Cytokine Profile as A Prognostic Parameter in Burn Patients

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Abstract_Burn injuries of the skin are one of the most common household injuries which vary depending on the way of induction and its severity. Common complications are sepsis of the wound and immunosuppression. A total of 44 burned patients were included in the study. Blood samples and a swab were taken from the location of burn from each patient. All cytokines (IL-5, IL-6, IL-10, G-CSF, TNF and IFN) were evaluated using the ELISA technique. Swabs were cultured and the bacterial growths were tested for sensitivity to 20 different antibiotic discs. Hematological tests were performed and compared to the healthy group control using the automated five parameter counter. The mean serum levels of the cytokines in both female and male patients were significantly higher when compared to the healthy control group. Hematological parameter level results showed an increase in WBC count in both female and male samples, eosinophil and lymphocyte levels decreased in both genders. Neutrophils have significantly increased in both female and male samples. Monocytes, basophils, and RBC count showed no significant difference in both female and male patient samples. The mean concentrations of Hb and platelets have significantly increased in males but no significant difference in female samples. In the bacteriological cultures, only 21 samples showed a bacterial growth. 14 were females and 7 were males. 7 Gram negative bacteria and 13 Gram positive bacteria were detected. 6 total bacterial species were identified (*Enterococcus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Enterobacter*, and *Pseudomonas*). Antibiotic sensitivity results showed that the number of sensitive bacteria was significantly higher than the resistant bacteria. 14 of the patients had 3rd degree burns. 5 had 2nd degree burns, and 1 had 1st degree burn.

Further immunological studies are required to interpret the high mortality rates in burned patients.

I. INTRODUCTION

Interleukin (IL-5) is an anti-inflammatory cytokine that has been demonstrated to be involved in cardiovascular diseases, including aortic aneurysm and heart failure [1]. Human IL-5 was found to selectively stimulate morphologica1 changes and the function of human eosinophils. This molecule is thus a prime candidate for the selective eosinophilia and eosinophi1 activation seen in the disease, immune consequences of burn injury [2]. IL-5 is a homodimer cytokine that plays a role in the differentiation, maturation, migration, growth, sustenance, transport, and effector function of eosinophils in blood and tissues, along with basophils and mast cells [2]. The cytokines of the IL-6 family are four-helical proteins that have been categorized into a single family due to their use of the gpl30 receptor subunit. The IL-6 family cytokines include IL-6, IL-ll, ciliary neurotrophic factor (CNTF), leukemia inhibitory factor (LIF), oncostatin M (OSM), cardiotrophin 1 (CT-l), cardiotrophin-like cytokine (CLC), and IL-27 [3]. The IL-6 family includes seven 4-helix cytokines that bind to the receptor complex, including gpl30 (IL-6, IL-l1, LIF, OSM, CNTF, CT-l, and CLC). Furthermore, IL-27 is a heterodimer cytokine made up of the 4-helix protein p28 and a soluble cytokine receptor known as EBI3, which is an Epstein-Barr virus-inducing gene [4]. IL-6 is one of the most important inflammatory cytokines; IL-6 is unique in signaling via a membrane bound and a soluble receptor. Intriguingly, these two pathways strongly differ in their biologic consequences [5]. IL-l0 is a cytokine that plays a vital role in restricting the host's immune response to pathogenic bacteria, which yields to prevent any kind

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of damage to the host. In addition to that, it maintains homeostasis. Any kind of irregularity of the IL-10 is the result of an infection with a high risk of developing auto-immune diseases [6].

Tumor necrosis factor-alpha (TNF-α) is a highly pleiotropic cytokine involved in a spectrum of physiological processes that control inflammation, anti-tumor responses and homeostasis through two receptors, TNF-R1 and TNF-R2 [7].

Interferon gamma (IFN-γ) is a proinflammatory cytokine produced by activated NK cells, NKT cells, effector T CD4+ cells (T helper 1 cells, Th1) and CD8+ T cells (cytotoxic T cells) during the immune response against intracellular pathogens [8].

Granulocyte colony-stimulating factor (G-CSF or GCSF), also known as colony-stimulating factor 3 (CSF 3), is a glycoprotein that stimulates the bone marrow to produce granulocytes and stem cells and release them into the bloodstream [9].

Skin is the primary physical and chemical barrier against pathogens, producing antimicrobial peptides in particular. Burns destroy this barrier, making the body more susceptible to infections due to a lack of vascularity, immune deficiency, and repeated surgery [10]. A burn is a sort of injury to the skin or other tissues caused by heat, cold, electricity, chemical products, friction, or radioactivity; superficial or first-degree burns affect only the top layers of the skin. They are red without blisters, and the pain usually lasts three days [11].

Skin burns are one of the most severe injuries, causing tissue damage that extends further than the wound to distal organs such as the gastrointestinal tract, liver, and lungs. The second leading cause of death after burns is a multiorgan failure and causes excessive systematic and localized inflammation, which contributes directly to end organ damage [12].

Aim of study

This study was conducted to evaluate the levels of the different cytokines (IL-5, IL-6, IL-10, G-CSF, TNF and IFN) in burn patients, as a reflection of heir immunological status.

II. MATERIALS AND METHODS

A. Patients and Controls

A total of 44 burn patients were included in this study. They were newly admitted patients to the "West Emergency Hospital" in Erbil City from November, 2019 till February, 2020. Another 40 apparently healthy individuals (20 males and 20 females) were also included in the study as the control group (C.G.) for the cytokines (IL-5, IL-6, IL-10, G-CSF, TNF and IFN) and hematological parameters.

B. Sample collection

Blood samples

A total of 44 blood samples were collected aseptically from the burn patients. 7 ml of blood were taken from each patient and control group by sterile disposable syringes. Blood samples were placed into Gel tube (5 ml) after that centrifuged at 2500 rpm for 15 minutes for serum collection, and EDTA tube (2 ml) for performing hematological parameters. The serum of each patient was stored in Eppendorf tubes at -20°C until use, for performing IL-5, IL-6, IL- 10, G-CSF, TNF and γ -IFN level by ELISA.

C. Swab samples and Cultures

In parallel with blood sampling, swab samples were taken from each patient from the burn area for bacteriological culturing and antibiotic sensitivity, using sterile swabs and transferred to the laboratory for processing.

All the media and solutions used in this work were sterilized using an autoclave at 121°C, 15lb/in^2 for 15 min. The medium was cooled after autoclaving to 45-50°C and poured in Petri dishes on a leveled surface to a depth of 4 ml. When the medium solidified, the petri dishes were placed in the incubator at 35-37 °C for 15-30 minutes to let the excess moisture evaporate.

D. Inoculation of the test plates

The samples of fresh swabs were prepared and labeled for each patient with their corresponding plates to avoid mixing. Thes swab sample was streaked on the plate once and the streak-plate method was used by sterile loop on the blood agar and MacConkey agar and placed in incubator at 37 °C for 24 hours, after that Growth identification.

E. Microscopic examination (Gram staining film) of the culture

Smears were prepared by taking a colony, with a bacteriological loop, of growth from the bacterial culture onto a glass slide and mixing with a drop of distilled water, stained with Gram stain and examined using oil immersion objective lens under 100X magnification. The Gram stain was used to differentiate between Gram positive (purple/blue

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color) and Gram negative bacteria (pink/red color) in according to the regulatory diagnostic system which are based on.

F. Cytokine Measurement

The Human Interleukins- IL-5, IL-6, IL- 10, G-CSF, TNF and γ –IFN tests (China) were performed for each sample after thawing the serum sample and bringing it to room temperature, using the ELISA test as mentioned in the kit instructions.

G. Hematological Tests

Complete blood count (CBC) determinations were made within l hour of sample co1lection using an automated 5-parameter (Total WBC, differential WBC, RBC, Hb, Platelets) mednic coulter (Convergys X3) for each patient and control group.

H. Diagnostic emphatic tests

The diagnostic emphatic tests for the bacteria were done by using API (Analytical Profi mdex) in Med 24 lab according to the regulatory diagnostic system which is based on [13].

Antibiotic sensitivity was made using the disc diffusion test with 20 different antibiotic discs on Mueller Hinton agar. The inhibition zone was read according to the standard of each antibiotic.

I. The Statistical Analysis

Data were evaluated; analyzed and statistically pooled SPSS version l8 was used for computer programming. Quantitative variables were compared using the Student t-test. Results were considered significant and highly significant, if the *p* value was 1ess than 0.05 and 0.0l, respectively.

III. RESULTS

A. Gender of the burn patients

Figure (1) represents percentage of the gender of burn patients. The numbers of patients were (44) 23 (52%) were females and 21 (48%) were males.

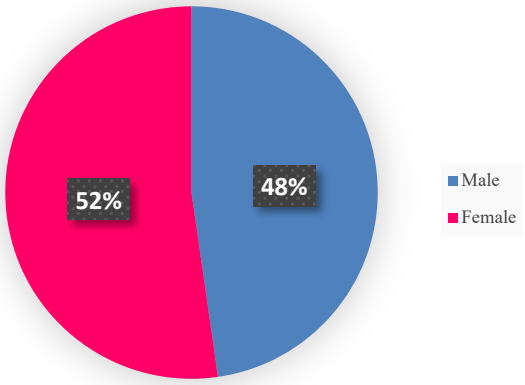

Figure 1: Gender of burn patients Evaluation of cytokine levels among burn patients and control group

The means of serum (IL- 5, 6, and10) and G-CSF levels in both male and female burn patients were significantly higher when compared to the healthy control ($p < 0.01$) (table 1).

While means of serum TNF-α and IFN-γ 1evels in male and female of the burn patients showed no significant decrease between the means of male patients and healthy group ($P \geq 0.05$), but reveals high significant dropping when female patients compare with healthy group ($P < 0.01$), presented more details in table (1).

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Table 1: Mean serum levels of (IL- 5, IL-6, IL-10, G-CSF, IFN-γ, and TNF-α) among males and females of burn patients and healthy group.

Parameter	Sex	No.	Burn Patients Mean ± SE	No.	Healthy Group Mean ± SE	T-test P value	P
IL-5 (pg/ml)	M	21	4.40 ± 1.66	20	4.74 ± 0.22	0.854	N.S
	F	23	4.50 ± 1.25	20	4.75 ± 0.21	0.863	N.S
IL-6 (pg/ml)	M	21	4.41 ± 0.91	20	8.35 ± 0.22	0.000	H.S.**
	F	23	0.84 ± 0.75	20	9.51 ± 0.23	0.000	H.S.**
IL-10 (pg/ml)	M	21	34.57 ± 11.81	20	4.95 ± 0.36	0.021	H.S.**
	F	23	29.77 ± 6.83	20	4.78 ± 0.32	0.001	H.S.**
G-CSF (pg/ml)	M	21	27.63 ± 3.84	20	42.80 ± 2.89	0.003	H.S.**
	F	23	54.16 ± 23.29	20	43.00 ± 2.89	0.639	N.S
IFN-γ (pg/ml)	M	21	36.12 ± 3.40	20	41.75 ± 2.87	0.214	N.S
	F	23	43.39 ± 7.82	20	41.10 ± 3.09	0.787	N.S
TNF-α (pg/ml)	M	21	5.99 ± 1.98	20	8.24 ± 0.66	0.294	N.S
	F	23	3.64 ± 0.42	20	9.83 ± 0.81	0.000	H.S**

P: Probability; P value ≥0.05: Non significant; *P va1ue <0.05: Significant; **P<0.0l: Highly Significant

B. Measurement of hematological parameters among burn patients and healthy group

Table (2) shows all means of hematological parameters versus healthy group mean levels. When the mean of total WBC count in male and female burn patients was compared to the healthy group, it showed a high1y significant increase (p<0.0l). While, mean level of lymphocytes and eosinophil counts of male and female burn patients reveals a highly significant decrease when compared to healthy group (p<0.0l), but the mean of neutrophil count of male and female burn patients presents a highly significant rise in comparison to the mean neutrophil counts of healthy group (p<0.0l). However, the mean counts of monocyte and basophils showed a non-significant difference between male burn patients and healthy group (p≥0.05), and female burn patients showed a slight significant change in comparison to the healthy group.

The meant count of RBC in both male and female burn patients showed a non-significant difference when compared to the healthy group (p≥ 0.05).

The mean concentration of Hb in male burn patients reveals a significant increase when compared to males in healthy group (p< 0.05), while the females showed a non-significant change in comparison to the healthy group (p≥ 0.05). Platelet mean count in male burn patients presents a significant rise when compared to the healthy group (p< 0.05). However in female burn patients it showed a non-significant increase in comparison to the healthy group (p≥ 0.05).

Table 2: Hematological parameters among male and female burn patients and healthy group

Hematological Parameters	Gender	Burn Patients No	Burn Patients Mean ± SE	Healthy Group No	Healthy Group Mean ± SE	T-test P value	P
Total WBC $10^3/\mu L$	M	21	11.64 ± 1.12	20	6.45 ± 0.13	0.000	H.S.**
	F	23	14.04 ± 1.67	20	6.58 ± 0.14	0.000	H.S.**
Lymphocyte $10^3/\mu L$	M	21	1.96 ± 0.19	20	2.85 ± 0.13	0.001	H.S.**
	F	23	1.19 ± 0.12	20	2.81 ± 0.13	0.000	H.S.**
Monocyte $10^3/\mu L$	M	21	0.43 ± 0.16	20	0.50 ± 0.06	0.692	N.S.
	F	23	0.97 ± 0.16	20	0.49 ± 0.06	0.014	S.*
Neutrophil $10^3/\mu L$	M	21	8.15 ± 1.08	20	4.08 ± 0.14	0.001	H.S.**
	F	23	12.58 ± 2.51	20	4.19 ± 0.14	0.003	H.S.**
Eosinophil $10^3/\mu L$	M	21	0.09 ± 0.01	20	0.20 ± 0.03	0.007	H.S.**
	F	23	0.02 ± 0.01	20	0.18 ± 0.02	0.000	H.S.**
Basophil $10^3/\mu L$	M	21	0.09 ± 0.01	20	0.10 ± 0.02	0.681	N.S.
	F	23	0.04 ± 0.01	20	0.11 ± 0.01	0.017	S.*
RBC $10^6/\mu L$	M	21	5.44 ± 0.30	20	5.05 ± 0.13	0.249	N.S.
	F	23	4.71 ± 0.27	20	4.98 ± 0.12	0.279	N.S.
Hb g/dL	M	21	15.62 ± 0.70	20	13.95 ± 0.13	0.029	S.*

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Platelet 10^3/µL	F	23	13.77 ± 0.77	20	12.95 ± 0.13	0.305	N.S.
	M	21	271.33 ± 20.21	20	217.50 ± 6.61	0.018	S.*
	F	23	269.47 ± 25.74	20	213.10 ± 6.12	0.062	N.S.

P: Probability; P value ≥0.05: Non significant; *P value <0.05: Significant; **P<0.01: Highly Significant

C. *Bacteriological culture and sensitivity*

From the 44 patients, 41 swabs were taken; it was not possible to take swabs from 3 patients. After 24 hours of incubation, 21 samples showed no bacterial growth, while 20 samples showed bacterial growth. No significant difference (p≥0.05) is found between both.

From the samples that had bacterial growth, 7 of the total isolates were Gram positive and 13 were Gram negative, the difference being highly significant (p<0.01). The bacterial species that were found were six, three Gram positive and 3 Gram negative. No significant difference (p≥0.05) was found in the number of cases of each bacterial species (table 3).

Table 3: Bacterial species found in burn swabs of patients.

Gram stain	Bacterial species	Number
Gram positive	*Enterococcus* sp.	2
	Staphylococcus aureus	3
	Staphylococcus epidermidis	2
	Total	**7**
Gram negative	*Escherichia coli*	4
	Enterobacter sp.	4
	Pseudomonas sp.	5
	Total	**13****

P: Probability; P value ≥0.05: Non significant; *P va1ue <0.05: Significant; **P<0.0l: Highly Significant

Out of the 20 patients that had a bacterial growth in their swabs, l4 were females and 6 were males, showing a highly significant difference (p<0.0l). The bacterial species found varied and Gram negative bacteria were significantly higher (p<0.0l) than Gram positive bacterial species among female patients, while in males it was equal (table 4).

Table 4: Bacterial species found in burn swabs of male and female patients.

Gender	Bacterial species	Number
Female patients	*Enterococcus* sp.	1
	Staphylococcus aureus	2
	Staphylococcus epidermidis	1
	Escherichia coli	4
	Enterobacter sp.	4
	Pseudomonas sp.	2
	Total	**14****
Male patients	*Enterococcus* sp.	1
	Staphylococcus aureus	1
	Staphylococcus epidermidis	1
	Pseudomonas sp.	3
	Total	**6**

P: Probability; P va1ue ≥0.05: Non significant; *P va1ue <0.05: Significant; **P<0.0l: Highly Significant

Concerning sensitivity of the bacterial species to the 20 antibiotics used, the number of sensitive bacteria were significantly higher (p<0.01) than those that were resistant to all antibiotics used (table 5).

Table 5: Antibiotic sensitivity and resistance of bacterial species found in burn swabs of patients

Antibiotic	Number of Sensitive species (%)	Number of Resistant species (%)
Amikacin [AK]	19** (95)	1 (5)
Amoxicillin/ Clavulinic acid [AMC]	12** (60)	8 (40)
Ceftraxone [CRO]	16** (80)	4 (20)
Levofloxacin [LEV]	15** (83)	3 (17)
Azithromycin [AZM]	9** (90)	1 (10)
Cephotaxime [CTX]	16** (94)	1 (6)
Ciprofloxacin [CIP]	14** (78)	4 (22)
Gentamicin [CN]	17** (100)	0 (0)
Nalidixic acid [NA]	10** (77)	3 (23)
Nitrofurantoin [F]	20** (100)	0 (0)
Norfloxacin [NOR]	8* (62)	5 (38)
Trometheprim/Sulfomethoxazole [SXT]	13** (65)	7 (35)
Tobramycin [TOB]	18** (90)	2 (10)
Meropenem [MEM]	12** (100)	0 (0)

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Imipenem [IPM]	12** (100)	0 (0)
Vancomycine [VA]	8** (100)	0 (0)
Clindamycin [DA]	8** (100)	0 (0)
Doxycycline [DO]	8** (100)	0 (0)
Rifampin [RA]	7** (100)	0 (0)
Tetracycline [TE]	5* (71)	2 (29)
P: Probability; P va1ue ≥0.05: Non significant; *P value <0.05: Significant; **P<0.0l: Highly Significant		

Antibiotic sensitivity profile of all bacterial species isolated is shown in figure (2).

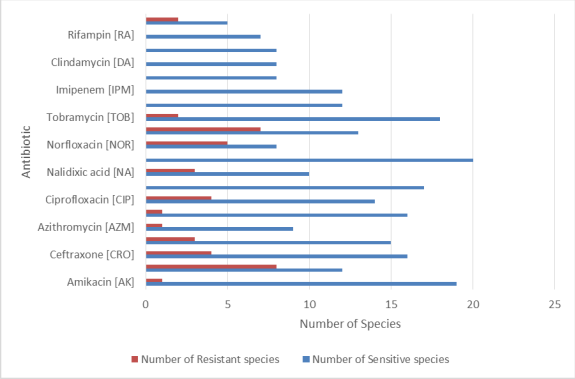

Figure 2: Antibiotic sensitivity profile of the bacterial species isolated from burn patients

D. Location of burns

Among the 20 patients that had bacterial growth in their swabs, 11 (55%) had whole body burns, while other patients had burns in different locations, making the difference between whole body and other locations highly significant (P<0.01) (table 6).

Table 6: Location of burns in patients who showed bacterial growth in their swabs

Burn location	Number
Whole body	11**
Legs	3
Hand/ leg	1
Thighs	1
Feet	1
Face/ neck/ hands	1
Limbs	1
Hips	1
Total	20
P: Probability; P va1ue ≥0.05: Non significant; *P va1ue <0.05: Significant; **P<0.0l: Highly Significant	

E. Degree of burns

For those patients who showed bacterial growth, 14 (70%) of them had 3rd degree burns, 5 (25%) had 2nd degree burns and 1 (5%) had first degree burns, making the difference highly significant (P<0.01) when comparing the 3rd degree burns to the other two degrees (table 7).

Table 7: Degree of burns in patients who showed bacterial growth in their swabs

Degree of burn	Number
1st	1
2nd	5
3rd	14**
Total	20
P: Probability; P va1ue ≥0.05: Non significant; *P va1ue <0.05: Significant; **P<0.0l: Highly Significant	

IV. DISCUSSION

Forty four burn patients were enrolled this current study, which included 23 (52%) females and twenty-one (48%) males. Similar results were obtained in a study conducted in India by [14], where more females (76.4%) were affected as compared to males (23.6%).

Burns are among the most prevalent and lethal types of trauma, affecting public health, causing millions of deaths annually [15].

According to the current laboratory results of the samples, the mean levels of hematological parameters among burn patients and healthy group, it is evident that the mean level of serum IL-10 in both female and male burn patients were remarkably higher in comparison to the healthy control mean level. It was evident that the increase of the serum IL-10 was detected in mostly non surviving patients with proven sepsis [16].

IL-10 is not the only signaling evidence to prove the presence of an infection. Furthermore, based on the current results, the mean of total WBC count to the healthy group, in both males and females showed an outstanding increase. This implies to WBC being a possible laboratory marker of a predictable infection thus foreshadowing a possible acute or chronic infection [17].

The mean level of lymphocytes and eosinophil of the male and female patients, when compared, showed that their numbers has drastically dropped. Since the evidence of the effect of the burn resembles the regulation of the ATCH (adrenocorticotropic hormone), sodium and chloride retention plus increased potassium loss occurs, in addition to carbohydrate metabolism, hyperglycemia,

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glycosuria, and lactic acid acidemia. In addition to that, it is also an indication of possibly having a longer stay in the hospital and these patients are 7 times more likely to develop an infection [18].

Serious burns have been shown to cause immunosuppression, making burn patients more vulnerable to infectious complications. Early reports of immunodeficiency following burns were linked to research on "burn toxins" [19].

IL-6 is a significant pleiotropic inflammatory cytokine that is elevated in the cardiovascular and wound sites. Higher IL-6 levels are also linked to poor burn patient outcomes [20]. IL-6 is a multi-functional cytokine capable of promoting both beneficial and detrimental outcomes depending on the level of IL-6 [21].

According to the stated that the mean IL-1β and IL-6 levels, white blood cell count (WBC), red blood cell count (RBC), percentage of lymphocytes (Lym%), mean corpuscular volume (MCV), and platelet count (PLT), and Procalcitonin (PCT) were significantly higher in people exposed to high magnetic field than in the unexposed group. On the other hand, mean serum levels of IL-6, IL-1β and some hematological parameters including WBC, lymphocytes, RBC and hematocrit showed the highest levels in those exposed magnetic field [22]. However, as compared to the in this research mean of burn patients for hematology factor of RBC for male burn patients was 5.44 ± 0.30 of burn patients while as compared to the female burn patients of Red blood cells (RBC) hematological factor mean was 4.71 ± 0.27 of burn patients as well as of mean of white blood cells (WBC) of burn patients for both genders (male and female) mean was 11.64 ± 1.12 for male burn patients and 14.04 ± 1.67 for female burn patients.

Granulocyte colony-stimulating factor or Granulocyte macrophage colony-stimulating factor therapy significantly increased infection recovery, but not all-cause mortality, in-hospital mortality, or adverse events at 14 or 28 days, according to this meta-analysis. It was not associated with a significant reduction in patients with sepsis. As a result, our current meta-analysis does not support the routine use of G-CSF or GM-CSF in sepsis patients [23]. To investigate mHLA-DR-guided G-CSF or GM-CSF therapy in patients with sepsis-related immunosuppression, large, prospective, multicenter clinical trials are required. Tissue damage caused by heat, chemicals, electricity, sunlight, or nuclear radiation is referred to as a burn. Hot liquids or vapors, building fires, and flammable liquids and gases are the most common causes of burns [24].

According to [25] demonstrate that (TNF) was produced in wound tissue, peaked on day one, and then returned to baseline levels. Even at maximum production, the increase in TNF levels was not statistically significant. This could be due to high TNF levels detected shortly after the wound (0 hours), which could mask the production of relatively small amounts of this cytokine. TNF was detected constitutively in skin tissue taken from untreated humans in a study, with an average of 5.99 for men and 3.64 for women with burns.

An identification that showed us that our research has similarities with the research of [26], that the predominant bacteria was *Pseudomonas* sp. (5 samples), but no *Acinebacter sp.*, *Proteus mirabilis*, *Klebsiella sp., Citrobacter sp., Klebsiella oxytoca, Proteus vulgaris* were found.

Conclusion

The mean serum levels of the cytokines in both female and male patients were significantly higher when compared to the healthy control group. Hematological parameter level results showed an increase in WBC count in both female and male samples, eosinophil and lymphocyte levels decreased in both genders. Neutrophils have significantly increased in both female and male samples. Monocytes, basophils, and RBC count showed no significant difference in both female and male patient samples. The mean concentrations of Hb and platelets have significantly increased in males but no significant difference in female samples. In the bacteriological cultures, only 21 samples showed a bacterial growth. 14 were females and 7 were males. 7 Gram negative bacteria and 13 Gram positive bacteria were detected. 6 total bacterial species were identified (*Enterococcus*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Enterobacter*, and *Pseudomonas*). Antibiotic sensitivity results showed that the number of sensitive bacteria was significantly higher than the resistant bacteria. 14 of the patients had 3rd degree burns. 5 had 2nd degree burns, and 1 had 1st degree burn.

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