

Diagnoses Bacterial Wound Infection and Detection of Some Immune Parameters in its

Shahrazad Ahmed Khalaf *

Department of Biotechnology, College of Science, Diyala University, Diyala city, Iraq.

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Abstract

This study aims to diagnose bacterial wound infection and to estimate the serum levels of TLR-3, CXCL8, TGF- β 1, IL-8, IL-17 and IL-22 in wound patients. This study had been included 140 samples (blood and swab) obtained from patients found in Baquba-Teaching Hospital from 15/ 6 /2023 to 20 /3 /2024. Swab samples taken from wound were culture on the blood agar and MacConkey agar, and then Bacteria diagnoses was done by using an automatic VITEK 2 system. The serum levels of immune parameters done by ELISA. The results shown that sixty isolates were grow in culture, the results indicated of 38(63.3%) isolates of gram-negative bacteria, represented by: 12 (20%) isolates *E. coli*, 18 (30%) *Pseudomonas aeruginosa*, 6 (10%) *Protus spp.*, 2 (3.3%) *Acinetobacter spp.* the results indicated of 22 (36.6%) isolates of gram-positive bacteria, represented by: 16 (26.6%) isolates *Staphylococcus aureus*, 4 (6.6 %) another *Staphylococcus spp.*, 2 (3.3%) *Enterococcus spp.* The study also shown the a increase in the concentration of TLR-3 , CXCL8 , IL-2 , IL-17 and IL-22 in the serum of bacterial patients compared with the healthy, Where the results of the current study show decreases in the concentration of TGF- β 1 among compared with healthy. In conclusion, it was discovered that the infection of bacteria in wound infection was lower in females than males. *Pseudomonas aeruginosa* and *S. aureus* were found to be the highly isolated bacteria wound infection, with increases levels of TLR-3, CXCL8, IL-17 and IL-22 in serum in patients and decreases level of TGF- β 1 in serum of patients.

Keyword: Wound; TLR-3; CXCL8; TGF- β 1; IL-17 and IL-22

1. Introduction

Irrespective of the kind of wound, infections are linked to patient morbidity and death, particularly in impoverished countries (1). Wounds that get infected are those that have been colonized by bacteria or other microorganisms that lead to degradation and a delay in the healing process. Stated differently, infections arise from compromised immune systems or from the body's inability to fight off common bacterial development. Surgery-related wound infections are a serious health risk, and since bacteria typically contaminate surgical wounds, prior research has shown that surgical site infections account for around 70% of patient fatalities following surgical procedures (2). Additionally, certain bacteria can enter a patient's skin by contact with contaminated objects outside of their body, water, vomit, the air, and the solid hands of medical personnel (3). *Streptococcus pyogenes*, *Proteus species*, *Streptococcus species*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Streptococcus aureus* are common bacterial pathogens linked to wound infection (4). One of the most prevalent Gram-negative pathogens linked to nosocomial infections is *P.aeruginosa*. Because of its inherent resistance to a variety of antimicrobial agents and its capacity to develop adaptive resistance during a therapeutic course, diseases caused by these bacteria are frequently life-threatening and challenging to treat (5).

* Corresponding author: Shahrazad Ahmed Khalaf

Cells of the innate and adaptive immune system cooperate with resident cells to provide integrated and sometimes redundant mechanisms to ensure proper wound repair (6). The immune system is activated by PAMPs and DAMPs that PRRs, such as TLRs, recognize. This interaction with TLRs activates intracellular signaling pathways that result in the production of inflammatory cytokines, such as IL-1, and some chemokines, such as CXCL1, CLCL5, and CXCL8 (7). Following an injury, pro-inflammatory cytokines such TNF- α , IL-1, IL-6, and IL-8 are released by keratinocytes, which encourage neutrophil and macrophage migration and proliferation near the wound site (8). In order to activate and attract immune system cells, keratinocytes emit a variety of pro-inflammatory cytokines, including IL-1, IL-6, INF- γ , and TNF- α , as well as chemokine, such CCL27 (9). Macrophages play a crucial role in tissue regeneration and wound healing. Resident macrophages serve as homeostatic sentinels in undamaged skin (6).

2. Material and Method

This study was conducted from Baquba Teaching Hospital between June 2023 and March 2024. The study included 140 sample (blood and swab from wound) from both sexes. All swab specimens were exam by microscope and only specimens that have infection diagnosed by cultured on blood agar and MacConkey agar plate and the species of bacteria was diagnosed by Vitek2 compact. Patients' blood samples (5ml) were collected in a clean plain tube with gel and allowing them to coagulate for 30 minutes at 37C° before centrifugation. The tubes were centrifuged for 5 minutes at 6000 rpm, and the serum was collected and the TLR-3, CXCL8, TGF- β 1, IL-8, IL-17 and IL-22 levels were measured by using Sandwich ELISA test according to the instructions obtained in the kit made by sunlong Biotech Company.

Statistical Package for Social Sciences program version 22 has been used to examine the data statistically.

3. Result

In this study, one hundred and forty-five clinical samples (blood and swab) isolated from Baaquba Teaching Hospital in Diyala. Including sixty isolates were grow in culture, the results indicated of 38(63.3%) isolates of gram-negative bacteria, represented by: 12 (20%) isolates *E. coli*, 18 (30%) *Pseudomonas aeruginosa*, 6 (10%) *Protus spp.*, 2 (3.3%) *Acinetobacter spp.* the results indicated of 22 (36.6%) isolates of gram-positive bacteria, represented by: 16 (26.6%) isolates *Staphylococcus aureus*, 4 (6.6 %) another *Staphylococcus spp.*, 2 (3.3%) *Enterococcus spp.* As shown in table 1 and 2.

Table 1 Prevalence of Organisms isolated from wound sites

Sample			Total	
			No.	%
Positive culture	G - ve	38 (63.3 %)	60	41.3
	G +ve	22 (36.6 %)		
Negative culture			85	58.6

Table 2 Bacterial isolates types from swab culture

Bacterial Isolates		Total (60 isolates)	
		No.	%
G - ve bacteria	<i>E. coli</i>	12	20
	<i>Pseudomonas aeruginosa</i>	18	30
	<i>Protus spp</i>	6	10
	<i>Acinetobacter spp</i>	2	3.3
G + ve bacteria	<i>Staphylococcus aureus</i>	16	26.6
	other <i>Staphylococcus spp</i>	4	6.6
	<i>Enterococcus spp.</i>	2	3.3

The infection rate in relation to sex of patients was highest among male patients (56.6%) compare to female patients (43.3%) (Table 3).

Table 3 Distribution of patients according to gender

Study groups	N		%
Patients	60	Female 26 (43.3)	66.6
		Male 34 (56.6)	
Control	30		33.3
Total	90		100

The study showed increase in the concentration of TLR-3 , CXCL8 , IL-2 , IL-17 and IL-22 in the serum of bacterial patients (346.01 ± 67.91) pg/ml, (83.12 ± 17.81) pg / mL, (36.11 ± 8.12) pg/mL , (54.02 ± 22.02) pg/mL , and (48.01 ± 19.1)pg/mL respectively compared with the healthy also the level in control group was(266.12 ± 53.21) pg / mL,(68.21 ± 21.01)pg/mL ,(32.31 ± 6.21)pg/mL, (39.11 ± 18.32) pg/mL and (29.21 ± 17.91) pg/mL as shown in table(4)(Figure1). Where the results of the current study show decreases in the concentration of TGF-β1 among patient (221.01 ± 36.1) pg/mL compared with healthy (301.3 ± 41.91) pg/mL.

Table 4 Serum levels TLR-3, CXCL8, TGF-β1, IL-17 and IL-22 among studied groups

Parameter	Patients (Mean ± S.E)	Control (Mean ± S.E)	P. value
TLR-3	346.01 ± 67.91	266.12 ± 53.21	<0.05
CXCL8	83.12 ± 17.81	68.21 ± 21.01	>0.05
TGF-β1	221.01 ± 36.1	301.3 ± 41.91	<0.05
IL-2	36.11 ± 8.12	32.31 ± 6.21	>0.05
IL-17	54.02 ± 22.02	39.11 ± 18.32	<0.05
IL-22	48.01 ± 19.1	29.21 ± 17.91	<0.05

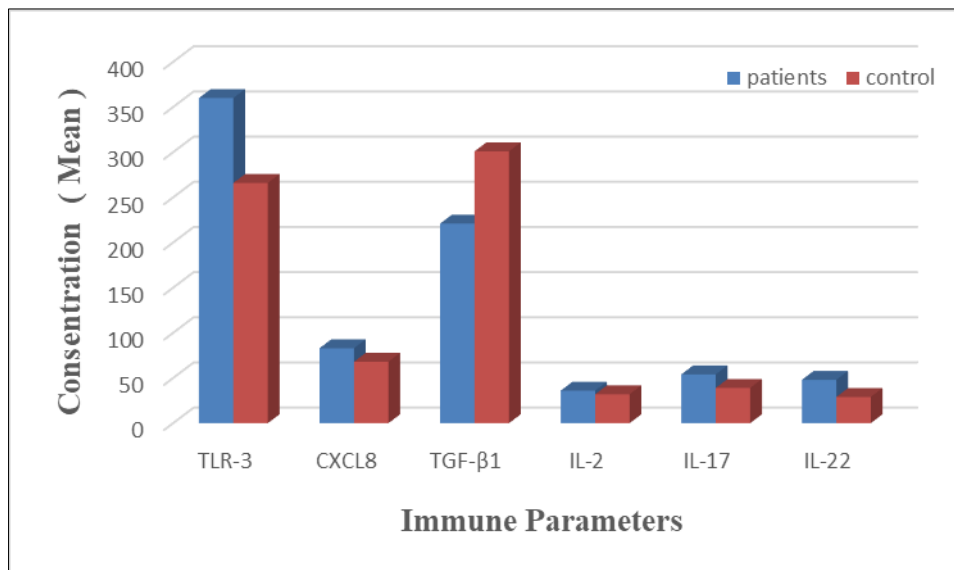


Figure 1 Serum levels TLR-3, CXCL8, TGF-β1, IL-17 and IL-22 among studied groups

4. Discussion

If surgical wounds with infection are not appropriately treated, they can result in metastasis through bacterial fluid lesions, suppurative skin responses, and subcutaneous nodules. Based on a surgical wound's vulnerability to microbial contamination, the risk of infection is often determined (10).

Gram-negative bacteria were more represented (57.9%) in respect to Gram-positives (36.6%), confirming the results reported in other studies (11). The spread of Gram-negative bacteria in environmental and clinical samples might be attributed to their resistance to a variety of antibiotics and their capacity to cause a variety of nosocomial illnesses. Nevertheless, other writers contend that endogenous channels, such as the body's own gut flora, rather than external routes, such as hospital staff hands and sink taps, may be the cause of Gram-negative infections (12).

There have been suggestions that the majority of wound infections stem from the endogenous flora present in the patient's skin or mucous membrane. Prolonged hospital stays, bacteremia, septicemia, and shock are linked to key bacterial species implicated in nosocomial wound infections, including *Staphylococcus aureus*, *E. coli*, and *Pseudomonas spp.* (13). *Pseudomonas aeruginosa* infections pose special challenges for patients because the organism can spread quickly and readily across compromised hosts' wounds. Additionally, the organism possesses inherent resistance to several drug classes and can develop resistance to all potent antimicrobial medications (14). *P. aeruginosa* was the Gram-negative more detected, which is also in agreement with other studies (11). *S. aureus* have several virulence factors, including invasions, hyaluronidase, catalase, coagulase, hemolysins, leukotoxin, and leucocidin, are released by *S. aureus* to cause virulence (11).

The predominance of male patients (56.6%) was observed in our study as has been noted in the majority of the other studies (15). Immune inflammatory cells, various signaling pathways, and the local cell environment are all involved in the intricate process of wound healing. Different growth factors, cytokines, and chemokine mediate and coordinate the repair process; these factors are engaged in every stage of wound healing, and their control and dynamic ensure the creation of a normal scar (16).

Chronic cutaneous wounds are connected with neutrophil chemoattractant CXCL8 (17). Neutrophils are actively drawn to the site of tissue damage when CXCL8 binds to and activates the neutrophil surface receptors CXCR1 and CXCR2 (18). These chemokines, such as CXCL8, bind glycosaminoglycans on tissue cell walls and in the extracellular matrix to form a signaling gradient that permits neutrophil movement in a clear, directed manner towards the injury (19).

TGF- β 1 produced from platelets promotes the growth of keratinocytes and the remodeling and regeneration of the epidermal layer (20). Tissue repair and neovascularization are mediated by PDGF, insulin-like growth factor 1 (IGF-1), VEGF, and TGF- β 1, and are carried out by pro-wound healing macrophages. Wound-associated macrophages emit PDGF- β and IL-10, which promote epithelial healing (17).

IL-2 is a cytokine that stimulates the production of T cells which play an important role in wound healing (21). IL-2 is a cytokine that participates in immune system signaling pathways, is vital to many immune system processes, and interacts with other cytokines to control the production and activation of immune cells—a process that may also have an effect on wound healing. The majority of CD8+ and CD4+ T-cells that are activated generate IL-2 (22).

Elevated levels of IL-17 can improve the recruitment of neutrophils and other immune cells to the wound site, which is important for fighting infections but can also cause tissue damage if improperly regulated. IL-17 is known to play a pivotal role in the host defense against pathogens by regulating the activities of various immune cells and the production of antimicrobial peptides (23).

It has been determined that IL-22 is a key regulator of wound healing and tissue regeneration (24). In order to promote the growth, migration, and differentiation of the cells involved in tissue repair, IL-22 plays a crucial role in wound healing (25). The findings revealed that *Pseudomonas aeruginosa* and *S. aureus* are the most dominant pathogenic bacteria found in wound. It was also discovered that the infection of bacteria in wound infection was lower in females than males. According to immunological parameters, patients with bacterial wound infection have high levels of TLR-3, CXCL8, IL-17 and IL-22 and decreases level of TGF- β 1 in serum of patients.

5. Conclusion

The study shows an increased incidence of wound infection due to bacterial pathogens, the most important of which are *P. aeruginosa* and *S. aureus*. The infection was higher among males than females. We also discovered an increase in the level of TLR-3, CXCL8, IL-17 and IL-22 in serum in patients and decreases level of TGF- β 1 in serum of patients.

Compliance with ethical standards

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Statement of ethical approval

It is declared that all ethical considerations were taken into account in the preparation of the submitted manuscript.

Authors' Contribution

The author conceived and designed the study, acquired the data, analyzed and interpreted the data, drafted the manuscript, critically revised the manuscript, and performed statistical analysis.

Data Availability

They support the findings of this study are available on request from the corresponding author.

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