



## THE ROLE OF IL-10 IN BURN OUTCOME WITH AND WITHOUT SEPTIC

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<p><b>Received:</b> January 1<sup>st</sup> 2022 <b>Accepted:</b> February 1<sup>st</sup> 2022 <b>Published:</b> March 8<sup>th</sup> 2022</p>	<p>From December 2020 to November 2021, 60 patients entered the Al Sadder hospital in Al Najaf province, Iraq. The patients were divided into two groups: 40 with <i>pseudomonas aeruginosa</i> and 20 uninfected. The purpose of this study was to identify the function of the anti-inflammatory cytokine interleukin 10 (IL-10) in the pathophysiology of burn injuries. The patients were divided into two groups based on gender (34 males and 26 females) and age (1-61) years. To eliminate patients who had received antibiotic medication during the sample collection. There was a significant difference between males and females in this study, with the highest frequency of patient age being 16-30 (36.6 %), followed by 31-45 (30 %), 1-15 (23.4 %), and 46-61 (10%). All patients divided according to total degree of burn. However, <i>P. aerogenosa</i> antibiotic sensitivity test, in contrast most bacterial isolates exhibited high resistance to Ceftazidime (90%) Tobramycin (80%) and Gentamicin (80%), whereas resistance to Ciprofloxacin (50%) Amikacin (40%) and Pipracillin (70%) was variable finally less resist to TC (20%) and no resistance toward IMP (0 %). The study found that patients with <i>P.aerogenosa</i> infection had a substantially higher mean blood concentration of IL-10 (<math>61.4 \pm 11.8</math>) than healthy controls (<math>4.58 \pm 0.77</math>), whereas non-infected patients had a concentration of (<math>18.21 \pm 3.5</math>) pg/ml and non-infected patients had a concentration of (<math>4.5 \pm 0.06</math>) pg/ml.</p>

**Keywords:** burn sepsis, *P. aerogenosa*, antibiotic sensitivity, IL-10.

### INTRODUCTION

Burns are skin, mucous membrane, and/or soft tissue injuries produced by heat, electricity, radiation, and caustic chemicals (American Burn Association, 2019). Burn injury increases susceptibility to infectious diseases that lead to increased morbidity and mortality, with higher rates of hospital admissions in both severe and minor burns (Duke *et al.*, 2017). Severe burns cause an immunological and inflammatory response, metabolic abnormalities, and distributive shock that can lead to multiple organ failure (Jeschke *et al.*, 2020).

Rech, (2019) mention that autopsy study appear more than 60% of deaths in patients with burn injury were attributable to infectious complications and nearly all had at least one associated organ failure that affects different organs including the kidneys, lungs, liver, gastrointestinal tract, heart and bone marrow.

Burns divided into 3 degrees depended on depth of skin damage: first degree (superficial only the surface epithelium is damaged with erythema, dry skin and heal in a few days) burns, second degree (partial

thickness) burns, and third degree (full thickness) burns (Graham-Brown, 2007).

Cytokines an important players in the post-burn pathophysiological process by keeping a precarious balance between resisting infection and minimizing tissue damage. elevation of pro- and anti-inflammatory cytokines alters immune response (Pileri *et al.*, 2008).

Burgener *et al.*, 2019 report that *Pseudomonas aeruginosa* is linked to increased morbidity, mortality, and poor quality of life in human diseases such as burns and lung infections. Due to its clinical relevance and the rise of antibiotic resistance, researchers are seek to learn how the immune system can remove *P. aeruginosa* from wound sites, and how the immune response evolves from acute to chronic infection (Serra *et al.*, 2015). This study aim to investigate the role of IL-10 serum level in burn pathophysiology and their associated with *Pseudomonas* infection.

### METHODOLOGY

A case-control study was conducted on 60 burn patients attending to burn center in Al\_Sadder



hospital in Al\_Najaf province - Iraq , during the period March- November 2020 . The patients divided into two groups 40 burn patients infected with *pseudomonas aeruginosa* and 20 patients not infected with any pathogens as well as all patients divided according to total degree of burn, both sexes with ages ranging from 1-61 years. According to gender, the patients divide in to 34 males and 26 female, as well as total of 20 healthy subjects volunteer were included as controls group with ages between 1-65 and gender match with patients group. The included criteria was all burn patients and excluded patients.

Swabs were taken from burn injury under aseptic condition and transferred to the laboratory for culturing on differentiated and enriched media, whereas blood samples are collected by withdraw 3 ml of Venous blood from each subjects by using disposable syringes, then 2 ml was placed in gel tubes allowed to clot at room temperature, centrifuged at 3000 rpm for 10 minutes and sera were dispensed into Eppendorf-tube, numbered and stored at -20 ° C until used for evaluate IL-10 by ELISA technique according to (Elabscience, USA) while 1 ml from residual blood is allocated to CBC test .

After identification of *P. aeruginosa* by biochemical tests antibiotics sensitivity test was done according to Kirby-Bauer disc diffusion method using Mueller-Hinton agar" as recommended by Clinical Laboratory Standard Institute . Zone size was compared to standard zones depending on clinical and laboratory standard institute (CLSI, 2019) .

**Ethical approval:** This study was ethically approved by the medical ethics committee in Al-Sadder medical city, Najaf, Iraq (approval NO.:8761). Moreover, all subjects gave the informed consent before they gave the samples.

### STATISTICAL ANALYSIS

The result of IL-10 level was expressed as arithmetic mean  $\pm$  SE. The comparison between patients and control was analyzed by T-test.

### RESULTS AND DISCUSSION

Demographic characteristics for 60 patients attending to burn center in AL-Najaf province by patients-control study revealed that male were 34(53.6)% and female were 26(46.3)% and the statically analysis revealed a significant difference between male and female, as shown in Figure (1):

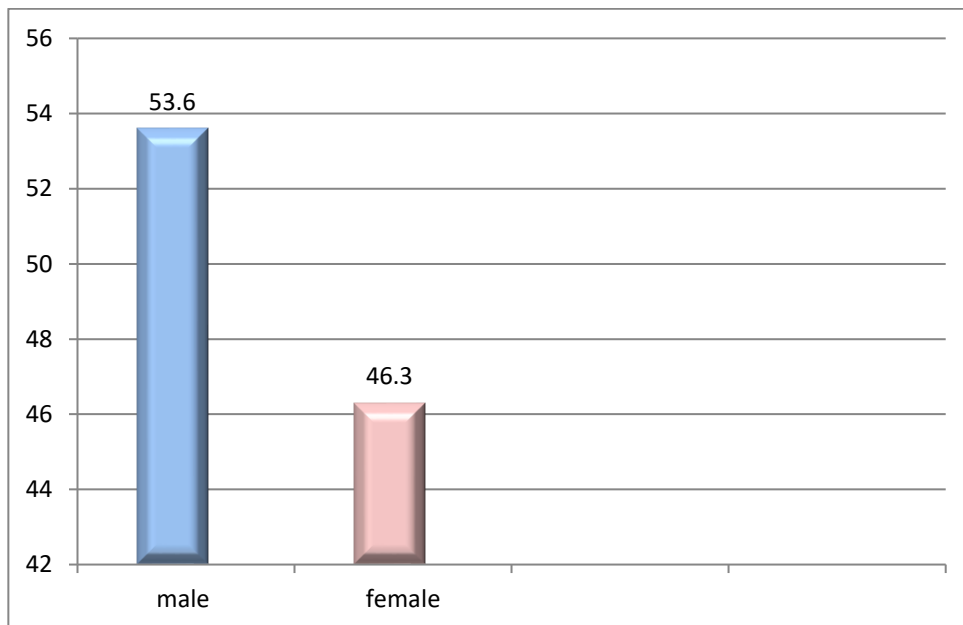


Figure (1) distribution of burn patients according to gender

The present study appear that classified the patients according to age ranges revealed there are 4 categories. The highest frequency of patient age was in 16-30which recorded ( 36.6%) followed by 31-45

(30%) ,1-15 (23.4%) , and lowest frequency were 46- 61 (10 %) respectively. The results revealed less number of older patients compared with high number in younger patients, as shown in Figure (2) .

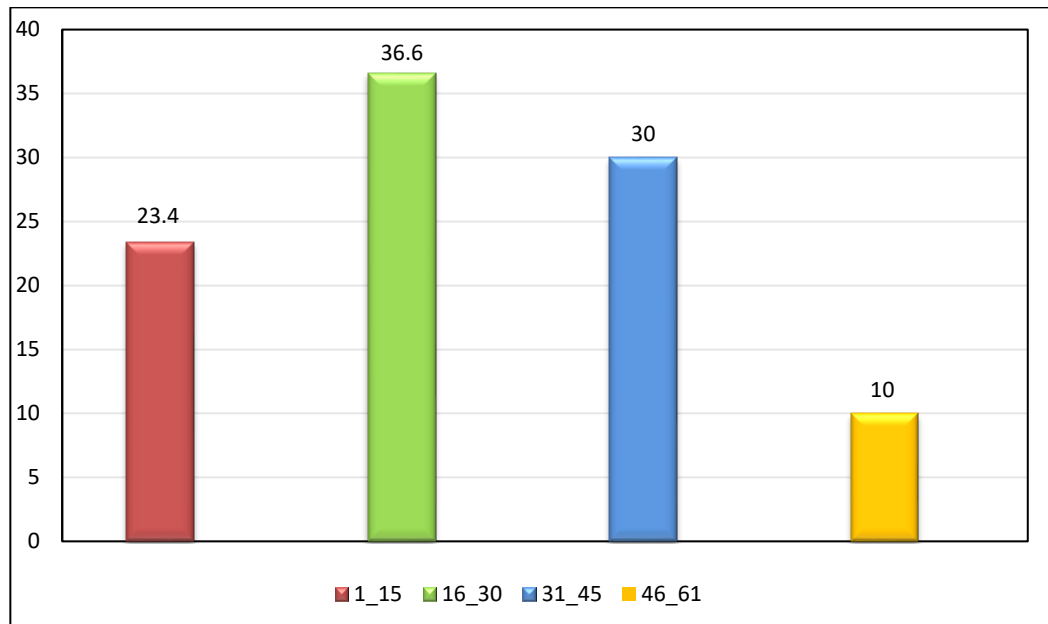


Figure (2) distribution of burn patients according to age

Tian *et al.*,2018 founded that the percent of males (71.3%) were more than females(28.7%) in China. Also, Zheng *et al.*, (2019) showed that (67.1%) of the affected cases were males and (32.9%) females.

The burns tend to occur more in a certain age group reflecting the particular behavioral patterns associated with age. In children, the lack of coordination and unawareness of dangerous substances play important roles in increasing the rate of burn exposure. In addition, The researchers found that family characteristics play a crucial role in increasing the likelihood of the injury. Lower maternal education, young age of mother and unemployment, and lone parenthood are identified risk factors of burns in children (Kawalec ,2015).

In adult, the burns occur at low rate because the adults are realize more than the children, but more burns are caused by flame, contact, electricity, chemicals, or explosion increased sharply when people reached working age (18.1–65 years old). The great majority of the burns were accidental and occurred at home (96.85%), especially in young children. In a total of 303 adult patients (18.1–65 years old), 48.40% were work related(Tian *et al.*,2018).

The study revealed that patients divided according to size of burn 38 (63.3 %) with injury > 50% and 22 (36.6%) the injury less than 50 % as show in figure (3).

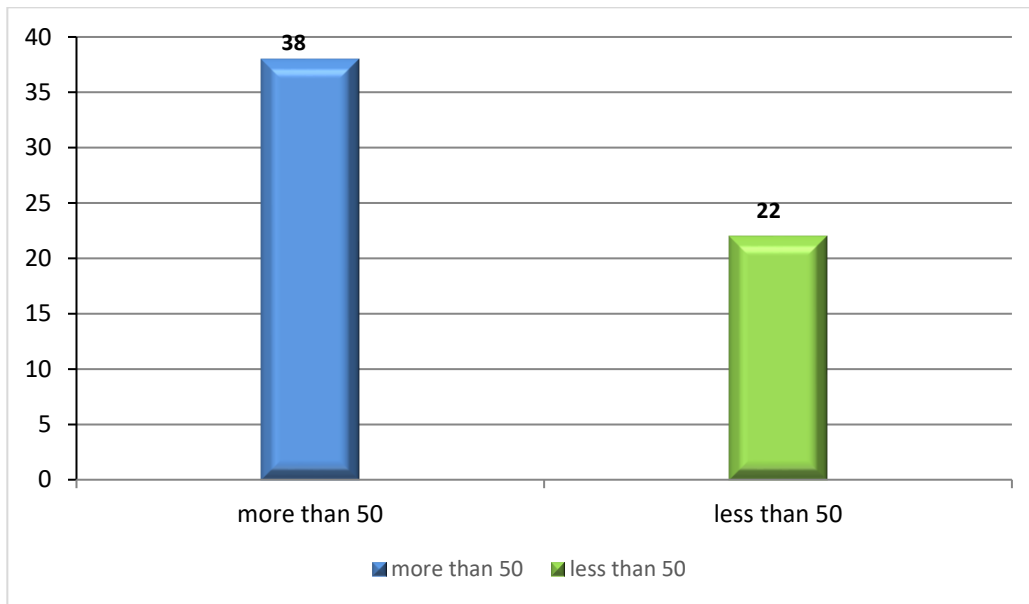


Figure (3) distribution the burn patients according to body surface injury

Nosocomial infection in the burnt patients is major challenge for a clinician (Ekrami and Kalantar 2007). It has been estimated that 75% of all deaths in burnt patients were associated with infections (Srinivasan *et al.*,2009). This agrees with a study in Kuwait by Bang *et al.* (2002); they revealed that in patients with burn size more than 40% of the (TBSA), most dead cases were related to septic. Besides, Aldemir *et al.* (2005) in Turkey found a positive correlation between the extent of the burn and mortality; the mortality rate risen as the burn extent increased. Prolonged use of antibiotics leads to the development as well as selection of multidrug resistant (MDR) bacteria which results in treatment failure and intensifies the complications. Thus, the information of microbial flora and the current antibiotic susceptibility patterns are important for the clinician treating burn sepsis(Gupta *et al.*,2019).

This agrees with a study shows significant thermal injuries induce a state of immunosuppression

that predisposes burn patients to infectious complications. Major burn injury is a type of trauma with high morbidity and mortality rates at all age groups. (Güldoğan *et al.*,2018).

The skin is a complex organ that has devised numerous strategies, such as physical, chemical, and microbiological barriers, to protect the host from external insults. In addition, the skin contains an intricate network of immune cells resident to the tissue, crucial for host defense as well as tissue homeostasis, deregulation of immune responses often leads to impaired healing and poor tissue restoration and function (Nguyen and Soulika, 2019).

#### **ISOLATION OF PATHOGENIC BACTERIA**

A total number of 60 swabs about 40 (66.6%) show positive culture of bacterial growth versus 20(33.3%) show negative for culturing table (1). In this result *Pseudomonase aerogenosa* appear in 40 positive culture that dividing according to degree of burn

Degree	Burn (No. of <i>p.aerogenosa</i> ) N=40	Burn without infection N=20
1 st	4 ( 2 isolates)	12
2 nd	12(3 isolates)	6
3 ed	18 (4 isolate)	2
4 th	6 ( 1 isolate )	



total	40 (10 isolate)	20
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This agrees with Vural *et al.* (2013) in Turkey who revealed that *Pseudomonas aeruginosa* (45.5%) was the most predominantly isolated bacteria from the wounds, and there was good concordance between the results of the surface swab and quantitative biopsy culture samples (78%).

Burns are one of the most common and devastating forms of trauma. Patients with serious thermal injury require immediate specialized care in order to minimize morbidity and mortality. Significant thermal injuries induce a state of immunosuppression

that predisposes burn patients to infectious complications (Church *et al.*,2006).

The results of antibiotic sensitivity test for *P. aeruginosa* Most bacterial isolates showed high resistance towards Ceftazidime , Tobramycin, and Gentamicin with percentage 90%, 80%, 80%, respectively, while The bacterial isolates showed a varying resistance to each of Ciprofloxacin 50%, Amikacin 40 %, Pipracillin 70% , in other word was less resist to TC 20% and no resistance toward IMP 0 % as shown in Figure (4)

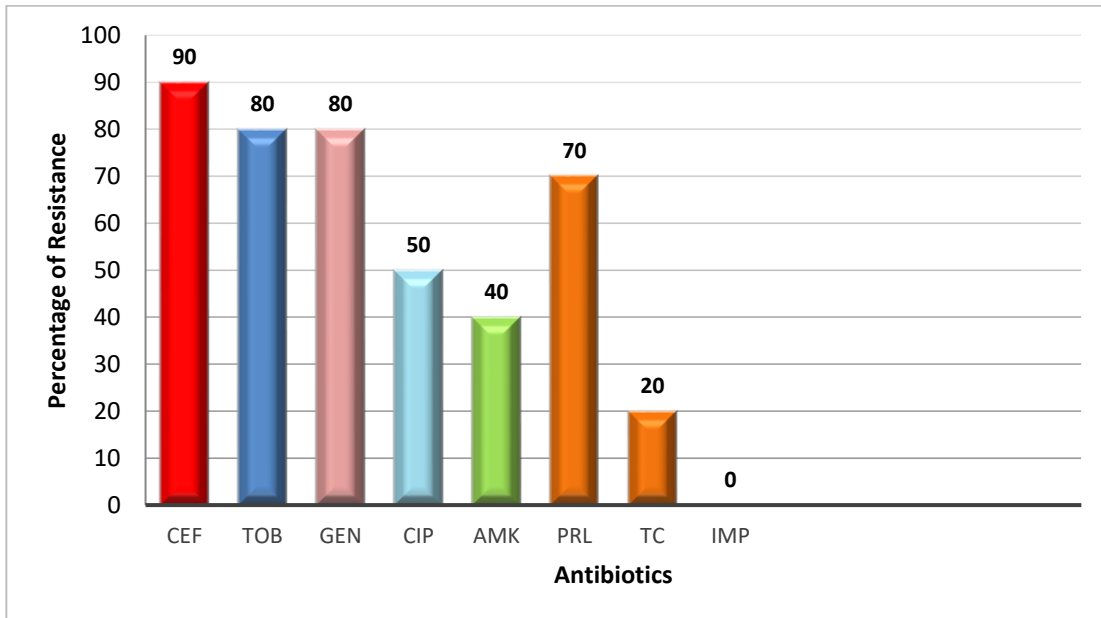


Figure (4 ) antibiotic susceptibility test against *P. aeruginosa* in burn patients

The results by Gupta *et al.*,2019 revealed that the emergence of extensively drug-resistant and pandrug resistant strains in burn patients and all isolates were exhibited resistance to the commonly used antibiotics as well as new generation antibiotics and recorded Meropenem (83.78%), Imipenem

(85.41%) Amikacin (87.03%) and Ciprofloxacin (89.19%) .

The study shows that the mean serum concentration of IL-10 in patients is increase significantly ( $61.4 \pm 11.8$ ) than in healthy subjects ( $4.58 \pm 0.77$ ) as control group, as shown in Figure (5)

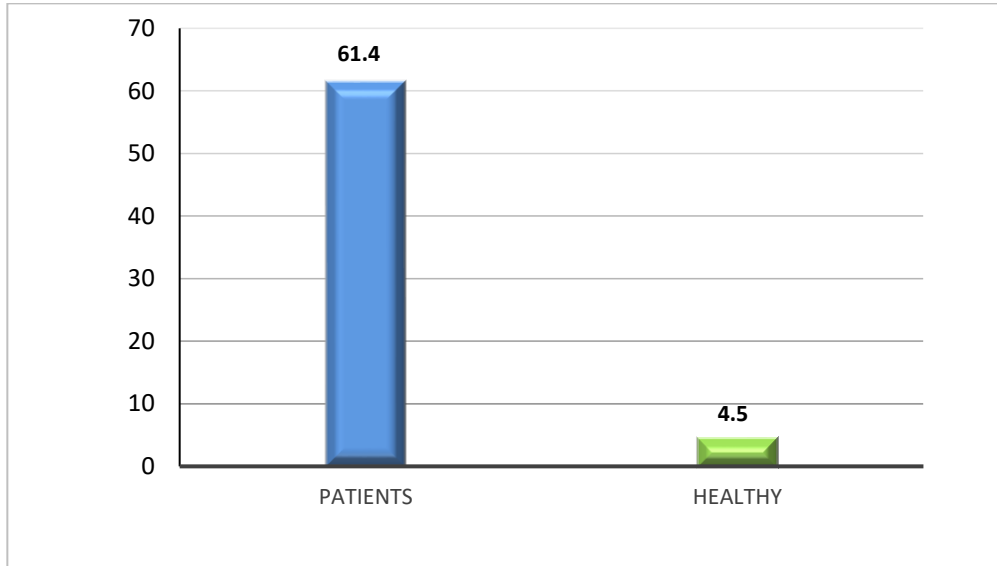


Figure (5) The mean serum concentration of IL-10 in patients.

Our study is closely agree with Pileri and his colleagues(2008) they indicated that .IL-10 levels were higher in septic patients than in non-septic patients. , the high value of circulating IL-10 on day 3 suggests that cytokine may discriminate between no survivor septic and survivor septic patients , as well as they noticed that, among the patients who developed sepsis, the analysis of IL-10 on day 3 was a determinant discriminator between survivors and nonsurvivors. This result suggests that, in addition to its anti-inflammatory effect, IL-10 also appears to be correlated with the development of septic complications.

Balouchi *et al.*, 2014 revealed that IL-10 level was significantly higher than healthy and mention that immunological abnormalities have been characterized in  $\beta$ -TM , many of which are linked to cytokines and this imbalanced immune condition involving inflammation and immunosuppression in patients.

The present study also observed that the concentration of IL-10 (pg/ml) in Burn patients with *P.aerogenosa* infection was  $43.77 \pm 11.9$  pg/ml, Burn patients without infection was  $18.21 \pm 3.5$  pg/ml and normal persons were  $4.5 \pm 0.06$  pg/ml as shown in Figure (6) .

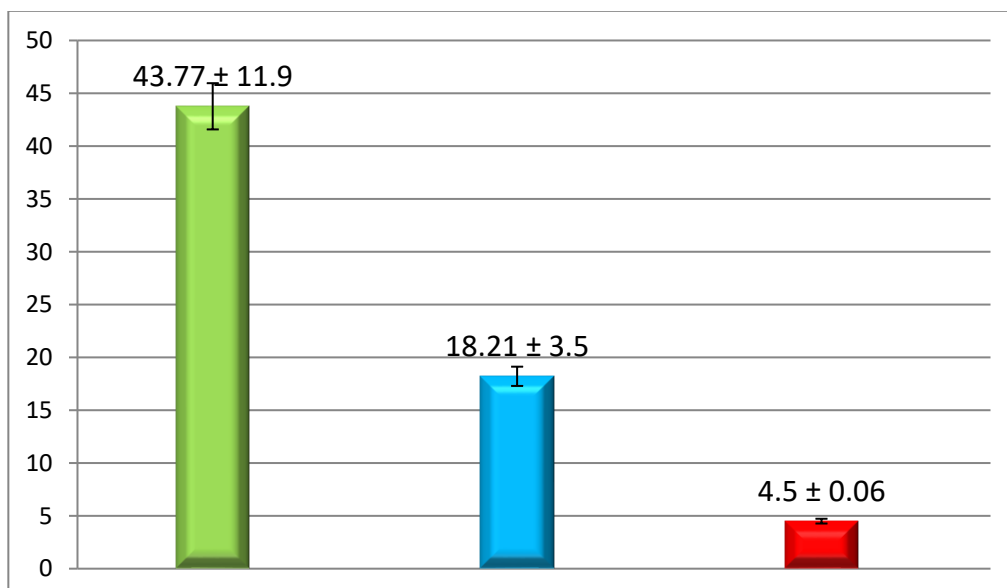


Figure (6 ) IL-10 serum level in infected burn patients and non infected patients



## CONCLUSIONS

Burn injuries occur more frequently in individuals aged 16-30 than in other age groups. The number of microorganisms that cause burn wounds rises with the burn percentage. Infections of burn wounds and blood are more commonly caused by Gram negative bacteria than Gram positive bacteria. Antibiotics that treat *P.aerogenosa* in vitro include imipenem. L-10 concentration rises in burn patients.

## REFERENCES

1. Tan JQ, Zhang HH, Lei ZJ, Ren P, Deng C, Li XY, et al. The roles of autophagy and apoptosis in burn wound progression in rats. *Burns*. 2013;39:1551–6.
2. Nisanci M, Eski M, Sahin I, Ilgan S, Isik S. Saving the zone of stasis in burns with activated protein C: an experimental study in rats. *Burns*. 2010;36:397–402
3. Rowan, M. Cancio, L.; Elster,E.; Burmeister, D.; Rose, L. and Natesan, S. (2015 ). Burn wound healing and treatment: review and advancements. *Crit. Care* 19 :243-248.
4. Vos T, Allen C, Arora M, Barber RM, Bhutta ZA, Brown A, (2016). Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015". *Lancet*. 388 (10053): 1545–1602.
5. Haagsma JA, Graetz N, Bolliger I, Naghavi M, Higashi H, Mullany EC. (2016) The global burden of injury: incidence, mortality, disability-adjusted life years and time trends from the Global Burden of Disease study 2013 *Injury Prevention*. 22 (1): 3–18
6. Herndon D, ed. (2012). "Chapter 1: A Brief History of Acute Burn Care Management". *Total burn care* (4th ed.). Edinburgh: Saunders. p. 1. ISBN 978-1-4377-2786-9. Burn Incidence and Treatment in the United States: 2012 Fact Sheet.
7. American Burn Association. 2012. Archived from the original on 21 February 2013. Retrieved 20 April 2013.
8. Chaganti P, Gordon I, Chao JH, Zehtabchi S (June 2019). A systematic review of foam dressings for partial thickness burns". *The American Journal of Emergency Medicine*. 37 (6): 1184–1190.
9. Gupta (2003). *Textbook of Surgery*. Jaypee Brothers Publishers. P. 42. ISBN 978-81-7179-965-7. Archived from the original on 27 April 2016.
10. Peck MD. (2011) Epidemiology of burns throughout the world. Part I: Distribution and risk factors. *Burns*. 37 (7): 1087–100.
11. Pileri ,D.; Palombo,A.A.;D'Amelio, L.; D'Arpa, N.; Amato, G.;Masellis, A.;Cataldo,V.; Mogavero,R. ; Napoli, B.; Lombardo, C. and Conte, C.(2008). Concentrations of cytokines IL-6 and IL-10 in plasma of burn patients: their relationship to sepsis and outcome. *Ann Burns Fire Disasters*2008 Dec 31;21(4):182-5.
12. Jeschke, M.G.; van Baar, M. E. ; Choudhry, M.A.; Chung, K. K. ; Gibran, N. S. and Logsetty, S .(2020).Burn injury. *Nat Rev Dis Primers*. 6(1): 11.
13. Duke, J.M.; Randall, S.M.; Wood, F.M.; Boyd, J.H. and Fear, M.W. burns and long-term infectious disease morbidity: a population-based study. *Burns*. 2017;43(2):273–81.
14. Burgener, E.B.; Sweere, J.M.; Bach, M.S.; Secor, P.R.; Haddock, N.; Jennings, L.K. Marvig, R. L. ; Johansen, H. K. ; Rossi, E. ; Cao, X. ; Tian, L.; Nedelec, L.; Molin, S. ; Bollyky, P.L. and Milla, C. E. . Filamentous bacteriophages are associated with chronic *Pseudomonas* lung infections and antibiotic resistance in cystic fibrosis. *Sci Transl Med* 2019;11:eaau9748.
15. Serra, R.; Grande, R.; Butrico, L.; Rossi. A.; Settimio, U.F.; Caroleo, B.; Amato, B.; Gallelli, L. and de Franciscis, S. Chronic wound infections: the role of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *Expert Rev Anti Infect Ther* 2015;13:605–613.
16. Tian , H.; Wang ,L., Xie, W.; Shen, C.; Guo, G.; Liu, J.; Han, C.; Ren, L.; Yi Liang; Tang, Y.; Wang, Y.; Yin, M.; Zhang, J. and Huang, Y. (2018) Epidemiologic and clinical characteristics of severe burn patients: results of a retrospective multicenter study in China, 2011–2015. *Burns & Trauma* volume 6, Article number: 14 (2018) .
17. Zheng, Y., Lin, G.; Zhan, R.; Qian, W.; Yan, T.; Sun, L. and Luo, G. (2019). Epidemiological analysis of 9,779 burn patients in China: An eight-year



- retrospective study at a major burn center in southwest China. *Experimental and Therapeutic Medicine*. EXPERIMENTAL AND THERAPEUTIC MEDICINE 17: 2847-2854, 2019.
18. Kawalec, A. Environmental factors of burns in children—Review. *Medycyna Środowiskowa - Environmental Medicine*. 2015;18(3):40-46.
  19. Church, D.; Elsayed, S.; Reid, O. ; Winston, B. and Lindsay, R. . Burn Wound Infections. *Clin Microbiol Rev*. 2006 Apr; 19(2): 403–434.
  20. Gündoğan, C. E. ; Kendirci, M.; Gündoğdu, E. and Yast, A. Ç. Analysis of factors associated with mortality in major burn patients *Turk J Surg*. 2019 Sep; 35(3): 155–164.
  21. Nguyen A. V. and Soulika, A. M. The Dynamics of the Skin's Immune System *Int J Mol Sci*. 2019 Apr; 20(8): 1811.
  22. Ekrami, A. and Kalantar, E. Bacterial infections in burn patients at a burn hospital in Iran. *Indian J. Med. Res*. 2007 Dec 1;126(6):541.
  23. Srinivasan, S.; Vartak, A.M.; Patil A. and Saldanha, J. Bacteriology of the burn wound at the BaiJerbaiWadia hospital for children, Mumbai, India—a 13-year study, Part IBacteriological profile. *Indian J. Plast. Surg*. 2009 Jul;42(2):213.
  24. Gupta, M. ; Naik, A. K.; and Singh, S. K.(2019). Bacteriological profile and antimicrobial resistance patterns of burn wound infections in a tertiary care hospital *Heliyon*. 2019 Dec; 5(12): e02956.
  25. Pileri, D.; Palombo, A. A.; D'Amelio, L.; D'Arpa, N.; Amato, G.; Masellis, A.; Cataldo, V. ; Mogavero, R.; Napoli, B.; Lombardo, C. and Conte, C. Concentrations of Cytokines Il-6 and Il-10 in Plasma of Burn Patients: Their Relationship to Sepsis and Outcome *Ann Burns Fire Disasters*. 2008 Dec 31; 21(4): 182–185 .
  26. Rech, M. A. (2019)Outcomes in burn- injured patients who develop sepsis. *J. Burn Care Res*. 40, 269–273.
  27. Bang, R.L.; Sharma, P.N.; Sanyal, S.C. and Al Najjadah, I. (2002). Septicaemia after burn injury: a comparative study. *Burns*, 28(8): 746-751.
  28. Aldemir, M.; Kara S.H.; Girgin, S. and G Loglu C. (2005). Factors affecting mortality and epidemiological data in patients hospitalized with burns. In Diyarbakir, Turkey. *SAJS*, 43(4): 193-196.
  29. Graham-Brown; Robin; Burns and Tony. *Burns, Dermatology*. 9th ed. 2007, Oxford: Blackwell publishing.
  30. Vural, M. K.; Altoparlak, U.; Celebi, D. and Akcay, M. N. Comparison of Surface Swab and Quantitative Biopsy Cultures Dependent on Isolated Microorganisms from Burn Wounds. *Eurasian J Med*. 2013 Feb; 45(1): 34–38.
  31. Balouchi S. ; Gharagozloo, M. ; Esmaeil, N. ; Mirmoghtadaei, M. and Moayedi, B . Serum levels of TGFβ, IL-10, IL-17, and IL-23 cytokines in β-thalassemia major patients: the impact of silymarin therapy. *Immunopharmacol Immunotoxicol*. 2014 Aug;36(4):271-4.