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The antigenicity of *Staphylococcus aureus* in rats

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Abstract

Staphylococcus aureus was isolated from patients of Hilla hospital for surgery .The killed antigen of *S. aureus* was prepared and mixed with incomplete Freund's adjuvant then injected in rats . The results revealed that the effect of *S. aureus* antigen in cellular immunity which including ; phagocytosis ,endocytic killing by neutrophils and ingestion of bacteria by phagocytes at different times of incubation in rats primed with bacterial antigen . The means of Phagocytosis were reached 15.2 ، 35.4 ،and 50.7 after 10 ,30 and 50 min of incubation respectively ،while the means of endocytic killing were reached 4.8 ،4.9 ،and 9.8 at the same times mentioned above . Furthere more ،the rates of ingestion were 0.48 ,0.178 and 0.196 at the same times respectively،while lower in above tests in control rats . The concentrations of total protein ،albumin and crude globulins were appeared lower in treated rats compared with control animals ،which reached 9.45 ,8.02 and 1.45 g/L, while it was 12.34 ,10.44 and 1.9 g/L for control group respectively .These results suggests that the killed antigen of *S. aureus* stimulated the cellular immunity ،while it was suppressive for humoral immunity in rats.

Keyword :- ***Staphylococcus aureus*. antigens . phagocytosis .Humoral . Immunity . Rats**

Introduction:-

Staphylococcus aureus is a gram positive bacterium that has remained a persistent pathogen (Vancey ،1999) for diverse spectrum of human and animal diseases (Garloni and Zanetti ،2005). The pathogenicity of this bacteria is mainly due to the production of a number of secreted and cell surface- associated proteins (Mullarky *et al*., 2001) ،furthermore ،this pathogen regarded as storage for toxin ،exoenzymes ،adhesions and immune-modulating proteins (Fournier and Philpotte,2005).The outer cell wall of *Staphylococci* is composed of exposed peptidoglycan and lipoteichoic acids and a range of other toxic secreted products (Sriskandan and Cohen,1999) ،and these components induced release of the TNF-α,IL-6 and IL-10 in human blood (Wang etal.,2000). The toll-like receptor 2 (TLR2) is a signaling receptor for peptidoglycan from *S. aureus* and *Streptococcus pneumonia* (Schmander *etal*. ، 1999 ; Yushimura *et al*.,1999) .In addition ،there is some evidence that Staphylococcal virulence was by impeding phagocytosis in the infected hosts (O'Riordan and Lee ،2004) .Phagocytosis of microorganisms is a key element in host defense against bacterial infections (Silverstein and Steinberg .,1989) . There are two principal mechanisms of phagocytosis ,the first is opsonin- dependent phagocytosis (Wright and Silverstein ,1986) ,while the second is opsonin- independent phagocytosis (Ofek *et al* ., 1995).Furthermore ,phagocytes such as macrophages and dendritic cells must differentiated infections self from non infectious agents and initiate an appropriate inflammatory response (Hoffmann *et al*.,1999).

Neutrophils are the principle line of defense during the initial stages of Staphylococcal infections ، (Paape *et al*.,1979) . Pincus *et al*., (1976) reported that the individuals with neutropenia are more susceptible to *S. aureus* infections . Defects that alter normal PMN cells function ،such as chronic granulomatus diseases predispose individuals to serious *S. aureus* infections (Lekstrom-Himes and Gallin ،2000) .

Albumin is a 69-KDa plasma protein with a variety of physiological functions including ; maintain the blood pressure ،transport to and from tissues of multiple substances and etc., (Emerson,1989 ;Peters ,1985) . Furthermore ،Iglesia *et al* . ،(1999) reported that albumin is a major serum survival factor through it's ability to inhibit of apoptosis by at least two mechanisms : carriage of lysophatic acid and scavenging of reactive oxygen species .

The increased levels of γ-globulin in serum related to chronic inflammatory diseases ، including infection ،liver diseases ،autoimmune diseases ،IgM in viral disease and early bacterial disease ،while the decreased levels of γ-globulins in serum related to immunodeficiency (genetic or acquired)(Johnson *et al*. ،2004)

We chose *S. aureus* a prototypical gram –positive microorganisms and an important cause of life threatening bacterial infections in human ،therefore ،the aim of this study is to investigate the cellular and humoral immune response of killed *S. aureus* antigen in rats .

Materials and Methods :-

1- Laboratory animals :-

Twenty mature native rats *Rattus rattus* which have been brought from Karbala university ،college of science ,biology department . Their blood were tested for bacterial infections and it was negative for bacterial pathogens and their respective specific antibodies . Rats were kept through out experimentation periods at libidum conditions of ration and housing (Schneider *eta*l . ،1990) . The weight of such animals ranged between 300-350 gm and their ages ranged from 4-6 months .

2- Bacterial Isolates :-

The *S. aureus* was isolated from respiratory of patients in Hilla hospital for surgery according to Macfaddin (2000) .The bacteria were cultured over night in nutrient broth to make certain that they will be in a logarithmic growth phase then washing by sterile normal saline (0.85%) and diluted to give suspension of a bout 1×10^7 cell/ ml .Cells was counted using neubeur chamber (Chruchshank *et al*., 1975).

3- Preparation of *S. aureus* antigen :-

The antigen of this bacteria was prepared according to the Bradshaw (1996).

4- Immunization protocol of rats :-

The rats were divided into two groups ,ten replicates for each group .The first group was treated with *S. aureus* antigen ,while the second group ,was treated with sterile normal saline (0.85%) .

The animals of the first group were injected subcutaneously four doses (0.8 ml) for 2 weeks intervals . These doses which include antigen mixed with incomplete Freund's adjuvant in ratio 1:1 ,then the doses above were divided into four parts (0.2 ml for each) ،and each of sub doses were injected in the two sides of the neck region and both sides of the groin region (Bradshaw,1996) .

5- Blood samples:-

The blood samples were collected directly from the immunized and controlled rats by heart puncture, seven days after the last injection . The samples were kept in sterile tubes containing anticoagulant (heparin) AFM –Dispo and placed in refrigerator at 4º C in order to measure the phagocytic activity and the concentrations of total protein ،albumin and crude globulins .

5-1:- Phagocytosis Test :-

The means of phagocytosis ,endocytic killing by neutrophils and rates of ingestion for *S. aureus* by phagocytes were measured .The test was done by mixed blood of immunized and controlled rats with suitable a mount of bacterial isolate ,then incubated at 37ºC for different times (10 ,30 and 50 minutes) ,after that prepare the slides ,stain and examine under oil immersion microscope .(AL-Barrak ،1997)

5-b:- Blood proteins ،Albumin ,Globulins concentration

The total protein ,albumin and crude globulin were estimated according to manual procedure of linear chemical procedure (company of Almacen Joaquim.costa ,Montgat ,Barcelona(spain).

6- Calculation :-

A. The positive percentages for neutrophils

$$= \frac{\text{Number of phagocytic cells for bacteria}}{\text{Total number}} \times 100$$

OR

$$= \frac{\text{The number of bacteria}}{\text{100 neutrophils}}$$

$$B.\quad \text{Endocytic killing} = \frac{\text{Bacteria (B)/Neutrophils (N)}}{\text{Time of incubation}}$$

(AL-Barrak ،1997)

7- Biometry:-

The results of the experiments were analyzed using completely randomized design (Donald,1989).

RESULTS :-

Table(1) showed a gradual increase in phagocytosis of *S. aureus* with the time of incubation of the whole blood- bacteria mixtures at times 10 ,30 and 50 minutes ،but these increase were higher in treated group (plate 1) compared with control group (plate 2). In immunized rats the mean of phagocytosis were 15.2 ،35.4 and 50.7 at time intervals of 10 ,30 and 50 minutes respectively ,while it was 25 ،29.6 and 43.6 in control animals at the same time intervals .

Table (1) :- The means of phagocytosis of *S. aureus* in rats primed with *S. aureus* antigen at different times of incubation .

Groups	number	phagocytosis mean± standard Error		
		10 min.	30min.	50min.
Treatment	10	15.200 ± 1.339	35.40± 3.419	50.70 ± 3.249
control	10	25.00 ± 0.948	29.60 ±1.630	43.60 ± 1.568

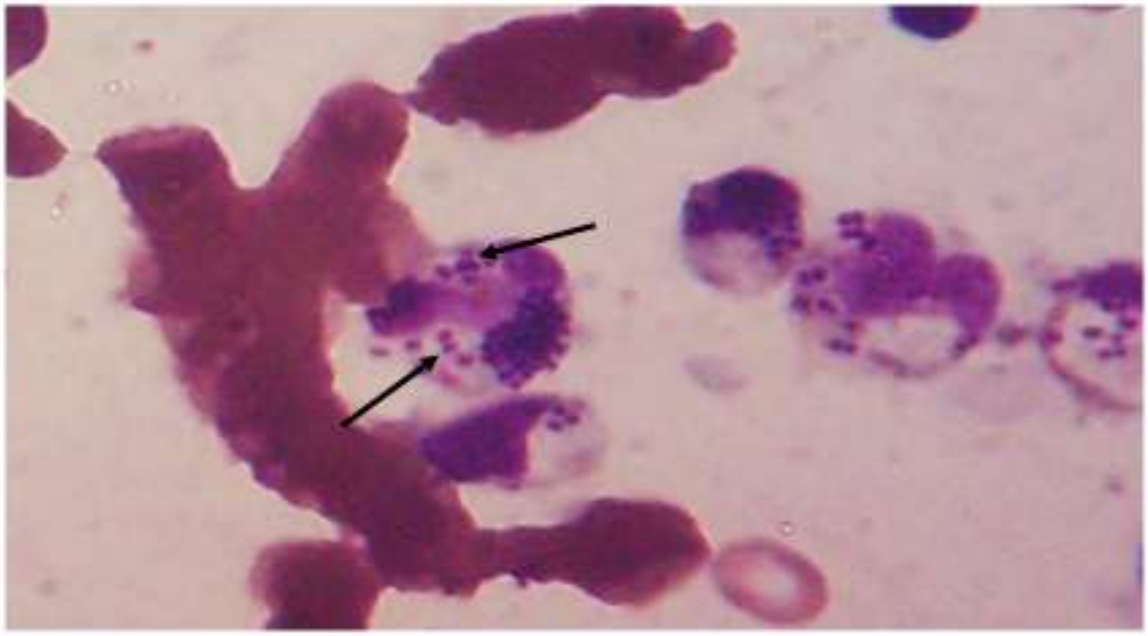

Plate (1): Phagocytosis of *S. aureus* by neutrophils (X100 stained by leishman)

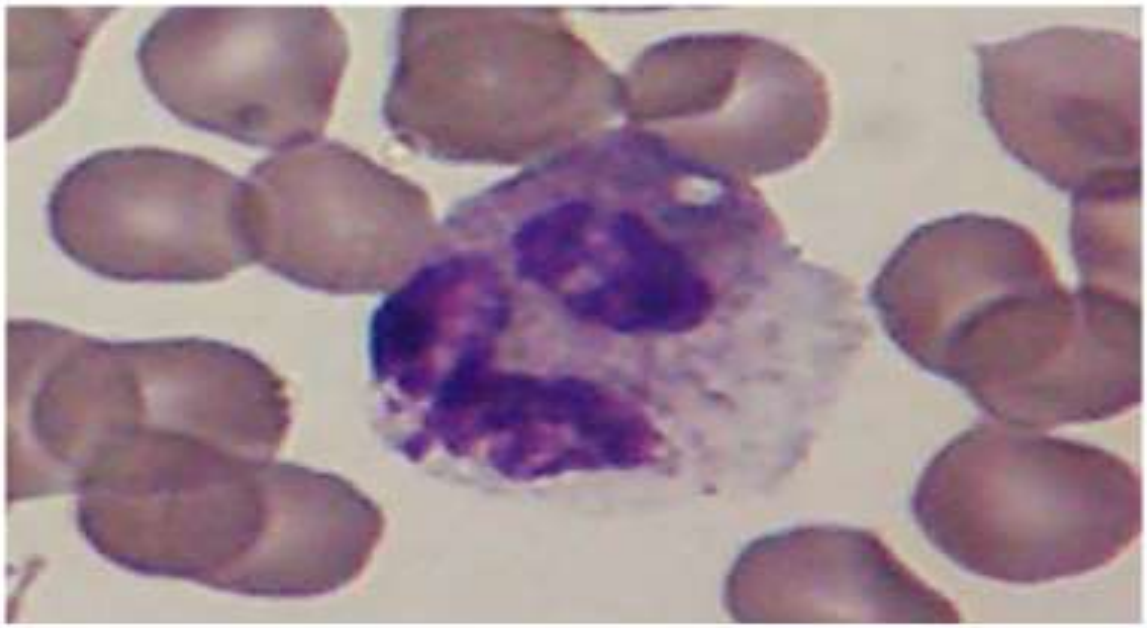

Plate (2): Neutrophil negative (X100 stained by leishman)

The endocytic killing of this bacteria by neutrophils also were increased with the time of incubation and higher in treated group than that in control group . The mean of endocytic killing reached 4.8 ،4.9 and 9.8 at times mentioned above consuctively in immunized rats ، while it was 3.4 ،4.4 and 4.6 at times 10 ,30 and 50 minutes respectively (Table 2).

Table (2):- The endocytic killing of *S. aureus* by neutrophils in rats primed with *S. aureus* antigen at different times of incubation

Group	number	B/N mean ± standard error		
		10 min.	30min.	50min.
Treatment	10	4.800± 0.61	4.900 ± 0.566	9.800 ±1.404
control	10	3.400 ± 0.244	4.400 ±0.509	4.60 ±0.509

Meantime ,the rates of ingestion of *S. aureus* by phagocytes ,were higher in rats immunized with bacterial antigen in comparison with control group ،and reached 0.48 ،0.178 and 0.196 in treatment at times mentioned above respectively ,while it was 0.136 ،0.150 and 0.116 in control rats at the same times respectively(Table 3).

Table (3):- The rates of ingestion of *S. aureus* by phagocytes in rats immunized with *S. aureus* antigen at different times of incubation .

Groups	number	Rate of ingestion mean ±standard Error		
		10 min.	30min.	50min.
Treatment	10	0.480 ±0.061	0.178 ±0.022	0.196 ±0.028
control	10	0.136 ±0.010	0.150 ±0.013	0.116 ±0.006

There were a decreased in mean of concentration of total protein in treated group (9.450 g/ml) compared with control (12.340 g/L) ،and these results showed a high significance difference between both groups (Table 4).

Table (4) :- The concentrations of total proteins (g/L) in rats primed with *S. aureus* antigen

Groups	number	Total protein (g/L) Mean ± standard error
Treatment	10	9.450 ± 0.351
control	10	12.340 ± 0.282

Meantime ,the concentrations of albumin in control group were higher than that in treated group ،also there was a high significance difference ($p< 0.01$) between two groups of animals (Table 5).

Table (5) :- The concentration of albumin (g/L) in rats primed with *S. aureus* antigen

Groups	number	Albumin (g/L) Mean ± standard error
Treatment	10	8.020 ±0.273
control	10	10.440 ± 0.112

Furthermore the mean of concentration of globulins in rats primed with *S. aureus* antigen were lower than that in control group ,it was reached 1.450 and 1.900 g/L in groups mentioned above respectively (Table 6) .

Table (6) :-The concentrations of globulins (g/L) in rats primed with *S. aureus* antigen .

Groups	number	Globulin (g/L) Mean ± standard error
Treatment	10	1.450 ± 0.118
control	10	1.900 ± 0.238

Discussion :-

Table 1 ،2 and 3 showed significant increases in means of phgocytosis ،endocytic killing and rate of ingestion of *S. aureus* in the rats primed with *S. aureus* antigen compared with the control animals. The peptidoglycan and lipoteichoic acids of *S. aureus* induce release of TNFα ،IL- 1B and IL-6 from cultured macrophages – monocytes (Bhakdi *et al* . ، 1991). However this bacterium have been shown to activate T – cell subset (Teisser *et al* .,1998) as well as provide protection by delay apoptosis in rat neutrophils (Moulding *etal* .,1999) . Silverstein and Steinberg (1989) illustrated that the roles of type I and II class a scavenger receptors (SR- AI/II) in innate immunity to bacterial infections by recognize their cell wall products (lipopolysaccharide and lipoteichoic acids) ،therefore ,mice deficient SR –AI/IIare more susceptible to intraperitoneal infection with *S. aureus* than control mice .In addition ،Thomas *et al* .,(2000) reported that the opsonin-independent phagocytosis of bacteria is a critical determinant of host survival in bacterial infection . The bacteria or bacterial products stimulate a systemic macrophages in reticulo- endothelial system (RES) and circulating leukocytes (Brandtzaeg *et al*.,1996) . Phagocytes recognition of foreign or

modified ligands ,such as bacteria and apoptotic cells ,is an essential component of both the innate and adaptive immune responses. Furthermore ,particle recognition and internalization is mediated by a variety of phagocytic receptors including the FcγR ,CR3 ,the mannose receptor and members of the surface receptors family (Harvey and Champe , 2008) .Sriskandan and Cohen (1999) showed that the Staphylococcal infection induces an influx of neutrophils . Indeed ، *S. aureus* is apyogenic pathogen capable of tissue invasion and evasion of phagocytosis by neutrophils .The innate immune system is capable of recognizing pathogens provides a first line of defense to the host and this system initiates a sequences of events that result in the production and secretion of awide range of inflammatory cytokines and chemokines ،the activation of macrophages/ monocytes ,and the initiation of adaptive immunity (Aderem and Ulevitch ,2000;Schnare *et al*.,2001) .The polymorphonuclear leukocytes are important for human innate immunity by killing most invading bacteria ,such as *S. aureus* and these pathogens avoid destruction by PMNs to survive (Voyich *et al*.,2005) .In addition ,neutrophils are a critical component of innate immunity and are essential for controlling bacterial infections (Lekstrom-Himes and Gallin,2000).

Results have showed a decreases in means of total protein ،albumin and globulins in primed rats compared with control animals . The inflammatory response initiated by peritoneal inoculation of dilutes *S. aureus* promotes the influx of neutrophils, monocytes and plasma proteins ,such as complement and antibodies (Thomas *et al* . ،2000),furthermore (Johnson *et al*. ،2004) illustrated that the clinical conditions that affect serum protein level :acute inflammation ،chronic inflammation ,protein loss and redistribution of body fluids .Meantime ،peptidoglycan is a human T-cell mitogen as well as an activator of B-cell because *S. aureus* contains super antigens (Rasanen and Arvilommi . ،1982). White blood cells can phagocytize kill and degrade the pathogen ،and the molecules resulting from this degradation are then presented to T-cells to activate adaptive immunity (Aderem and Ulevitch ,2000; Schnare *et al*., 2001). Thereby ،the adaptive immune system recognize pathogens by antigen receptors that are expressed at the surface of B and T lymphocytes ,these receptors are characterized by specificity and memory (Takeda and Akira, 2003).

The decreased levels of albumin in serum related to acute phase response ,liver disease ,increased concentrations of immunoglobulin and genetic an albuminemia ،while the increased level of albumin in serum related to acute dehydration(Johnson *et al* .,2004).

The significant of this paper lies in the investigation of the humoral and cellular immune response of *S. aureus* in rats that mimics the *S. aureus* infection and their antigenicity in human.

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الخلاصة

تم عزل بكتريا المكورات العنقوديه الذهبيه من مرضى مستشفى الحله الجراحي . حضر المستضد المقتول لتلك البكتريا ومزج مع مساعد فرويند غيرالكامل ، ثم حقن في الجرذان البيض.اظهرت النتائج تأثير مستضد البكترياعلى المناعه الخلويه والتي تضمنت البلعمه ,القتل داخل خلوي بواسطه العدلات وهضم البكتريا بواسطه الخلايا البلعميه في اوقات مختلفه من الحضانه في الجرذان الممنعه بالمستضد البكتيري ، وبلغ معدل البلعمه ١٥,٢ ,٣٥,٤ و ٥٠,٧ في اوقات الحضانه ١٠،٣٠ و ٥٠ دقيقه على التوالي ، في حين كان معدل القتل داخل الخلوي ٤,٨ ,٤,٩ و ٩,٨ في الاوقات المشار اليها اعلاه على التوالي ، بالاضافه الى ذلك وصلت نسبه الهضم ٠,٤٨ ,٠,١٧٨ و ٠,١٩٦ في ذات الاوقات على التوالي ، بينما كانت النتائج منخفضه للفحوصات اعلاه في جرذان السيطره . اظهرت تراكيز البروتين الكلي ، الالبومين والكلوبيولينات الخام انخفاضا في جرذان المعامله مقارنه مع حيوانات السيطره حيث بلغت ٩,٤٥ ,٨,٠٢ و ١,٤٥ غم /لتر على التوالي , بينما كانت ١٢,٣٤ ,١٠,٤٤ و١,٩ . غم /لتر في حيوانات السيطرة . يتضح من هذه النتائج ان المستضد المقتول لتك البكتريا هو محفز للمناعه الخلويه ، في حين انه كان كابحا للمناعه الخلطيه في الجرذان.