Trop J Nat Prod Res, May 2023; 7(5):2859-2862 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Upregulation of Interleukin-33, Human B-Defensin 2, and Toll-Like Receptor 2 in Response to *Staphylococcus aureus* Furunculated Patients

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ARTICLE INFO

Article history:
Received 06 January 2023
Revised 12 May 2023
Accepted 15 May 2023
Published online 01 June 2023

ABSTRACT

A furuncle is an infection of the skin and subcutaneous tissue that involves hair follicles and ultimately results in the formation of an abscess. Furunculosis is most frequently caused by *Staphylococcus aureus*. interleukin-33, human β defensin 2, and Toll-like receptor 2 (*Toll like receptor 2*) play a significant role in the innate immunity of the host. The present study aims to determine the level of the *interleukin-33*, *human β defensin 2*, and *Toll like receptor 2* gene expression in staphylococcal furunculosis. A case-control study includes 50 patients and 50 control were evaluated for the level of interleukin 33, human *β* defensin and Toll like receptor 2 by enzyme and qPCR, respectively. The results indicated that furunculosis was most prevalent in people between the ages of 25 and 45 ($P > 0.05$). However, there was no significant difference between males and females ($P > 0.05$). The serum levels of *interleukin-33* were observed to be significantly higher in FP compared to HC. *Toll like receptor 2* and *human β defensin 2* were elevated in PF, but much more in female patients than in male patients ($P < 0.05$). In conclusion, the findings of the present study reveal that it is more likely that *Toll like receptor 2*, *human β defensin 2*, and *interleukin-33* play a significant role in the host's defense against *S. aureus* infection.

Keywords: Furunculosis, *human β defensin 2*, *interleukin-33*, *Staphylococcus aureus*, *Toll like receptor 2*.

Introduction

Staphylococcus aureus causes many skin-related infections. The frequency of skin infections caused by *S. aureus* mirrors the conflict between the skin's immune defenses and the bacteria.[1] *S. aureus* predominantly inhabits the naris of 28-30% of healthy individuals.[2]
Furunculosis is an abscess that develops when a hair follicle becomes infected, causing pus to accumulate and possibly necrotic tissue as well. *Staphylococcus aureus* is the most frequent etiological agent for this infection, which manifests as tender, swollen nodules that are red and occur on skin regions with hair.[3]
Furunculosis immunological and inflammatory responses depend on the pathogenicity of *S. aureus* as well as innate and acquired immunity.[4] Typically, patients with chronic furunculosis are *S. aureus* carriers; interestingly, the *S. aureus* strains isolated from their skin and nose showed similar characteristics.[5]
Toll like receptor *2* and Toll like receptor 4 are associated with the innate immune reactions brought on by *S. aureus*, but their exact functions are still unknown.[6]
Numerous disorders, including asthma, rheumatoid arthritis, tissue damage, and other infections, have been linked to interleukin-33 (*interleukin-33*). It is controlled by a mechanism to improve the host's immunity against cutaneous bacterial infections.

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Citation: Al-Halaq AA and Utba NM. Upregulation of Interleukin-33, Human B-Defensin 2, and Toll-Like Receptor 2 in Response to *Staphylococcus aureus* Furunculated Patients. Trop J Nat Prod Res. 2023; 7(5):2859-2862 http://www.doi.org/10.26538/tjnpr/v7i5.2

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

A significant protective role is played by *interleukin-33* throughout the infection process through the stimulation of type 2 immune responses or by promoting the proliferation and recruitment of neutrophils against wound infections caused by *S. aureus*.[1] However, several structural elements of Gram-positive bacteria, like peptidoglycan and lipoteichoic acid, may use *Toll like receptor 2* as a signal-transducing receptor.[7]
All the four types of human β-defensins are expressed by skin epithelial cells.[8] In addition to antibacterial activity, defensins can also control apoptosis, wound healing, and immunological responses.[9] There is a paucity of information regarding *human β defensin 2* and *Toll like receptor 2* gene expression in patients with furunculosis.
The present study was, therefore, conducted to investigate the potential roles of *interleukin-33*, *human β defensin 2,* and *Toll like receptor 2* genes in staphylococcal furunculosis.

Materials and Methods

Ethical approval
Ethical approval for this study was obtained from the ethics committee of the College of Science at the University of Baghdad (Ref. CSEC/1120/0061). An informed consent form was given to each participant to sign.

Specimen collection
A total of 50 blood samples were collected from 50 patients with staphylococcal furunculosis (FP) admitted to AL-Kindy hospital and others, private clinics, and laboratories in Baghdad, Iraq, were obtained under the supervision of a dermatologist. In addition, 50 blood samples were collected from 50 healthy individuals; thus, considered as a control group (HC).

Estimation of interleukin-33 level in the study groups
The human interleukin-33 ELISA kit was used to estimate the serum levels of *interleukin-33* in the blood samples from the study groups according to the manufacturer's instructions.

Quantitative real-time polymerase chain reaction

RNA was extracted from the blood samples using the TRIzol™ Reagent, following the manufacturer's instructions. The RNA concentration was determined by a Quantus Fluorometer. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed to evaluate *human β defensin 2* and *Toll like receptor 2* gene expression levels by dye-based gene expression assay. The assay employed the primers for *Toll like receptor 2*; [10] and *human β defensin 2*.[11] In addition, TEGT primers were used as an internal control. [12] 2X GoTaq® 1-Step RT-qPCR System (Promega, USA) was used for all reactions. The reaction mixture contained 5 µl qPCR Master Mix, 0.25 µl of RT mix, 0.25 µl of $MgCl_2$, 0.5 µl of the primer pair (10 pmol/µl), 2.5 µl of nuclease-free water (Promega, USA), 1 µl of RNA (300 ng). The amplification was performed using the Mic qPCR Cycler (BioMolecular System, Australia) as it is mentioned in Table 1. The melting curve was performed at 65–95°C. Folding was used for data analysis, employing the formula $2^{-\Delta\Delta CT}$.[13]

Statistical analysis

To test for normality, the Kolmogorov-Smirnov test and the Shapiro-Wilk test were performed using IBM SPSS Statistics 26 (SPSS Software, Chicago, USA). Chi-square analysis was used to compare age and sex frequencies. The results were presented as a median and range for non-normally distributed data, and the Mann-Whitney U test was used to determine the significance of the difference between the medians. The unpaired T-test was used for comparing the gene expression between the FP and HC groups. Spearman rank-order correlation was done to determine the correlation between *interleukin-33* serum level, *human β defensin 2*, and *Toll like receptor 2* gene expression. Significant differences were considered when P<0.05.

Table 1: qPCR protocol

Step	Temperature (°C)	Time (min:sec)	Cycle
Reverse transcription	37	15:00	1
Initial denaturation	95	05:00	1
Denaturation	95	00:20	40
Annealing	60	00:20	cycles
Extension	72	00:20	

Results and Discussion

Age and sex frequencies of the study groups

As shown in Figure 1, most of the patients with furunculosis (72%) were between the ages of 25 and 45, while 18 and 10% of them fell into the categories of people who were over 45 and those who were under 25, respectively. Regarding the controls, 10 and 30% of them were under 25 years old and 60% were between the ages of 25 and 45. Interestingly, the differences between the FP and HC age groups were highly significant (P<0.01). The frequency of furunculosis was insignificantly (P=0.08) higher in males than in females (72 vs. 28%).

Staphylococcus aureus is the primary pathogen implicated in recurrent furuncles. However, once the host's defenses are weakened, furuncles may develop.[4] The present findings indicated that the frequency of furunculosis according to age did not show any significant differences (Figure 1). Paller and Mancini[14] pointed out that any individual can be infected with furunculosis irrespective of his/her age and sex. Nevertheless, it was reported that furunculosis is most frequently prevalent among male teenagers and young adults. Also, the results of the present study revealed that the males were infected with furunculosis more than the females. Similarly, Demos *et al.*[15] observed that all patients with furunculosis were between the 20 and 44 years old, with an average age of 29 years; among them, 16 (76%) were males and 5 (24%) were females. Ibler and Kromann [3] noted that although the incidence of furunculosis is rising globally, patients under the age of 18 are experiencing the greatest rise. According to Narain *et al.*[16] there were 735 patients, with a mean age of 54.8 ± 2.1 years, and male dominance over females (62% vs 38%).

Serum levels of interleukin 33 in the study groups

A significant increase in the interleukin-33 serum levels was observed in FP compared to HC (532.64 vs. 491.93 ng/L, P= 0.0043), with a range of 277.94 – 2700.64 ng/L for FP and 264.38 – 796.72 ng/L for HC (Figure 2). However, the results presented in Figure 3 revealed non-significant differences in the level of *interleukin-33* serum between age groups in FP (p = 0.97) and HC (P= 0.98). Figure 4 illustrates the insignificant differences in *interleukin-33* serum levels between males and females in FP (505.49 vs. 700.68, p =0.08) and HC (494.13 vs. 474.71, P = 1.0).

It is still unclear how the host immune system works because of the complexity of the responses that depend on both its humoral and cellular arms. [17,18] Innate immunity serves as the skin's initial line of defense and guards it against various diseases. This role is played by several innate immunity cytokines, among which is interleukin-33, [17] but the primary complicated mechanism by which interleukin-33 is controlled to increase host defenses against bacterial skin infection remains largely unknown. interleukin-33 serum levels were increased in FP in this study, which agreed with Li *et al.* [18] who reported that interleukin-33 was abundantly increased in the skin of *S. aureus*-infected patients.

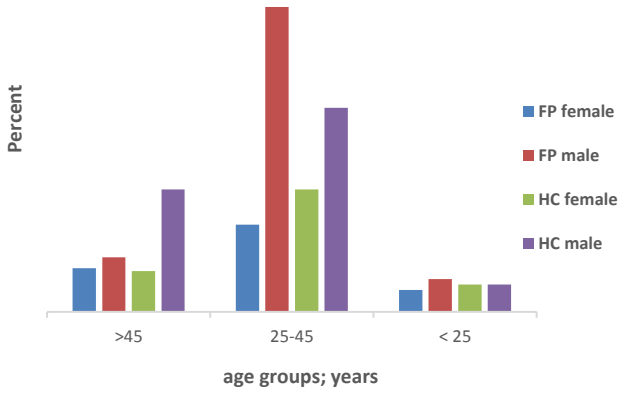

Figure 1: Furunculosis patient and healthy control groups according to age and sex.

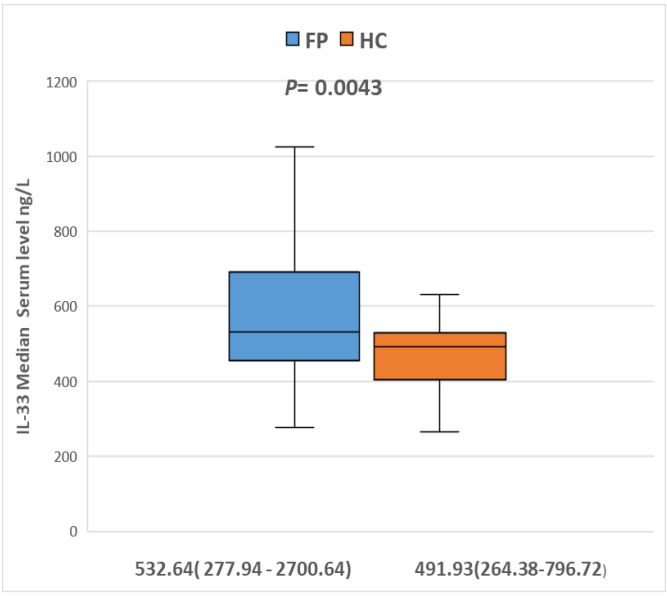

Figure 2: IL-33 serum level in furunculosis patient and healthy control groups.

Expression of human β defensin 2 and Toll like receptor 2 genes in the study groups

The expression of the *human β defensin 2* gene (8.0 ± 3.90) was upregulated in the PF group. It was interesting to observe that *human β defensin 2* gene expression was elevated much more in female patients than in male patients (17.84 ± 12.8 *vs.* 4.18 ± 2.11, respectively, P < 0.05). *Toll like receptor 2* gene expression was upregulated in the PF group (9.26 ± 4.4). However, it was insignificantly (P>0.05) higher in female than in male patients (21.45 ± 14.23 *vs.* 4.52 ± 2.49, respectively).

Spearman rank-order correlation was performed to estimate the correlation coefficient (Rs) between *interleukin-33* serum level, *human β defensin 2*, and *Toll like receptor 2* gene expression. The analysis revealed that non-significant and negative correlations were observed between *interleukin-33* and *human β defensin 2* (rs= 0.07, P = 0.627) and between *interleukin-33* and *Toll like receptor 2* (Rs = -0.096, P = 0.505). Regarding the control group, a positive non-significant correlation was found between *interleukin-33* and *human β defensin 2* . Similarly, a negative non-significant correlation was found between *interleukin-33* and *Toll like receptor 2*.

The expression of *interleukin-33* by macrophages was induced by staphylococcal peptidoglycan (PGN) and lipoteichoic acid (LTA). This induction is via activation of *TLR*2–mitogen-activated protein kinase (MAPK)-AKT-signal transducer and activator of transcription 3 (STAT3) signalling pathway. PGN and LTA failed to induce interleukin-33 in *TLR*2-deficient peritoneal macrophages, and MAPK, AKT, STAT3 inhibitors significantly decreased PGN- or LTA-induced interleukin-33. Therefore, interleukin-33 acted on macrophages to induce microbicidal nitric oxygen (NO) release. This induction is dependent on inducible nitric oxide synthase (iNOS) activation, as treatment of macrophages with an inhibitor of iNOS, aminoguanidine, significantly decreased *interleukin-33*-induced NO release. Moreover, aminoguanidine significantly blocked the capacity of *interleukin-33* to inhibit the growth of *S. aureus*, and *interleukin-33* silencing in macrophages significantly increased the survival of *S. aureus* in macrophages. Furthermore, the administration of *interleukin-33*-neutralizing antibody to mouse skin decreased iNOS production but increased the survival of *S. aureus* in the skin. These findings reveal that *interleukin-33* can promote the antimicrobial capacity of dermal macrophages, thus enhancing antimicrobial defense against skin bacterial infections.[18]

The skin is the ultimate exterior barrier. As a result of this exposure to several pathogens, various defense mechanisms have been developed. It can produce phenol-soluble modulins (PSMγ and PSMδ), which when activated by *TLR*2, cause the formation of *hBD*2 and *hBD*3, which have potent antibacterial properties against *S. aureus*.[19] The dominance of males over females (2.57:1) in the present study can be attributed to the low expression of *hBD2* and *TLR2* that was observed in their blood. Such results indicated the important role of *hBD2* and *TLR2* genes in furunculosis. Lai *et al*.[20] indicated that *S. epidermidis* increased the expression of *hBD2* and *hBD3* via *TLR*2-induced p38 mitogen-activated protein kinase (MapK) signalling, demonstrating that normal flora of the skin can improve the innate immune response.

Bernard and Gallo[21] found that *hBD*2 is generally controlled by a variety of mechanisms in response to infections. The small sample size used in this investigation may have contributed to the negative and non-significant association between the previous parameters. A larger scale of studies is required to assess the significance of these parameters in *S. aureus* infections. However, The usage of applied molecular techniques, such as PCR, It was implemented in many areas of medicine [22-30], as a result, it is highly suggested in order to achieve accurate results in the context of pathogenic microorganisms.

Conclusion

The findings of the present study reveal that the male outnumbered the female in frequency percentage of furunculosis. Furthermore, the level of interleukin 33 was higher in male more than female. Also *human β defensin 2* and *Toll like receptor 2* are upregulated in the blood in response to *S. aureus* furunculosis.

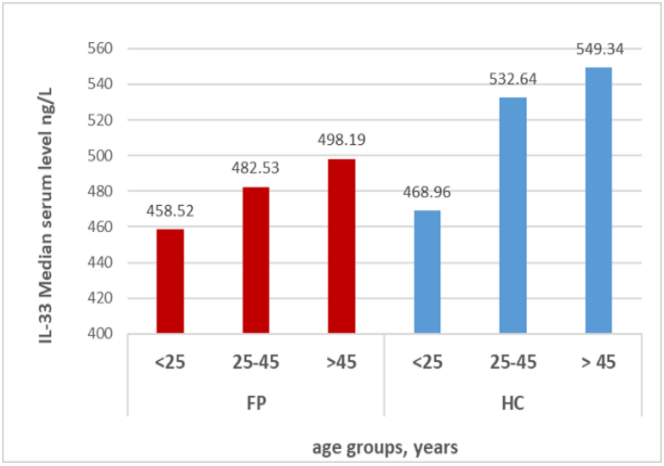

Figure 3: IL-33 serum level distribution according to age in furunculosis patient and healthy control groups.

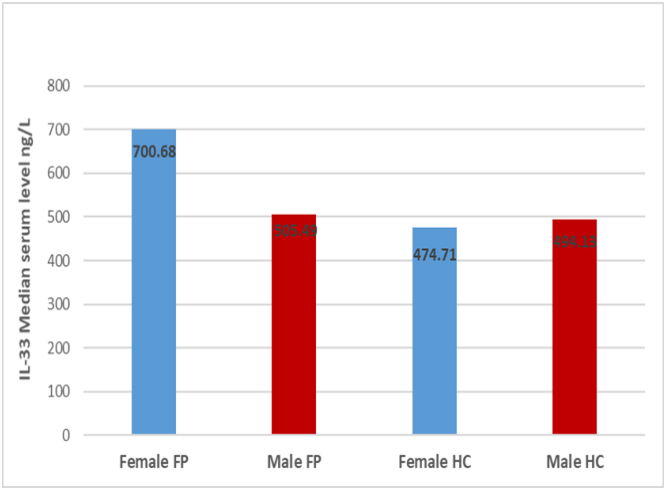

Figure 4: IL-33 serum level distribution according to gender in furunculosis patient and healthy control groups.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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