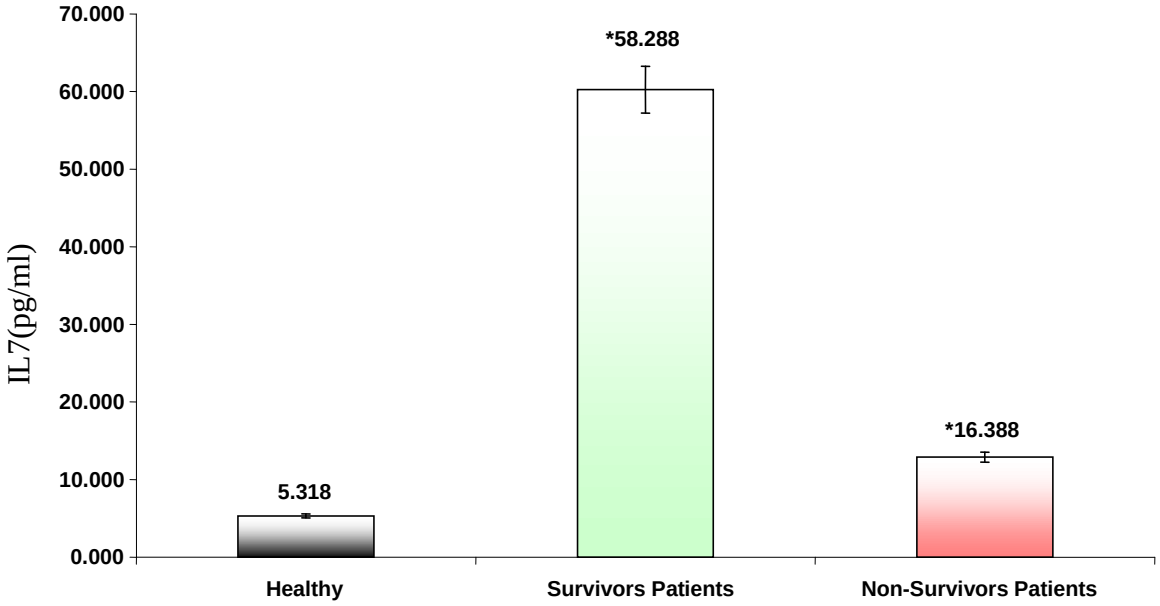
AK- Amikacin AMP-Ampicillin CAZ-Cefmetazole CN- Gentamicin CIP- Ciprofloxacin CTX-Cefotaxim F –Nitrofurantion FEP-Cefepime IPM-Imipenem MEM-Meropenem NOR-Norfloxacin PRL-Piperacillin SXT- Trimethoprim-Sulfamethoxazole TOB-Tobramycin

Type of bacteria	Type of antibiotics													
	AK	AMP	CAZ	C	CN	CIP	CTX	CX	F	FOX	NOR	SXT	TOB	VA
S. aureus (5)	5 (100)	5 (100)	5 (100)	0 (0)	5 (100)	5 (100)	5 (100)	5 (100)	0 (0)	5 (100)	5 (100)	5 (100)	5 (100)	0 (0)
S. epidermidis (4)	0 (0)	3 (75)	4 (100)	0 (0)	0 (0)	0 (0)	4 (100)	4 (100)	0 (0)	1 (25)	0 (0)	1 (25)	0 (0)	0 (0)
Total (9)	5 (55.5)	8 (88.8)	9 (100)	0 (0)	5 (55.5)	5 (55.5)	9 (100)	9 (100)	0 (0)	6 (66.6)	5 (55.5)	6 (66.6)	5 (55.5)	0 (0)

Table IV: Antibiotic Resistance of Gram Positive Bacterial Isolates [Number (%)]

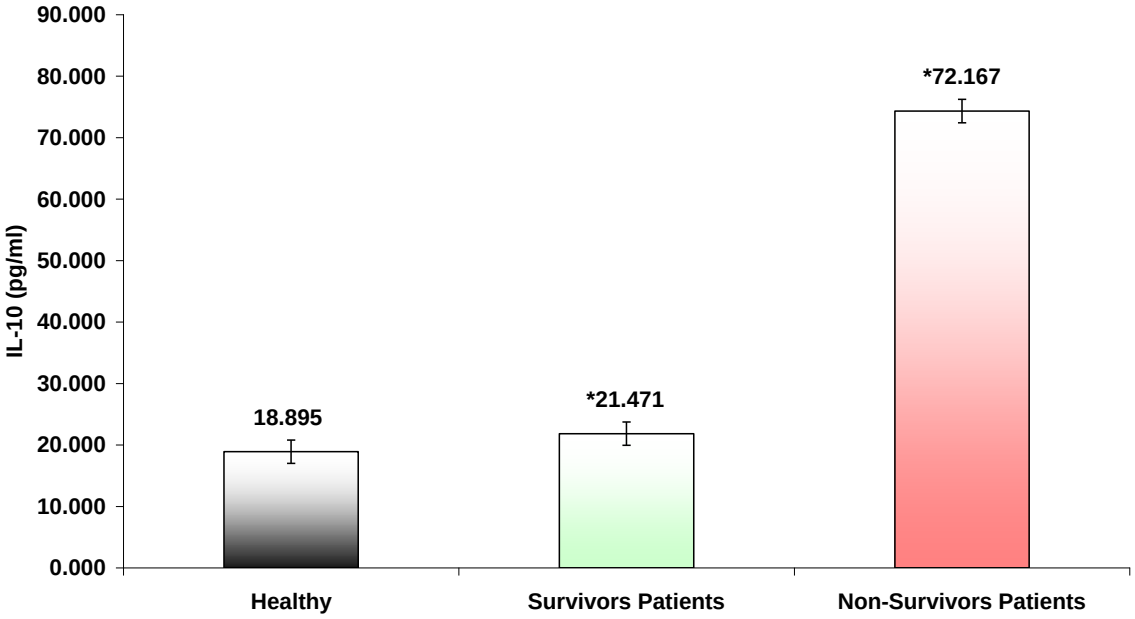
AK- Amikacin AMP-Ampicillin CAZ-Cefmetazole C-chloramphenicol CN- Gentamicin CIP-Ciprofloxacin CTX-Cefotaxim CX-Cefoxitin F –Nitrofurantion FOX-Oxacillin NOR-Norfloxacin SXT- Trimethoprim-Sulfamethoxazole TOB-Tobramycin VA-Vangomycin

Most bacterial isolates showed high resistance towards ceftazidin, cefotaxim, ampicillin, trimethoprim, sulfamethoxazole, tobramycin, gentamicin and nitrofurantion. Gram negative bacteria were completely resistance to cefepime and piperacillin. Gram positive bacteria were very resistance to cefoxitin and oxacillin. The ELISA results revealed that the concentration of IL-7 and IL-10 in burn patients were of significantly elevated compared to control group as showed in figure I and II.



*** Significant difference (P<0.01) between healthy group and patients .**

Figure1. Comparison between Serum IL-7 in Healthy Control and Patients Groups.



*** Significant difference (P<0.01) between healthy group and patients.**

Figure2. Comparison between Serum IL-10 in Healthy Control and Patients Groups.

The concentration of IL-7 showed high elevation in survived burn patients and low elevation in non-survivors. The concentration of IL-10 showed less elevation in survived burn patients and high elevation in non-survivors. The coefficient for IL-7 was positive, indicating that the risk of mortality decreased and the result of IL-10 was indicator for the risk of mortality among burn patients.

DISCUSSION:

The percentage of the burn is considered as important risk factors for septicemia and mortality. In this study, the reasons for this high prevalence of Gram negative bacteria may be due to resistant of these bacteria to many types of antibiotics or due to the virulence factors. The most frequently isolated organisms from burn patients were Pseudomonas species followed by K. pneumonia and S. aureus. The prevalence of S. aureus may be due to the virulence factors such as the production of coagulase by S. aureus which protects these bacteria from phagocytosis by coating the cell with fibrin (Langley et al, 2003).

In present study, P. aeruginosa were highly resistant to many antimicrobials, but were shown intermediate resistant (66.6%) toward amikacin and (77.7%) toward norfloxacin, ciprofloxacin, meropenem and imipenem. K. pneumoniae was resistant to some antibiotics and sensitive to another, where was little resistant toward imipenem (71.4%), norfloxacin (42.8%), amikacin (28.5%), nitrofurantion (17.4%) and ciprofloxacin (14.2%). S. aureus was completely sensitive to chloramphenicol, nitrofurantion and vangomycin and resistant to other antibiotics. S. epidermidis was completely resistant to cefmetazole, cefotaxim and cefoxitin and sensitive to other antibiotics. In such case the reason of some bacterial isolates was resistant to antibiotics may be due to development new resistant genes or by mutation through previously exposure to these antibiotics. There are three mechanisms of antibiotics resistance including: reduced uptake or decreased cell permeability, alterations at the ribosomal binding sites, or production of modifying enzymes (Lundström et al, 2012).

IL-7 plays many essential roles in human health and disease. The activation of IL-7 signalling pathway results in survival, proliferation, differentiation and maturation of haematopoietic cells (Cesur et al, 2011). The results of IL-7 were corresponds to earlier results in U.S.A. that provided a significant increase in the concentration of IL-7 in the serum of burn patients after (6-7) of burn injury (Ponchel et al, 2005). In non-septic and in survivors septic patients, the levels of IL-10 were lower than those of non-survivors septic patients. As in experimental models, trauma, burn, and major surgery induce IL-10, the presence of endotoxin in septic burn patients might induce this cytokine production (Scott Brittney et al; 2013)..

CONCLOSION:

Septic and mortality cases increased with the increase of burn percentage. Gram negative bacteria found to be more predominant as causative agent for bloodstream infections than the gram positive bacteria. P. aeruginosa is the most frequent species among bacterial isolates. ciprofloxacin, norfloxacin and amikacinwere found to be the most effective antibiotics against gram negative bacteria; whereas chloramphenicol, vangomycin and nitrofurantion found active against gram positive bacteria. Increase level of IL-7 plays an important role in the normal development and maintenance of the human immune system while the elevated level of IL-10 is considered as pointer to septic and fatality.

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REFERENCES:

1) Vale, E.C.S, Primeiro atendimento em queimaduras (2005). A abordagem do dermatologista. A Bras Dermatol 80: 9-19.
2) Church D, Elsayed S, Reid O, Winston B, Lindsay R (2006). Burn Wound Infections. CLIN MICROBIOL REV 19: 403-434.
3) Forner L, Larsen T, Kilian M, Holmstrup P (2006). Incidence of bacteremia after chewing, tooth brushing and scaling in individuals with periodontal inflammation. J CLIN PERIODONTOL 33: 401- 407.
4) Santucci S G, Gobara S, Santos C R, Fontana C, Levin A S (2003). Infections in a burn intensive care unit: experience of seven years. J HOSP INFECT 53: 6-13.
5) Murray P R, Baron E J, Jorgensen J H, Pfaller M A (2003). Mannual of clinical microbiology, (Washington, D.C.).
6) Gauglitz G G, Finnerty C C, Herndon D N, Mlcak R P, Jeschke M G (2008). Are serum cytokines early predictors for the outcome of burn patients with inhalation injuries who do not survive. CRIT CARE 12: 75-81.
7) Ponchel F, Verburg R J, Bingham S J, Brown A K, Moore J (2005). Interleukin-7 deficiency in rheumatoid arthritis: consequences for therapy-induced lymphopenia. ARTHRITIS RES THER 7: 80-92.

8) Pileri D, Accardo P A, D'Amelio L, D'Arpa N, Amato G, Masellis A, Cataldo V, Mogavero R, Napoli B, Lombardo C, Conte C (2008). Concentrations of Cytokines IL-6 and IL-10 in plasma of burn patients: their relationship to sepsis and outcome. Annals of Burns and Fire Disasters 12: 182-185.
9) Asadullah K, Sterry W, Stephanek K, Jasulaitis D, Leupold M, Audring H, Volk H D, Döcke W D (1998). IL-10 is a key cytokine in psoriasis: proof of principle by IL-10 therapy a new therapeutic approach. J CLIN INVEST 101: 783-794.
10) Moore, K.W, de Waal, M.R, Coffman, R.L. and O'Garra A (2001). Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 19: 683-765.
11) Baron E J, Peterson L R, Finegold S M (1995). Bailey and Scott's Diagnostic microbiology. (C.V. Mosby Company).
12) Collee J, Fraser A G, Marmian B P, Simmon S A (1996). Mackie and McCartney Practical Medical Microbiology (Churchill and Livingstone, New York).
13) MacFaddin J F (2000). Biochemical test for identification of medical bacteria (Williams and Wilkins-Baltimor).
14) Langley, R, Arcus, V. and Fraser, J (2003). Virulence Factors from Staphylococcus aureus: Tools to Study Innate and Adaptive Immunity. J Australian Biochemist 34: 11-18.
15) Lundström W, Fewkes N M, Mackall C L (2012). IL-7 in human health and disease. J Seminars in Immunology 24: 218-224.
16) Cesur S, Şengül A, Kurtoğlu Y, Kalpakç Y, Özel SA, Bilgetürk A, Erdem H, Aslan T, Kınıkl S, Eyigün C P, Bıyıkl Z (2011). Prognostic value of cytokines (TNF-α, IL-10, Leptin) and C-reactive protein serum levels in adult patients with nosocomial sepsis, Journal of Microbiology and Infectious Diseases 1: 101-109.
17) Scott Brittney NV, Derek J Roberts, Helen Lee Robertson, Andreas H Kramer, Kevin B Laupland, et al (2013). Incidence, prevalence, and occurrence rate of infection among adults hospitalized after traumatic brain injury: study protocol for a systematic review and meta-analysis. TRANSPORT RES REC 2:68.