Study of Some Clinical, Bacteriological and Immunological Aspects of Patients with Burn Injury in Hilla

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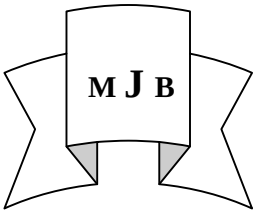

Abstract

During the period between November, 2005 and May, 2006 , a total of 78 skin swabs, 48 blood specimens from 78 burn patients, 30 swabs from the burn unit at Al-Hilla General Teaching Hospital, and 12 blood specimens from normal healthy subjects(controls) have been bacteriologically and immunologically studied.

It has been found that Gram negative bacteria are more frequent than Gram positive type in skin, blood and burn unit specimens. *Pseudomonas aeruginosa* is the most frequent species among the Gram negative bacteria in skin and burn ward. On the other hand, *Staph. aureus* is the most frequent isolate among Gram positive bacteria.

The level of Boyd index is more than (80) for the dead cases.

It was observed that there is no significant difference($p>0.05$) between the effect of silver sulphadiazin(1%) and silver nitrate(0.5%) on bacterial skin isolates. The most affected bacterial species by SSD is the *Staph. epidermidis* and the least susceptible is *Staph. aureus*, whereas the most affected bacterial species by SN is *P.aeruginosa* and the least affected are *Enterobacter* and *Staph. aureus*.

Immunologically, there is a significant decrease($p<0.05$) in the mean level of IgG and IgA. IgM is not increased($p>0.05$) during bacterial infection of burn victims. Likewise, C3 and C4 complement components are not increased as a mean level for burn victims when compared to that of controls($p>0.05$).

الخلاصة

شملت هذه الدراسة التي استمرت للفترة من تشرين الثاني (2005) إلى أيار (2006) (78) مسحة جلد، (48) عينة دم من مرضى الحروق و (30) مسحة من ردهة الحروق التابعة الى مستشفى الحلة التعليمى العام, إضافة إلى (12) عينة دم من الأشخاص الأصحاء للمقارنة وخضعت جميع العينات للدراسة البكتريولوجية والمناعية. كانت البكتريا السالبة أكثر شيوعا من البكتريا الموجبة في عينات الجلد والدم وردهة الحروق كما وأن بكتريا(*Pseudomonas aeruginosa*) كانت أكثر الأنواع عدداً من بين البكتريا السالبة في عينات الجلد. أما بكتريا(*Staphylococcus aureus*) فهي الأكثر عدداً من بين البكتريا الموجبة.، إن مستوى مؤشر (Boyd) لحالات الوفيات بسبب الحروق كان أكثر من (80).

أظهرت الدراسة بأنه لا يوجد أي فرق معنوي (قيمة الاحتمالية > 0.05) بين تأثير السلفرسلفاديازين1% والسلفرنايتريت 0.5%على عزلات بكتريا الجلد وان اكثرانواع البكتريا تأثرا بالسلفرسلفاديازين هى *Staph. epidermidis* واقلها تأثرا *Staph. aureus* بينما اكثرانواع البكتريا تأثرا بالسلفرنايتريت هى *Pseudomonas aeruginosa* واقلها تأثرا *Enterobacter* و *Staph. aureus* .

كان معدل مستويات الكلوبيولينات المناعية نوع IgG ، IgA قليلا في المرضى المصابين باخماج الحروق. أما معدل مستوى IgM فأنه لم يظهر أية زيادة فى حالات اخماج الحروق(قيمة الاحتمالية >0.05) وبالمثل فأن معدل مستويات مكونات نظام المتمم C3 ، C4 لم يزدد في مرضى الحروق

بالمقارنة مع الأشخاص الأصحاء (قيمة الاحتمالية > 0.05).

Introduction

Burns are one of the most common and devastating forms of trauma. They induce a state of immunosuppression that predisposes burn patients to infectious complications[1].

Burn injury destroys the physical skin barrier that normally prevents the invasion of microorganisms and consequently, this injury provides novel sites for bacterial colonization, infection and clinical sepsis[2].

Initially the burned area is considered free of major microbial contamination. However, Gram positive bacteria in the depth of sweat glands and hair follicles may survive the heat of initial injury and unless topical antimicrobial agents are applied, these bacteria heavily colonize the wounds within the first 48 hours post-injury.

The organisms that predominate as causative agents of burn wound infection in any burn unit change over time where Gram positive organisms are initially prevalent and then gradually superseded by Gram negative opportunists[3].

This study aims to isolate and identify the aerobic bacteria from burn patients as well as from burn unit, study the effect of silver sulfadiazine and silver nitrate on bacterial skin isolates, clarify some clinical parameters for burn patients and the Boyd index for the dead cases, and study some humoral immunological factors in burn patients.

Patients and Methods

1- Patients

A total of seventy eight(78) thermally burned patients(36 males and 42 females) whose ages range between(2-65) years were included in this study which lasted from November/2005 to May/2006. Those patients were clinically diagnosed by specialist doctor as having burn wound infection and were admitted to the burn unit at Al-Hilla General Teaching Hospital. They were suffering from second to third degree (flame and scald) burn injury and their burn percentage was ranging from 10-80% from the total body surface area(TBSA).

2- Controls

Twelve apparently healthy subjects (clinically assessed by specialist doctor) were included as controls in this study. Their ages range between (10-40) years.

3- Specimens Collection

Skin and Burn Unit Swabs

Skin swabs were taken from the pus of the burned area of all patients before the bathing of the affected area(before hydrotherapy). Thirty swabs were taken from the burn unit(medical appliances). Each swab was placed in a sterile tube containing normal saline till reaching the laboratory to be inoculated on culture media(Blood agar, MacConkey agar and Nutrient agar) and incubated aerobically for 24- 48 hours at 37C˚[4].

Blood Samples

Out of the (78) burned patients, 48 patients were subjected for blood sampling. Blood samples were applied for aerobic bacterial cultivation [4]. Those patients who were positive regarding blood culture were included in the immunological assays by using their sera.

Bacterial Diagnosis

Isolation and identification of bacteria were carried out according to [4-8].

The Effect of Silver Sulphadiazin and Silver Nitrate on Bacterial Growth

The effect of silver sulphadiazin(SSD)(1%) and silver nitrate(SN) (0.5%) on the growth of bacterial isolates was carried out according to **[**9].

Determination of Immunoglobulin and Complement Levels

This is done according to [10] by the single radial immunodifusion(SRID) test.

Statistical analysis:

Mean, standard deviation, and T-test [p-value (0.05)] were used as statistical parameters in this work [11].

Results

Burn Injury and Mortality

The results shows that both the positivity of skin culture in relation to the total number of cases for each percentage of burn and the number of the deaths increase with the increment of the percentage of burn. The number of dead cases is increased when burn percentage is equal or more than 40% and reaching maximum when burn percentage is 60% and more.

In this study, all the dead cases were symptomatic and have positive blood culture for bacteria namely *Enterobacter*, *Pseudomonas* and *Staph. aureus*. The death occurs during hospitalization and specifically in the second week of admission to the hospital. Their symptoms were fever more than 38.5 C˚, hypotension, pain, tachycardia, tachypnea, oliguria and disorientation. They were suffering from second degree deep partial thickness to third degree full thickness burns. The Boyd index (Table1), which is the result of the summation of the age of burn patients in years and the percentage of the burn, is a good predictor for the severity of burn injury. If the Boyd index is 80 or more means that there is a high probability of death from burn injury [12].

Table1 Boyd Index for The Dead Cases

No. of Deaths	Bacterial Blood Isolates	Age (year)	Percentage of burn	Boyd Index
1	*Enterobacter* spp.	2	25	27
2	*P. aeruginosa*	9	40	49
3	*Staph. aureus*	13	40	53
4	*Staph. aureus*	65	50	115
5	*P. aeruginosa*	20	60	80
6	*P. aeruginosa*	18	70	88
7	*Enterobacter* spp.	29	75	104
8	*Enterobacter* spp.	18	80	98
9	*Enterobacter* spp.	18	80	98

Isolation of Bacteria

The results shown in Table(2) revealed that 108 samples(69.2%) gave positive bacterial culture whereas 48(30.8%) showed no bacterial growth(negative bacterial culture). Regarding skin swabs, 67:78 (85.9%) were positive bacterial cultures consisting of single growth 56(83.6%), and mixed bacterial growth 11(16.4%). Meanwhile, no bacterial growth was found in 11:78 (14.1%) of skin swab cultures.

In this study, all the controls were negative for bacterial blood culture.

Table2 Number and Percentage of Bacterial Isolates from Burn Patients and Burn Unit

Result	Source of Culture			
	Skin No. (%)	Blood No. (%)	Burn Unit No. (%)	Total of Samples No. (%)
Culture Positive	67 (85.9%)	24 (50%)	17 (56.7%)	108 (69.2%)
Single Growth	56	24	10	90 (83.3%)
Mixed Growth	11	0	7	18 (16.7%)
Culture Negative	11 (14.1%)	24 (50%)	13 (43.3%)	48 (30.8%)
Total	78 (100%)	48 (100%)	30 (100%)	156 (100%)

Types of Bacterial Isolates

As shown in Tables(3) and (4) which showed the frequency of bacteria in skin swab, blood and burn unit cultures, it is clear from the total number of isolates that Gram negative bacteria are more frequent than Gram positive type.

Table 3 Frequency of Gram Positive Bacteria

Skin		Blood		Burn Unit	
Bacterial Isolates	Frequency	Bacterial Isolates	Frequency	Bacterial Isolates	Frequency
S. aureus	4	*S. aureus*	7	*Enterococcus faecalis*	4
S.epidermidis	2	β-haemolytic streptococci	2	*S. aureus*	3
Enterococcus faecalis	1	*S.epidermidis*	2	*S.epidermidis*	1
Total	7	Total	11	Total	8

Table 4 Frequency of Gram Negative Bacteria

Skin		**Blood**		**Burn Unit**	
Bacterial Isolates	**Frequency**	**Bacterial Isolates**	**Frequency**	**Bacterial Isolates**	**Frequency**
P.aeruginosa	27	*Enterobacter spp.*	7	*P.aeruginosa*	6
Enterobacter spp.	20	*P.aeruginosa*	6	*Enterobacter* spp.	3
E.coli	12	——	——	*E.coli*	3
K.pneumonae	7	——	——	*A. baumannii*	2
A.baumannii	3	——	——	*K.pneumonae*	2
Proteus spp.	2	——	——	——	——
Total	**71**	**Total**	**13**	**Total**	**16**

Effect of Silver Sulphadiazin and Silver Nitrate on Bacterial Isolates of Skin

The effects of SSD and SN are shown in Table (5). There is no significant difference between the effect of SSD and SN on all the studied bacterial isolates (P>0.05).

The most affected bacterial species by SSD is the *Staph. epidermidis* and the least susceptible is *Staph. aureus*, whereas the most affected bacterial species by SN is *P.aeruginosa* and the least affected are *Enterobacter* and *Staph. aureus.*

Table 5 Effect of Silver Sulphadiazin and Silver Nitrate on Bacterial Isolates from Burned Skin

Bacterial Isolates	Diameter of Inhibition Zone(mm)		Significance
	SSD 1%	SN 0.5%	
Staph. aureus	**13**	**13**	Not Significant P>0.05
Staph. epidermidis	**23**	**18**	Not Significant P>0.05
P. aeruginosa	**17**	**19**	Not Significant P>0.05
***Enterobacter* spp.**	**15**	**13**	Not Significant P>0.05
K. pneumoniae	**18**	**14**	Not Significant P>0.05
E.coli	**20**	**18**	Not Significant P>0.05

Humoral Immune Response of Burn Patients

The results expressed in Table (6) show that there is a significant decrease in the serum level of IgG of burn patients in comparison with the controls (P<0.05).

The mean serum levels of IgA are also significantly decreased in the burn victims in comparison with the control individuals(P<0.05). Regarding the mean serum levels of IgM there is no significant difference(P>0.05) between burn patients and normal control individuals.

Table 6 Concentrations of Immunoglobulins IgM, IgG and IgA(mg/dl) in Burn Patients and Controls

		IgM	IgG	IgA
Patient	**M**	**152.1789**	**1422.6070**	**90.0632**
	SD	**39.1567**	**802.2898**	**44.7629**
Control	**M**	**138.0200**	**2343.3960**	**348.9000**
	SD	**40.2094**	**476.4445**	**80.0047**
Significance		**Not Significant P>0.05**	**Significant 0.05< P**	**Significant 0.05<P**

Regarding the complement components: C3 and C4 levels Table (7) the results of this study revealed that there is no significant difference (P>0.05) between the mean level of serum C3 in patients and in the controls. The same results are obtained regarding serum C4 estimation for both patients and control individuals.

Table 7 Concentrations of Complement Components C3 and C4 (mg/dl) in Burn Patients and Controls

		C3	C4
Patient	**M**	**133.1684**	**31.0211**
	SD	**54.4345**	**15.5358**
Control	**M**	**122.3200**	**43.5600**
	SD	**33.0349**	**14.2532**
Significance		**Not Significant P>0.05**	**Not Significant P>0.05**

M= mean
SD= standard deviation

Discussion

The Boyd index explains that both the age and the percentage of the burn are important risk factors for mortality. The socioeconomic factors, especially poverty, are important determinant of the occurrence of burn injury, but patient factors such as age are the major determinant of survival[13].

The risk factors for septicemia in burn patients including patient factors like extent and depth of burn, the age of patient, pre-existing diseases and the physical environment of the wound itself and microbial factors that influence the balance between resistance and susceptibility to infection[14].

All the dead cases in this work were females. This may be attributed to the immunosuppression effect of female sex hormones because estrogen mediates a sex difference in the post burn period; this immunosuppression increases the susceptibility to sepsis[15].

The predominance of Gram negative bacteria is clear from the high frequency of *P.aeruginosa* in each source of the cultures. This agrees with [16,17]. The reasons for this high prevalence may be due to factors associated with the acquisition of nosocomial pathogens in patients with recurrent long term hospitalization complicating illnesses, prior administration of antimicrobial agents and the immunosuppressive effects of burn trauma[18,19].The source of *Enterobacter* bacteraemia is either from burn wound or as a result of bacterial translocation which is caused by failure of the gut barrier, the imbalance of intestinal flora and impaired host immune defenses[20]. It is clear that the burn wound can be contaminated by microorganisms that migrate from the gastrointestinal, urinary and respiratory tracts [21]. This indicates the idea of autoinfection that the burn patients suffer from in addition to the infection acquired from the burn unit itself[22]. *Klebsiella*, *Proteus*, *Enterococcus faecalis, E.coli*, *Acinetobacter* and others were isolated from burned patients in frequencies less than that of *Pseudomonas* and *Staph. Aureus*[23,24].

Pseudomonas colonized in the intestinal tract of burn patients is noxious and can be fatal as a pathogen of post-burn infection. Furthermore

selective digestive decontamination (decontamination of endogenous pathogens in the intestinal tract) is essential in preventing post-burn infection associated with bacterial translocation [25].

In this study the most frequent Gram positive bacteria isolated from the blood is *Staphylococcus aureus* followed by β-hemolytic streptococci and then *Staphylococcus epidermidis*. These results were approximately fitted with that of [26] who found that MRSA comprised 92% of the Gram positive bacteria isolated from blood of burn patients. Whereas the most common bacteria isolated from blood culture were *Staph. aureus* followed by coagulase negative Staphylococci(CoNS). Furthermore, CoNS should be considered as an important pathogen for sepsis in burns. The organism, being ubiquitous in a hospital environment, and burn wounds being the ideal medium for its multiplication, it is hardly surprising that this bacteria would be the cause of 20.7% of septic episodes [27].

Although *Staph. epidermidis* is usually non-pathogenic, it is an important cause of infection in patients whose immune system is compromised, or who have indwelling catheters[28,29].

It was found that 0.5% SN and 1% SSD had an effect in the reduction of the incidence of burn wound sepsis[30]. Besides, the SSD was the drug of choice for prophylaxis of burn wound infection in most burn patients[31],

This low level of IgG expressed in this work agrees with[32,33].This low level of IgG may predispose burn patients to bacterial infection[34].

The serum level of IgM in burn patients may remain within the normal levels.; this may be interpreted as there is failure of IgM-B-cells to produce an increased level of IgM in these patients. This may indicate that the rate of IgM is not so enough to overcome the bacterial infection and this may be caused by the suppressor effect of burns on humoral immunity [35-37]. The improvement of immune state of burn victims is now considered as one of the most important ways in the treatment of burn infection[38].

Furthermore, the recognition of immunoglobulin deficiencies: IgM, IgG and IgA in burn patients may permit focused therapy, such as specific replacement of these proteins[39].

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