Measuring the levels of C5a in induced wound infection with *Staphylococcus aureus*

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Abstract

Animal models are standard tools for studying a wide range of traumatic wound infections. This paper aims to study the effect of *Staphylococcus aureus* (SA) in the wound and how the innate immune system interacts with this infection, by measuring C5a, and there are roles on wound healing. Fifty-one (females) Albino Rats at the age 6-9 week. The animals were divided into three groups (17 animal in the group) Group I (healthy control), Group II (control wound), and Group III (infection contamination, wound group), To mimic clinically occurring infections caused by SA infection, excision wound was made on the dorsal side of the animals, and SA 2×10^8 (CFU)/mL was inoculated on the wound site in infection contamination group, The blood was collected after 24hours post-infection for three days (24, 48, 72 hours) post-infection, ELISA technique was used for Measure C5a levels . Immunological assay results of this study showed that the serum level for C5a was statistical significant between different values at different times with ($P \leq 0.04$). The study successfully established the role of C5a in wound infection with SA.

***Keywords*:** *Bacterial inoculation, C5a, Staphylococcus aureus.*

قياس مستوى C5a في الجروح المصابة بالمكورات العنقودية

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الخلاصة

الحيوانات المختبرية هي من الأدوات القياسية لدراسة مجموعة واسعة من التهابات الجروح الرضية. تهدف هذه الورقة البحثية إلى دراسة تأثير المكورات العنقودية الذهبية في الجروح وكيفه تفاعل الجهاز المناعي الفطري مع هذه العدوى، وذلك بقياس مستويات C5a، ودراسة الدور الذي تلعبها في التئام الجروح. واحد وخمسون من اناث الجرذان البيض في سن 6-9 أسابيع. تم تقسيم هذه الحيوانات إلى ثلاث مجموعات (17 حيوان في المجموعة) المجموعة الأولى (السيطرة الصحية)، المجموعة الثانية (السيطرة على الجرح)، والمجموعة الثالثة (مجموعة الجرح الملوثة بالمكورات العنقودية الذهبية)، لمحاكات الالتهابات التي تحدث سريريًا والناجمة عن عدوى المكورات العنقودية، تم إجراء جرح استئصال على الجانب الظهري للحيوانات، و تم تلقيح المكورات العنقودية الذهبية 2 × 108 (CFU) / مل في موقع الجرح في مجموعة الجروح الملوثة ،ومن ثم تم جمع الدم بعد 24 ساعة بعد الإصابة لمدة ثلاثة أيام (24 ، 48 ، 72 ساعة) ، استخدامات تقنية إليزا في قياس مستويات C5a. أظهرت نتائج الفحص المناعي لهذه

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الدراسة أن مستوى مصل C5a كان ذا دلالة إحصائية بين القيم المختلفة و في أوقات مختلفة (P ≤0.04). نجحت الدراسة في تحديد دور C5a في عدوى الجرح بالمكورات العنقودية الذهبية.

الكلمات المفتاحية: *التلقيح البكتيري، C5a، المكورات العنقودية الذهبية.*

Introduction

The skin is the largest organ of the body **,** It conceder as the first line of innate immune defense , losing of the integrity of large portions of this barrier -as a result of injury or disease- may lead to Skin infections **(Treuting, et al., 2018)** . Any microorganism that colonizes the skin or is introduced to a wound can cause an infection. Skin wounds infection with Staphylococcus aureus are a major public health problem (Tsige, et al., 2020). it have the ability to express many virulence factors and it escape, During skin infection MAMPs of S. aureus are recognized by PRRs of the host, leading to activate host defense system [1], as complement protein chemokines and growth factors implicated in early wound repair stage such as IL-1, TNF, TGF-α, TGF-β, platelet factor IV (PF4) [2]. *S. aureus* has many virulence factors they can secrete various immune evasion factors, and these factors are help its attachment to host tissues, breaking or avoid the host immunity, Tissue invasion. [3] It including cytotoxins (hemolysins, cytolytic peptides, leucocidins), immunomodulatory proteins (superantigens, complement-inhibitory proteins), and factors that prevent immune cell recognition (SPA, among others) [4]. Anaphylatoxins C3a and C5a are cleaved from serum C3 and C5 during complement activation. It plays an important role in inflammation. It is generated in response to a serious bacterial infection, triggering degranulation of mast cells and basophils, resulting in the release of potent inflammatory mediators [5,6]. C5a has many biological functions it includes Expression and Activation of Adhesion Molecules on neutrophils monocytes, and endothelial cells, Chemotaxis, activate neutrophils and macrophages to release antimicrobial compounds, induced TGF-β, Cytokine Production by stimulating macrophages to synthesize and release TNF, IL-1, IL-6 [7]**.**

Material and Methods

Bacterial strain and culturing

Inoculate *Methicillin-resistant Staphylococcus aureus (MRSA)* strain from a frozen glycerol stock into sterilized Tryptic Soy Broth (TSB) media at 37ºC for 16-18 h when the bacteria reach the late stationary phase of growth. Then the bacterial sedimentation were measured by using a spectrophotometer, at 600 nm optical density (OD 600) when the bacterial sedimentation reaches 2.3 it equal to the concentration of 2×10^8 colony-forming units (CFU)/ml [8].

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Bacterial inoculation

The wounds was inoculated with 0.1 mL bacterial suspension after 24 hours from wounding using insulin sterile syringe (1ml) with 27-gauge needle. The bacterial suspension equal to 2×10^8 CFU/mL, were injected into the wound, the wound site were cover with adhesive Wound dressing, the dressing were change daily.

Laboratory animals

Rat animal model, 6-9 week old female (51) albino Rats and was placed in a controlled environment, The animals were purchased and placed in the animal house in the Faculty of Veterinary Medicine, University of Kufa, and the animal was divided to three groups Group I (healthy control), Group II (control wound) , and Group III (infection contamination, wound group).

Excision wound experiment

Animals were anesthetized prior to wound creation by using combination ketamine (50mg/ml) solution with 2:1 and Xylazine (20mg/ml) sequentially at a dose from 0.10 to 0.15 ml/100g body mass [9]**.** Excision wounds was made on the dorsal side of the rats. And the animal's fur was shaved after applying hair removal from veet with manual Razor, two kinds of antiseptic were apply (alcohol 70%, povidone-iodine) on the area where the wound was made, sterilizing wound area for the first time with alcohol 70% and that repeat for three times and then using povidone-iodine [10], A full-thickness excision wound was made using a surgical blade, forceps, and pointed scissors. The wound entirely was lifted opine [11].

Blood Collection

A total of 51 blood samples were collected; from albino rats at specific time line (24, 48, 72) hours after wound infection with MRSA, the sample collection happen after 24hours from contemning with *Staphylococcus aureus*, blood collected using 5ml disposable plastic sterile syringe from the heart in cardiac puncture technique and the animals were under anesthesia , the blood were collected into serum gel tube (without anticoagulant) Before centrifugation for 15 minutes, It is necessary to allow samples to clot for two hours in room temperature. At 3000× rpm , finally, each serum sample was divided into five small part (200 µl) each until used and kept at deep freeze (-20°C) until used [12].

Serological Tests

Enzyme Linked Immune Sorbent Assay (ELISA)

In our research, we have used the sandwich- ELISA kit company Bt lab.

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Statistical Analysis

Data of the present study were translated into codes by using a computerized data program Statistic Package for the Social Sciences (SPSS version 23) in order to be statistically analyzed into graphs. The statistical program we depend on P value level, when (P value more or equal to 0.05) $p \leq 0.05$, were considered statistically significant.

Ethics approval

The experimental processes protocols was approved, by Research Centre's Ethic Committee's suggestion, of the of Kufa University, Council on Guidelines to ensure that experimental animals are properly cared for and used are. (Authorization from the Animal Experimentation Ethic Agency; Ethic no. 12- 7-2021).

Results

Serological Tests

Serum concentration C5a protein produced from Group I, Group II, and Group III in the albino rat at different times

The serum concentration of C5a at different time intervals of Group III such as 24, 48, and 72 hours post-infection were measured. The results indicated that the average concentration of C5a at 24hrs was 1.63 pg/ml. The statistical analysis revealed a Standard deviation of 1.63± 0.90, at 48hrs, which was 1.61 pg/ml and a Standard deviation of 1,61±0.89, and at 72hrs, which was 0.32pg/ml with a mean of 0.32±0.40.

The C5a serum concentration of at of 24, 48, and 72 hrs. post wound for the Group II. The results indicated that the average concentration of C5a at 24 hr. of was 0.14 pg/ml and a Standard deviation of 0.14 ±0.04, whereas the average concentration at 48 hrs. It was 0.13 pg/ml and a Standard deviation of 0.13 ±0.03, but 72 hr., the average mean concentration was 0.12 pg/ml and a Standard deviation of 0.12±0.02. The serum concentration of C5a for the Group I shows that the average concentration of C5a was 0.15 pg/ml and a mean of 0.15 ± 0.00.

The statistical analysis showed that there is a significant difference between different values at 24 hrs. for Group III when the average concentration vs. 24 hr for Group II when the average concentration is -1.491 pg/ml, at 24 hr for Group III vs. 48 hrs. Group II when the average concentration is 1.50 pg/ml, and at 48 hr Group II vs. 48 hrs. Group III when the average concentration is -1.48 pg/ml ($P < 0.04$) (Figure 1-4).

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Table (1-1): Serum concentration of C5a produced in Group I, Group II, and Group III at deferent Time.

Post wounding	Group I (Mean ± SD)	Group II (Mean ± SD)	Group III (Mean ± SD)
24 hour	0.15 ± 0.00	0.14 ±0.04	1.63± 0.90
48 hour		0.13 ±0.03	1,61±0.89
72 hour		0.12±0.02	0.32±0.40

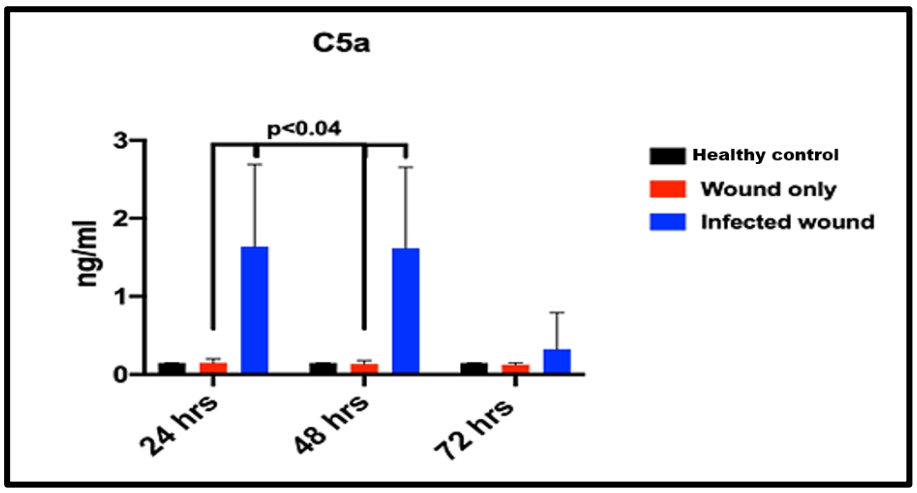

Fig. (1): The statistical analysis of C5a Serum concentration that produced in Group I, Group II, and Group III at deferent Time.

Discussion

Serum concentration C5a protein produced from Group I, Group II, and Group III in the albino rat at different times

The result shows that the mean serum concentration of C5a for the infection group was higher than the control wound group at all times of the experiment indicate that the C5a decrees in the control wound group as a sign to accelerated healing due to decrees in inflammatory cells (macrophages and neutrophils) that lead to the shortened inflammatory stage and also increase of mast cells that stimulate re-epithelialization as well as angiogenesis [13]. And that is obvious in the result when it shows decrees of the mean serum concentration of C5a from 0.14 pg/ml at 24hr. to 0.12 pg/ml at 72hr. this data was compared with the infection group that shows the obvious increase and mostly at 24hr. Post-infection 1.63 pg/ml that indicate the presence of infection due to that the serum concentration of C5a increase and this agree with [14]. that shows in both humans and animals, sepsis can cause complement activation, resulting in increased amounts of C5a and causing immune cells to migrate in the direction of C5a. As a result, it enhances immune response [15]. The result also shows

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a decrease in C5a in serum concentration in the healthy control group compared to the infection group and control wound group, which approve with [16]. that shows Both healthy and asthmatic people produce C5a in their lungs. In a Healthy state at low concentrations, C5a produced locally in pulmonary tissue, however in inflamed asthmatic lungs, C5a is found in high concentrations. The statistical analysis showed that there is a significant difference between different values $P < 0.04$.

Conclusions

The study successfully established the role of C5a in wound infection with SA, by accelerated healing due to decrees in inflammatory cells that leading to the shortened inflammatory stage and also increase of mast cells that stimulate re-epithelialization as well as angiogenesis, and also established the presence of C5a in healthy condition, our results can be useful in further study in animal model experiment.

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