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Effect of Ag nanoparticles on expression of *fnbA* gene in *S. aureus* and evaluation of IL-10 and IL-17 levels among burn patients

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ABSTRACT

Silver nanoparticles are widely recognized for their antibacterial properties; IL-10 and IL-17 are among the cytokines that inflammatorily respond to burn injury. The objective of this study was to investigate whether Ag NPs and OX antibiotics have a synergistic effect on the expression of the *fnbA* gene in S. aureus isolated from burn patients and to identify early alterations in the levels of IL-10 and IL-17 in those patients. During the period from August to October 2023; 160 samples were collected from burn patients; and blood samples were collected from burn patients, while 15 healthy individuals as the control group for the evaluation of IL-10 and IL-17. The current results recorded only 35 isolates were identified as *S. aureus* (21.9%). According to the ELISA results, burn patients infected with *S. aureus* showed a significantly increased level of IL-10 and IL-17 (267.7, 83.96) compared to the healthy group (113.4, 29.15), respectively. Also, serum IL-10 and IL-17 level in patients infected with Gram negative bacteria were increased a significantly (380.1, 41.6) than in the control group (113.4, 29.15) for IL-10 and IL-17, respectively. The q-PCR, the expression of the *fnbA* gene was a significant decrease with Sub-MIC concentration of Ag NPs (0.580-fold, p.0.001), while the synergistic effect of Ag and OX was a highly decreased significantly (p.< 0.001, 0.271-fold) than control group. In conclusion, synergistic effect of silver NPs with antibiotics to decrease the expression of *fnbA* gene in *S. aureus*.

Keywords: gene expression, Ag NPs, IL-10, *S. aureus* IL-17, *fnbA*

Introduction

Burns are a serious global matter of public health since they are one of the leading causes of trauma-related mortality globally [1]. However, it is important to note that the risk of infections and infection-related complications remains a serious concern, potentially leading to fatal outcomes for patients [2]. Burn injuries have an increased susceptibility to infections due to the compromised integrity of the wounded skin, which provides a pathway for the entry of bacteria and fungi. Furthermore, the production of biofilms in burn injuries is a considerable concern in effectively managing microbial infections in patients with burn wounds [3]. Gram-positive bacteria, such as S. aureus, and Gram-negative bacteria, such as E. coli, Proteus spp., Klebsiella spp., and Pseudomonas spp., were found to be causative agents of infections in burn wounds [4]. Various types of cells release interleukin-10 within both adaptive and innate immune systems [5]. The role of tissue protection is a major functional motif observed in the IL-10 family of cytokines. One perspective is that cytokines

play an important role in reducing the detrimental effects of bacterial and viral infections, as well as pro-inflammatory reactions, by preventing excessive tissue damage [6]. IL-10 is among the cytokines that act as a negative feedback regulator of the pro-inflammatory response to burn injury. IL-17 has been shown to have contrasting roles, exhibiting both destructive and protective effects in different diseases, particularly infectious diseases. The IL-17 cytokine family contains IL-17A-F. The source of interleukin-17 is the CD4 and Th17 cells. Most experts agree that IL-17F and IL-17A have biological functions [7]. The activity of Th17 cells may be modified after a burn injury, which might potentially result in the occurrence of systemic infections. This is because Th17 cells play a crucial role in the immune response of mucosal and epithelial linings [8]. Silver nanoparticles are widely recognized for their antibacterial properties and are commonly utilized as a biocide in various products such as cosmetics, medications, and clothing [9]. Studies have shown that Ag NPs effectively reduce bacterial biofilm volume and biomass [10]. The antimicrobial action of silver nanoparticles involves binding silver to bacterial proteins, generating reactive oxygen species (ROS) within the cells, and restricting biological activities including DNA replication and transcription [11]. Therefore, the penetration of silver nanoparticles into bacterial cells may disrupt many biological processes, such as gene expression. Analyzing gene expression helps understand the molecular mechanisms of toxicity, which may assist in detecting the biological effects of contaminants even at extremely low concentrations [12]. The aim of this study was to investigate whether Ag NPs and OX antibiotics have a synergistic effect on the expression of the *fnbA* gene in *S. aureus* isolated from burn patients, as well as to identify early alterations in the levels of IL-10 and IL-17 in those patients.

Materials and methods

Ethical Approval

The study followed the ethical guidelines established by the Declaration of Helsinki. The committee of researchers at the Thi-Qar Health Directorate (No. 2023/165 on 8/8/2023) has viewed and approved this study. The person's informed consent was obtained in hospitals, clinics, and when visiting them in their homes to collect samples.

Study design and setting

The present research was carried out on 160 burn patients attending the burn center of Al-Nasiriyah Hospital in Iraq, Thi-Qar Province, throughout the time span of August to

November 2023, as well as 15 healthy volunteers serving as the control group. Swabs from burn injuries were collected under aseptic conditions and then transferred to the laboratory to be cultured using differential and enriched media, including blood agar, mannitol salt agar (Biolab, Hungary), and chrome agar (HI Media, India). Also, burn patients and healthy persons used to draw 3 ml of venus blood for blood samples. Two milliliters of blood were used to collect the serum that was transferred into Eppendorf tubes and kept at -20°C until used for evaluating IL-10 and IL-17 (Bioassay Technology, China) by enzyme-linked immunosorbent assay (ELISA technique) (Biotek, USA). And 1 ml of remaining blood was used for a complete blood count (CBC) test. After being identified as *S. aureus* based on its morphological traits and through biochemical tests such as catalase, coagulase, and DNase tests using DNase medium (micromedia, UK).

Minimum Inhibitory Concentration (MIC) of Ag Nanoparticles

To determine the MICs of the tested agents, in accordance with the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI), Ag NP was evaluated in a 96-well plate using the broth micro-dilution method [13]. Then, different gradient concentrations were prepared from the stock solution. (25, 12.5, 6.25, 3.125, and 1.562 mg/ml). The MICs of the Ag NPs that stop the observable growth of bacteria were determined and defined as the MIC values.

Incubation of Bacteria with Nanoparticles and Antibiotic

Four groups of isolated bacteria were cultured in Brain Heart Infusion (BHI) broth for a duration of 18 hours at a temperature of 37°C. Each group is made up of 10 bacterial isolates. The first group of fresh bacterial suspensions was separately added to Eppendorf tubes with sub-MIC concentrations of nanoparticles; the second group of bacteria was mixed with 5μg/ml of OX antibiotic. The third group's S. aureus was put into Eppendorf tubes that already had 5µg/ml of antibiotic and a sub-MIC of Ag NPs in them. while the control group consists of only bacterial suspension, Then, all bacterial groups are incubated for 24 hours at a temperature of 37 °C until they are used for the next step.

RT-qPCR reactions and analysis of gene expression

The use of RT-qPCR technology to investigate the effects of Ag NPs alone and the synergetic effect of Ag NPs with OX antibiotics on the expression of the fnbA gene in S. aureus. Total RNA was isolated using the GENEzol™ TriRNA Pure Kit (Geneaid UK). The extracted RNA was analyzed using a Nanodrop (Thermo Scientific). The RNA was

transformed into cDNA using the transScript two Step gDNA Removal and cDNA Synthesis SuperMix (TRAN; China), following the instructions provided by the manufacturer. A quantitative real-time PCR system (Stratagene Mx3000; USA) was performed using the SYBR Green method (Syber Green Master Mix Kit; Promega, USA). The specific primer pairs sequence of fnbA gene as following: forward:5'GACCACCACCTGGGTTTGTA -3' and reverse: 5'TGGATAGCGAAGCAGGTCAC-3' [14]. In addition, the 16SrRNA gene was analyzed in order to normalize target gene expression measurements as an internal control. the specific sequence of 16SrRNA gene was, forward: 5'CCGGTGGAGTAACCATTTGGA-3' and reverse: 5'GTCCGGATACCATTTTACGACTTTT -3' [14].

A reaction volume of 20µl was used, which contained 4µl of cDNA and 12.5µl of SYBR Green master mix. 1µl of each forward and reverse, and completed the volume with nuclease-free water and was run according to the following program: an initial activation step at 94 °C for 4 minutes, 40 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 20 s. qPCR procedure was performed, in duplicates. Folding = 2^-ΔΔCT, ΔΔCT = ΔCT treated - ΔCT control, ΔCT = CT gene - CT housekeeping gene [15]. The threshold cycle (CT) method was employed in relative expression calculation for quantitative RT-qPCR.

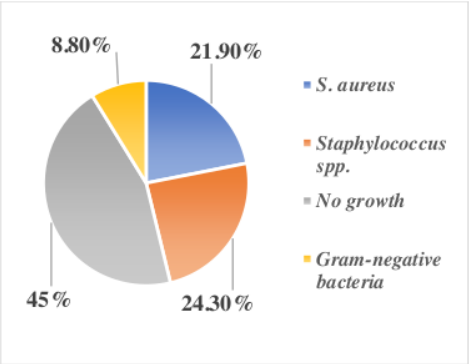
Statistical Analysis

The data of the current study was statistically analyzed using SPSS, based on non-parametric Mann-Whitney U for independent, Chi-square, and one sample test at p value < 0.05.

Results

Distribution of Bacterial Groups in Burn Patients

Out of a total of 160 swabs from burn patients, approximately 88 (55%) exhibited positive bacterial growth in cultural media, while 72 (45%) showed no growth on those media. The *Staphylococcus* spp. revealed 39 (24.3%), whereas *S. aureus* had 35 isolates (21.9%), as exposed in Figure (1). There was a significant difference at (p.<0.001) among the bacterial groups.



CalX2= 431, TabX2=7.81, df=3, p. value < 0.001

Figure 1. Distribution of bacterial groups in burn patients

Determination of IL-10 and IL-17 level in burn patients and control groups

To study the circulating immune mediators in burn injury patients, the levels of two interleukins (IL-10 and IL-17) in the serum of burn patients were screened to highlight significant changes in burn wound patients. The present study revealed a significant increase in the average serum levels of IL-10 in burn patients who were infected with S. aureus (267.7) than in healthy subjects (113.4), as shown in table (1).

Table 1. Serum level of IL-10 and IL17 in burn and control groups infected with *S. aureus*

	S. aureus infected Patients No. 20	Control No. 15	p. value
	Mean + rang		
IL-10	267.7 (36.5-696.0)	113.4 (27.6-411.6)	0.049
IL-17	83.96 (13.2-231.1)	29.15 (8.72-151.1)	0.001

Also, the present results showed that the average level of IL-10 in the serum of burn patients infected with Gram negative bacteria was increased significantly (380.1) than in healthy group (113.4), as revealed in table (2).

Table 2. Serum level of IL-10 and IL-17 in burn and control groups infected with Gram negative bacteria

	G-ve infected Patients No. 14	Control No. 15	p. value
	Mean + rang		
IL-10	380.1 (40.2-785.0)	113.4 (27.6-411.6)	0.049
IL-17	41.6 (19.2-136.6)	29.15 (8.72-151.1	0.001

To determine the IL-17 level in the serum of both burn and healthy groups, the mean concentration of IL-17 in the serum of burn patients infected with *S. aureus* was increase significantly 83.96 (13.2-231.1) than in the control group (29.15; 8.72-151.1), as shown in table (1). The existing data recorded a significant increase in IL-17 level in burn patients infected with Gram negative bacteria (41.6) compared with the control group (29.15) as shown in table (2).

Gene expression Analysis of *fnbA* gene

The results of the q-PCR demonstrated that the expression of the *fnbA* gene decreased in treated samples in the presence of a sub-MIC concentration of Ag NPs (fold change: 0.50± 0.254), which showed a significant decrease compared to the control samples (p. value. 0.001). Also, the gene expression level of *fnbA* in the presence of the OX was non-significant decreased (folds. 0.855; p. value. 0.147), while the synergistic effect of both Ag nanoparticles and the OX antibiotic was a highly significant difference decreased. (p value < 0.001, 0.271 ± 0.380-fold) than other groups, as Figure 2.

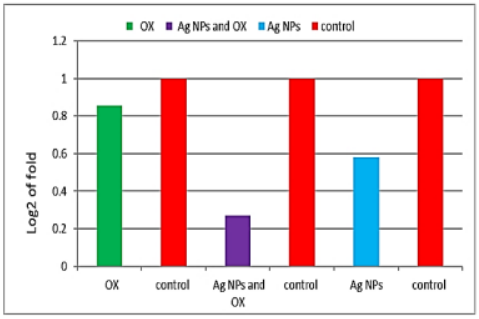


Figure 2. The gene expression of *S. aureus fnbA* gene in in Sub-MIC concentration of Ag NPs and OX

Discussion

In burn centers, the introduction and spread of methicillin-resistant *S. aureus* (MRSA) leads to poor outcomes such as prolonged hospitalization, bacteremia or sepsis, and even mortality [16]. Sepsis and invasive burn wound infection are both significantly influenced by *S. aureus*, which was the primary cause of early burn wound infection [17]. Similarly, Gram-positive bacteria were among the initial microbes to colonize burn wounds, while Gram-negative bacteria were typically present first. [18]. *S. aureus* was frequently found in burn wounds due its ability to quickly colonize burn sites within the first 48 hours [19,20]. Moreover, *S. aureus* can adopt a planktonic or biofilm mode of growth [21].

The current results recorded that *S. aureus* was only detected in (35; 21.9%). These results were closeness with locally study conducted in Misan, Iraq, which revealed that (18/105;17.14%) *identified as S. aureus* based on Multiple investigations have shown that *S. aureus* is the predominant bacterium colonizing burn wounds. [22]. Other studies conducted in the USA revealed that coagulase-negative *Staphylococci* and *S. aureus* have the highest prevalence among their overall patient population (47.1% and 44.1%, respectively), and Gram-negative bacteria (26.5%), which represent the lowest growth [23].

The results of study performed by Latifi and Karimi, [24] whom recorded that *Staphylococcus* spp. were (55.1%) As the predominant bacterium identified in burning site cultures, followed by *P. aeruginosa* (14.29%), *Enterococcus* sp. (12.24%). Recent results disagreed with local research conducted by Hassan *et al.* [25], which found that *P. aeruginosa* appeared in 40 positive cultures, the highest distribution of isolates. Furthermore, the study was done within Turkey, which demonstrated that *P. aeruginosa* was the most frequently isolated bacterium from burn injuries (45.5%) [26]. The present results recorded that the percentage of *Staphylococcus spp.* was 39 (24.3%). The study performed by Vural *et al.* [26] showed *Staphylococcus spp.* was found in (28.6%). The ability to cause the disease of coagulase-negative *staphylococci* (CoNS) remains unknown.

These results agreed with several studies, like: [27] who discovered that serum IL-10 levels, measured in hospitalized patients and within 24–48 hours after the burn, exhibited predictive value. Also, the animal experiments showed elevated levels of IL-10 for a duration of 84 days following the burns [28]. The present results of the IL-10 level were closely agreed with the locally study (25) recorded that the average IL-10 levels in patients' serum were significantly (61.4 ±11.8) than in the healthy group (4.58 ± 0.77) as control group. Likewise,[29] and Dehne *et al.*, [30] who, shortly after burn injuries, found elevated levels of IL-10 in their patients. Depending to current results can be interpreted as burn injuries caused an increase in proinflammatory cytokines. This needs to be suppressed by anti-inflammatory cytokines represented by IL10. Nevertheless, the involvement of IL-10 in the initial stages of immune-depression following serious injuries was still a subject of controversy. Finnerty *et al.*, [31], observed that septic episodes are more common in patients with elevated IL-10 levels.

The elevated level of IL-10 in the burn group infected with Gram-negative bacteria closely agreed with locally study conducted in AL-Najaf province [25]. This study found that the amount of IL-10 in burn patients with Gram-negative bacteria was 43.77±11.9 pg/ml, compared with normal persons (4.5 ± 0.06 pg/ml).

Some studies have observed long-lasting increases in IL-17 levels throughout the body in the bloodstream of patients [32]. The activity of Th17 cells may be modified after a burn injury, which might potentially result in the occurrence of systemic infections. This is due to Th17 cells are very important to the immune system. of mucosal and epithelial linings [8]. Not only IL-10 and IL-17 were elevated in burn patients, but also different cytokines such as TNF-α and IFN-γ as cellular immune responses, and there was a significant elevation in serum total IgG as a humoral immune response [33].

The current results of IL-17 level were closely agreed with the study conducted in Korea (34) which revealed that the median concentrations of IL-17 Serum levels in burn patients were markedly elevated compared to those in the control group. Whereas, these results were disagreed with other studies, like: Jeschke et al., Davis et al., [32,35] whom recorded that the IL-17 level remaining unchanged.

The current results were closely agreed with local study in AL-Najaf province which revealed that IL-17 concentration in burn patient infected with Gram negative bacteria were (129.22± 32.47), [36]. The explanations of interleukins levels (IL-10 and IL-17) in burn individuals might be diverse and affected by several factors, including: age, burn degree, the duration of burn damage, the presence of other medical conditions or consequences, in addition to the level of white blood cell. Following burn injury, it is probable that higher cytokine level in the blood were induced by interactions within a complex network of cytokine-related pathways rather than by a single factor.

Antimicrobial resistance is a fundamental issue in the treatment and management of infections. [37]. Bacteria have developed resistance to commonly used antibiotics on a worldwide basis in recent years. Reports have reported the rise of antibacterial resistance in hospital wards and society worldwide, possibly due to misuse of antibacterial treatments [38]. Nanoparticles exhibit minimal toxicity to the ecology, making them a viable option for combatting pathogens [14] showed that bacteria that were resistant to antimicrobials were extremely sensitive to silver nanoparticles.

The q-PCR results recorded a high decrease in the expression of the *fnbA* gene in the presence of Ag NPs with antibiotics (0.271-fold); these results were agreed with the results of [14], which showed that the expression of the *fnbA* and *fnbB* genes in the presence of Ag NPs decreased from 0.46 to 0.06-fold, and silver NPs were more effective than ZnO nanoparticles. Metal nanoparticles have been considered as potent antibacterial agents in recent years. Examining the impact of silver nanoparticles and their synergy with antibiotics on planktonic Staphylococcus spp is reported as an effective antibacterial agent. [39,40]. The current results of the bacterial group treated only with OX antibiotic showed elevated folding compared with groups treated with Ag NPs alone or with antibiotic; this may be related to using sub-MIC concentrations of nanoparticles, which have not inhibited the growth of the bacteria but only had an effect on the expression of genes. studies of gene expression investigate the impact of

antimicrobial agents by exposing cells to a sub-inhibitory concentration of the antimicrobial, which promotes growth and gene expression without triggering cell death in the presence of the toxicant [41]. The AgNP induce oxidative stress in planktonic bacteria [42]. also, another study [43] showed that only two genes of biofilms were down-regulated. Also, several genes associated with biofilm survival (*carA, carB, pyre, pyrF, trap, finbB and sarS*) were downregulated in presence of Ag+ [44].

Conclusions

The synergistic effect of Ag NPs with antibiotics to decrease the expression of the *fnbA* gene in resistant isolates of *S. aureus*, Nanoparticles have a promising use as antibacterial biofilm agents in burn centers. even though required to many studies, especially on animal models, The elevated titers of IL-10 and IL-17 in burn patients. may implied that those cytokines had an anti-inflammatory impact and were associated with the emergence of septic consequences. It might be suggested as a potential biomarker of prognosis in a clinical setting.

Disclosure

None

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