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Research Article

EVALUATION THE IMMUNE STATUS OF THE BURN PATIENTS INFECTED WITH BACTERIA *PSEUDOMONAS AERUGINOSA* IN KARBALA CITY

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ABSTRACT

The present study aimed to release frequency pseudomonas aeruginosa bacterium Pseudomonas aeruginosa among burns patients in the province of Karbala ratio. With shown immunological changes to the patient due to various injuries, burns with the aggravation of the injury that the germ and the evolution of the case of septicemia and death, so it was in the period from 08/16/2014 to -16 2-2014 Leather collect swabs from 64 patients of burn patients admitted to the Hussein Teaching Hospital In Karbala, and after that the cultivation of P.aeruginosa isolated by 45% of all burns swabs Group. When examining some immunological criteria for patients with these bacteria Was clearly evident and clear rise in the number of white blood cells in patients where proportional rise with the degree of burning and intensity, reaching the preparation of cells to mm3) / 103) 10.44, 13.222 and 15.955 for each of the patients with simple and moderate to severe burns group, respectively, as was the rise in particular for neutrophil white blood cells compared to other cellular species.

The concentrations of both acute phase protein and supplemented significant increase was found in the acute phase Brocaat values and which is growing directly proportional to the severity of the burn for up to 63.4 in simple burns while reached 95.2 and 121.8 for each of moderate to severe burns respectively.
The concentrations of complement protein C3 has statistical analyzes showed a non-significant increase complement each level of the third degree burns and Statistics by mgldL 125.45, medium and severe 137.5 mgldL 140.15 mgldL compared with healthy people 122.32 mgldL. While was significant rise in motor values cellular IL-6 for up to 118 pg / ml, 86 pg / ml, 33 pg / ml for each of the patients Statistics burns and moderate to severe respectively. On the contrary, it has recorded kinetic values cellular TNF higher higher in patients with severe burns, reaching 600.45 pg / ml compared to people healthy 175.15 pg / ml..
Keywords: *Pseudomonas aeruginosa*, burn patients, Brocaat values, complement protein C3.

INTRODUCTION

It provided burns and is one of the most forms of skin bruise time period as a result of the loss of his job and the defense, which result in the possibility of injury septic therefore require rapid intervention needed to reduce injuries and deaths among burn patients for medical care (Ekrami & kalantar, 2007). A patient who suffered a severe thermal injury burns, especially second-degree burns and the third suffers to large immunological and physiological changes where it is immune changes the main reasons that make the patient susceptible to blood sepsis where you get an increase in the effectiveness of the macrophage after obtaining thermal injury severe, leading to increase the effectiveness of those cells, which in turn lead to stimulate the inflammatory mediators initial production (Proinflammatory mediators) such as prostaglandins, TNF, IL-6, IL-1 and other inflammatory factors other also to increase these factors role in suppressing the immune system cells and thus the patient

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becomes susceptible to injury bloody sepsis as a result of what is accompanied by the burning of failure in many events physiological members of the patient's body, which is considered one of the major causes of morbidity and death in these patients (Meakins, 1990 Harris & Gelfand, 1995; Yamada etal., 2000;).

Upon entering the strange nurse to the human body, the immune system to distinguish those nurses through receptors on the surface of macrophages of cells granular A granulocyte cells only Monocyte cells, Macrophage, which is the first step to create an immune response against the nurses cells, while the second step is to activate all of the tracks Complement Complement system)) and inflammatory mediators Interlukin)) that operate on the overlap and organize the work of immune cells specialized, such as white blood cells neutrophil cells and macrophage then activate the immune response of cellular gained and immune cell helper T first two types, and the second (T helper cell 1 & 2) (Sadikot et al; 2005).

In thermal injuries, burns contaminated with germs note there is an increase in the number of white blood cells (Leukocytosis) to attack the bacteria involved in the thing's body infected, which depends on the presence of high movement of these defensive cells and they are attracted to the site of the injury quickly, leading to a decrease in these blood cells in the bloodstream thing which makes the body has difficulty in getting rid of pathogenic microbes hyphen to the blood thus the deterioration of the patient's condition and his death(Gamelli et al., 1986)).

acute phase protein one of the proteins that increase their level during the presence of inflammation in the body, so the high level of this protein in Patients serum is a sign of the presence of inflammation as the acute phase proteins are a group of proteins that already exist in the plasma of human blood low concentrations susceptibility to sedimentation the presence of calcium and increasing emancipation under certain conditions such as tumors and tissue damage burns and injuries germ even respiratory effects possible affect on levels in human blood plasma (Dannacco & sansonno, 2005).

CRP and acute phase proteins and the detachment of the cells of the liver inflammation due to infection, which affects the body causing raise the levels of cytokines produced by macrophages in the blood. In addition to that this protein is one of the immune proteins as a level less than 1 mg / L in the normal case and its level rises in the acute inflammatory conditions is evidence of inflammation (Yu, 2014).

Adding that the level of the complement proteins significantly change after various burns where reduced its focus in proportion to the severity of the burn and then return to rise above the natural level of it after a limited period as the increase C5a levels and C3a result in changes in blood pressure and vascular permeability as well as significant changes in the the functions of the egg cells, it was found that small amounts of the complement protein C5a leads to increase the functional activity of the cells of eggs, while the excess of it amounts lead to curbed and this thing also applies to the C3 b (Bengtson and Heideman, 1987; Yurt and Pruitt, 1986).

Play other immune proteins known as interleukins an important role in determining the effectiveness of the immune system in patients with burns like interleukin 6 (IL-6), which give rise for glycoprotein Glycoprotein molecular weight of 26 kDa and is produced from a single cell nucleus, macrophage , lymphocytes , endothelial cells , cells of B and T and the cornea cell in skin and others. Future IL-6R consists of two series of proteins first unit low familiarity series Alpha (chain) output and the complex will be linked with beta chain (chain) in excess of the value of linking and transmission of signals into the cell When for damage in the tissues of the body injury will begin of Cellular immunity by inflammation of topical response through the ability to Langerhans island cells in cooperation with the T and its neighboring keratinocytes in the epidermis area norepinephrine many immune components such as IL-6, which has an important role in the body's immunity and to be those components immune to the skin, called tissue fibro-related skin device (Parslow et al., 2001). The Alpha TNF is a mediator inflammatory first Proinflammatory liberated mainly of the only machrophage cells that are the main source of liberation. There is also a wide range of cells can heading such as mast cells and cells lymphatic T, B, NK cells NK, cells and neutrophil and endothelial cells and smooth muscle and cardiac fibroblasts and bone-building cells and this factor as one of the important media is to transmit signals and perform many of the immunological events that outgoing signals result in a wide range of cellular responses of programmed cell

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death(Bouwmeester et al., 2003; Brodley & combridge). In general cause immune to decline due to severe burns fundamental changes in the balance between the helper cells ratio of the first to the second helper cells occur as well as between the percentage of cells lymph assistance (CD4 TH - lymphocytes) cells ballasts (CD8 suppresser T cell) (Guo et al 0.2003). Therefore During the first week of burning observed decline in the totals Bcell B lymphocytes that are commensurate with the severity of the burn, leading to reduced production immune IgG that does not get low in the formation of antibodies and other B-cells by itself and in these circumstances be the body's reaction firing (Prostoglandin) coupled with the low level of IL-12 which has a synergistic effect in the differentiation of helper cells first to the second aid that promotes humoral immunity by producing dynamics of cellular such as IL-6 and IL-4 and IL cells - 10 B which in turn encourages the production of immune cells (Gharogozalla et al., 2004; Guo et al., 2003).

MATERIAL & METHODS :

Sample collection

Were collected 64 Leather tinge of burn patients admitted to the Teaching Hospital Hussein in Karbala province for the period Mn16-2-2014lgaah 16-2014-8 ages and from different samples were collected by the scanners sterile disposable which after only two hours planted on different agricultural circles (general and selective) for the purpose of isolating the bacteria Pseudomonas aeruginosa. Has also been collecting blood samples from all patients under study by syringes medical sterile then transfer 2 ml of blood in EDTA containing tubes while leaving 4 ml of blood in plastic tubes to clot, and blood expel central to separate serum, which saves in the pipeline epnidroff under freeze 18. Until an immunological tests of each of the CRP, C3, IL-6 and TNF

Isolation and diagnose the bacteria Pseudomonas aeruginosa

The samples were planting on the media nutritioni agar, blood agar and macounky agar then dishes were incubated degree of 37°C for 24 hours then I studied the characteristics of the developing colonies on the circles AGRO recipes bacterial cells under an optical microscope compound after dab with Cram dye and observe the shape, arrangement and color that studied the biochemical primary qualities of the isolates Proceeds for characterization biochemically confirmed the results of these tests using the system 20 and Api Alvaateix

Calculation of the total count and differential white blood cells

Total count of WBC

Use the full complet blood count device (CBC) used in the blood disease unit at Al-Hussein Teaching Hospital for the analysis of blood and automatically calculate the total count and differential of white blood cells

Identify some of the humoral immune parameters such as:

• C-reactive protein of Boditech company to measure the qualitative part of the effective protein.

• measuring the serum level of the complement proteins C3 and by spreading the immune radiographic single mode Single radial immunodiffusion using cooked dishes from EASY RED company.

• estimate the level of TNFα in a way adsorption linked immunosorbent Elabscience

• estimate the kinetic cellular level (IL-6) 6 way linked immunosorbent adsorption of BOSTER company

RESULTS AND DISCUSSION:

1. Isolation and diagnose the bacteria Pseudomonas aeruginosa

Planting Wipes group of patients with burns and environment on media mackcouncy agar and nutritious agar for the initial isolation of the bacteria pseudomonas aeruginosa and after the end of the lap amounting to 24 hours at temperature 37 ° m examined developing colonies, which were pale at the center of mackconcy agar a because it is fermented sugar lactose, while the colonies on the central Nutrient large agar her appearance is high and the edges of the flat as well as similar smell the smell of the grape (Grape like odor) and color greenish the ability to produce pigment Pyocyanin, then quoted the developing germ to the center of strmaid agar for being the center of selectively to those bacteria that contend Certrimide that inhibit inhibition other bacteria but pseudomonas aeruginosa, after incbation colonies appeared cleard on the surface that the middle indication as pseudomonas aeruginosa bacterium,

All isolates were holding her many biochemical tests for the bacteria developing biochemical tests results as compatible with approved diagnostic systems (Collee, 1996). And after

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the confirmation of the results was the adoption on both API20 and Vetix system.

The results Showed after completion of initial and confirmatory that tests 35 isolation (45%) were the bacteria pseudomonas aeruginosa from swabs from patients infected burns The findings come with matching results Alghanimi (2014), which showed that the highest percentage of isolated bacterial in burn patients had the bacteria pseudomonas aeruginosa , knowing that the thing that increases the chance of injury that MRSA is a high ability to stay in disinfectants and liquid medicines or treatments, such as eye drops, as well as anesthesia masks and floor galleries and other processes Ryan and Ray, 2004; Qarah, 2004)). Also, stay in the hospital for a long time may lead to a high percentage of the presence of these bacteria and the increasing risk of the especially in patients with severe burns Pollack, 2000; Tielker et al., 2005; Japoni et al., 2009)).

2. Complet and differential count for white blood cells in patients with burns

The total number of blood cells, increased in burn patients as compared to control as shown in Figure (1), where he was high for each of the group Statistics burns and moderate to severe for up to ((10.44, 13.222 and mm3) 15.955 / 103)), respectively, compared to the range control the amount (7.87 mm3 / 103) also increase the number of white blood cells fit with the severity of the burn and the size of the psychological pressure on the person burned as the psychological effects associated with the burning of a large variation in the effect is clear in the immunological criteria (Demling, 2004)

Results also showed a clear increase in the number of blood cells neutrophil as shown in Table (1), where the significant increase at the level of probability ($p < 0.05$) for all grades simple Incineration (7.35) and average (9.25) and severe (11.09) and the control group (3:41). This means that there is an increase in the macrophagic activity in neutrophils in patients two to three times more bad eggs, which indicates to the evolution of the case of acute burn patients (Paraslow et al., 2001). As lymphocytes has recorded a significant decline, especially in cases of burns severe, reaching attribute to 1.379 when compared with the control group (3.02). The results show the presence of high non-moral in the preparation of the only cell nucleus either all of acidophilus cells and grassroots there were not significant changes where it is believed that the reason for this is due to the fact that cells stimulate or increase when there is parasitic injury and hypersensitivity only sensitive and not related to bacterial injury in General (Doan etal., 2008; Mehta and Hoffbrand, 2009).

3. C3 complement protein level in burn patients

Results also showed a clear increase in the number of blood cells neutrophil as shown in Table (1), where the significant increase at the level of probability ($p < 0.05$) for all grades simple Incineration (7.35) and average (9.25) and severe (11.09) and the control group (3:41). This means that there is an increase in the macrophagic activity in neutrophils in patients two to three times more bad eggs, which indicates to the evolution of the case of acute burn patients (Paraslow et al., 2001). As lymphocytes has recorded a significant decline, especially in cases of severe burns,

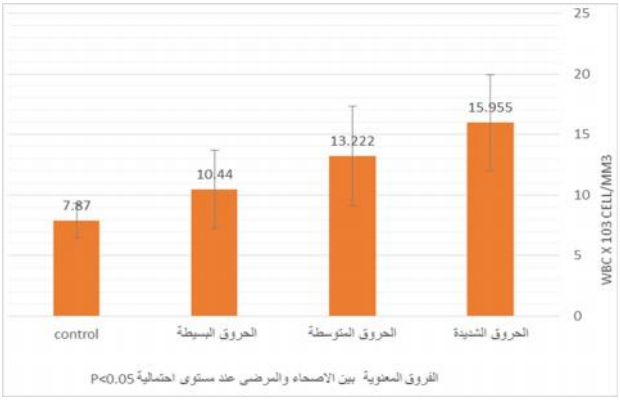

Figure 1: Shows the total count of white blood cells Leukocyte in patients and the control group proportion to the degree of the burn. [L.S.D(0.05)= 2.33]

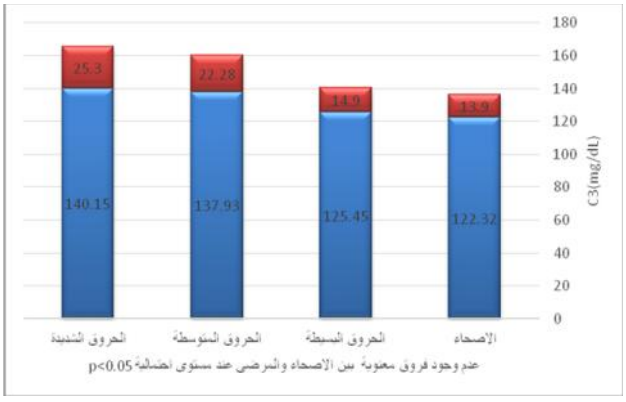

Figure 2: Complement C3 concentration in healthy subjects and patients with burns proportion to the degree of the burn. [L.S.D(0.05)= 12.928]

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Table 1: Differential count of white blood cells in healthy individuals and patients with burn ratios to the point of burning.

Patients			control	Types of WBC cell
Burn degree			M ± N	
Sever burn M ± N	Intermediate burn M ± N	Simple burn M ± N		
11.095±2.231	9.255±3.125	7.353±2.477	3.41 ±1.4	Neutrophils
1.379±0.526	2.077±0.953	2.487±0.804	3.02±0.24	Lymphocytes
1.472±0.395	0.947±0.344	0.823±0.451	0.621±0.17	Monocytes
0.067±0.066	0.075±0.038	0.081±0.063	0.199±0.12	Eosinophils
0.12±0.043	0.144±0.074	0.17±0.086	0.11±0.01	Basophils

reaching attribute to 1.379 when compared with the control group (3.02). The results show the presence of high non-moral in the preparation of the only cell nucleus either all of acidophilus cells and grassroots there were not significant changes where it is believed that the reason for this is due to the fact that cells stimulate or increase when there is parasitic injury and hypersensitivity only sensitive and not related to bacterial injury in General (Doan etal., 2008; Mehta and Hoffbrand, 2009).

4. The acute phase C-reactive protein

Concentration in burn patients increased acute phase protein values in the sera burn patients compared with healthy people showed in figure (3), has revealed the results of statistical analysis of the existence of significant differences for each degree burn (simple and moderate to severe burn) with healthy people at the level of probability(P <0.05).

The reason for increasing the concentration of the acute phase protein to increase the concentration of plasma proteins, which increases its focus in cases of inflammation and tissue damage. He has indicated a study by Jeschke and others (2013) that the high concentration of CRP is linked with the type, size and the severity of those burns.

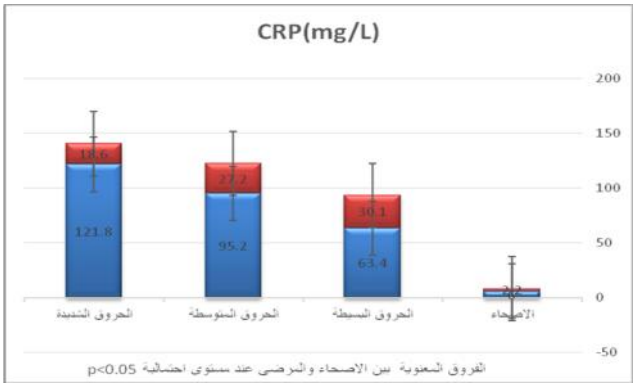

Figure 3: Acute phase protein concentration (CRP) in healthy subjects and patients with burns proportion to the degree of the burn. [L.S.D. (0.05)= 15.81]

Results of our study are consistent to the findings of a beautiful mechanism (2014) and (kingesely & jones., 2008) who indicated that there is a clear rise in the levels of acute phase proteins in patients with burns.

5. Cell kinetic level 6 ((IL-6 in patients with burns:

The results showed of the current study for a rise in cell kinetic values 6 (IL-6) in the sera infected burns compared Bash. Also revealed the results of the statistical analysis of the existence of significant differences at the level of probability (p <0.05) in IL-6 from minor burns and patients with moderate to severe 118,86,33) pg / ml values), respectively, and the control group pg / ml (15) as shown in the table and (Figure 4). Current results agreed with Jubouri (2008), which confirmed the presence of a rise in the level of cellular motor 6 in patients with burns.

Some empirical studies have indicated that the tumor factor necrotic and IL-1 both types of alpha, beta and IL-6 and IL-10 and IL-8 is put up in large quantities in cases of severe burns inflamed and the level of asking those cytokines vary depending on the type that burns and wounds and the size of those burns, as well as the patient's age(Kim etal., 2012).

Infection is an inflammatory response acute with dysfunction is a complex process of cellular events to control the infection (Cheung et al., 2008) Kmaan role of developing germ stimulate macrophage cells mediated by microbial signals help her launch of inflammatory mediators such as IL-I and IL-12 and TNf- and INF- and IL-8, which induce localized inflammation then will increase blood flow topically to attract neutrophil cells to the area to kill germs (Munford and Pugin, 2001)

Also, most of the cells in the skin can produce cellular dynamics such as stratum and T , macrophages and fibroblasts, So the impact topically and then travel to the Hungarian bloody ((Patricia et al., 1999 and this was

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confirmed by researcher Ross (2002) when injected mice laboratory under the skin with fatty polysaccharide isolated from negative bacteria gram and which led to the evolution of the case of inflammation the appearance of large amounts of IL-6 in the bloodstream thing that could be due to the introduction of these quantities produced topically to the Hungarian General the injury severe burns lead to stimulate the inflammatory response Preliminary to increase production and liberation of cytokines inflammatory initial of each of the TNF-α IL-1β, and IL-6, which is working on the alert to prevent the entry of bacterial action pathogen so can be seen rising these factors after 12) -24) hours of injury, trauma or burn (Gauglitz etal. , 2008; Maass etal., 2002).

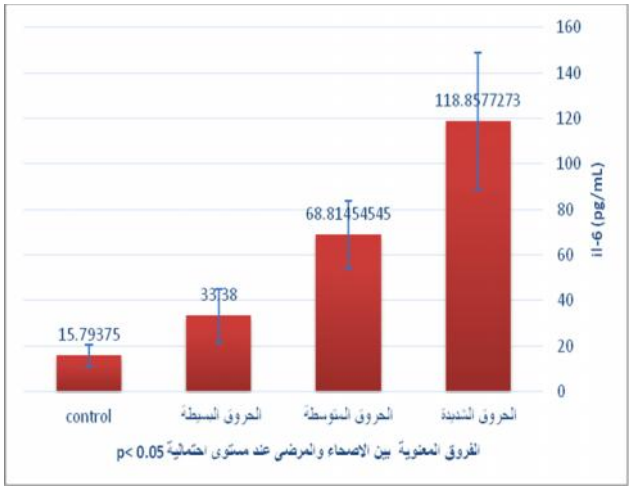

Figure 4: The level of cellular motor 6 (IL-6) in patients ratios to the point of burning. [L.S.D. (0.05) = 11.804]

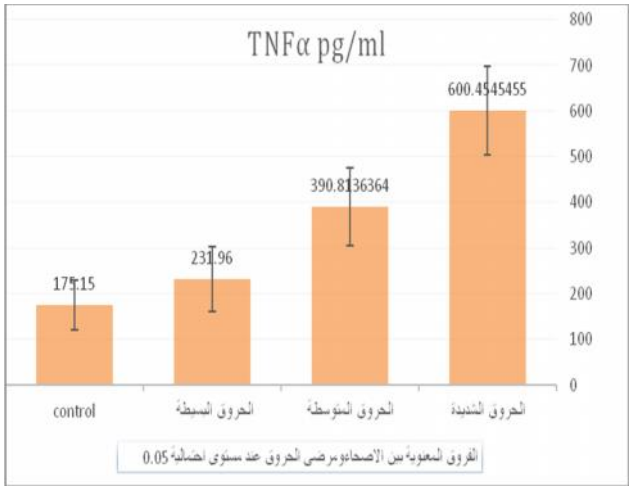

Figure 5: Shows the level (TNF) in patients ratios to the point of burning. [L.S.D. (0.05) = 50.663]

6. level of kinetic cellular -TNF in burn patients:

The results of the current study showed increase the values of kinetic cellular -TNF in sera infected burns compared with healthy people shown in the figure(5) and) recorded the highest increase in his patients with severe burns reaching(600.45 pg / ml) compared to people healthy (175.15 pg / ml) as statistical analysis of the existence of significant differences at the level of probability of detection ($p < 0.05$) in TNF between healthy and groups of patients who suffer from simple burns and moderate to severe values.

When you get bruises on the skin bruising example, the macrophage cells and cells of Langerhans provide antigen (Ag-presentation) to T Tcell cells which works to motivate and then start doubling quickly under the influence of the type of cytokines is IL-1 stimulate cellular dynamics by inflammation, such as IL - 6, IL - 8, IL-2 and IL-4 and IL-5, which works to stimulate and doubled the B cells produce antibodies antibody also launches keratinocytes cells, macrophages also factor nostril of tumors (TNF-), which contribute to the lifting of the inflammatory response and the expression of complex compatibility Histological (MHC) and thus helps to antigen presenting cells inflammatory (Garcia et al., 2005) in addition to the fact that the type IL-1 is a factor chemical attraction (chemotaxis) Direct-white cells, it is in collaboration with TNF- search expression of adhesion molecules between cells (ICAM) Intercellular adhesion molecule on epithelial cells and fibroblasts t that cause adhesion of the white cells with epithelial cell surfaces (Schumann et al., 1998; Dinarello, 1997).

Some studies have also indicated that the level of some interleukin be linked with the size of those burns whenever there was a large-sized high levels of cytokines and this speech not for all interleukin. In a study conducted at Children's Hospital in the state of Texas that the level of interleukins , INF IL-4, IL-1B, IL-2 was not affected by the concentrations with increasing size burn area, while the interleukin other IL-6 level, TNF and IL-8 recorded high concentrations compared with healthy controls in the case of children suffering from severe burns (Jeschke etal., 2007).

Some empirical studies have indicated that the tumor factor necrotic and IL-1 both types of alpha, beta and IL-6 and IL-10 and IL-8 is put up in large quantities in cases of severe burns inflamed and the level of asking those cytokines vary

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depending on the type that burns and wounds and the size of those burns, as well as the patient's age Kim etal., 2012)). Also, the role of bacterial infection in cells stimulate macrophage-mediated microbial signals help her launch of inflammatory mediators such as IL-I and IL-12 and TNF- and INF- and IL-8, which induce localized inflammation then the blood flow will increase topically to attract neutrophil cells to the area to kill germs (Munford and Pugin, 2001)

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