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The Role of Staphylococcus Aureus Types and Toxin-Producing Ability in Pediatric Atopic Dermatitis and its Association with Disease Severity

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Abstract

Background: The skin of patients with atopic dermatitis has a high susceptibility to staphylococcus aureus (*S. aureus*) colonization known to produce toxins with super antigen (SAgs) activity which are a family of potent immune-stimulatory exotoxins and may aggravate AD. The aim of this research was to evaluate the role of staphylococcus aureus types and toxin-producing ability in pediatric atopic dermatitis and its association with disease severity.

Materials and Methods: In this cross-sectional study, fifty-two patients with AD were evaluated for clinical severity of disease using severity scoring of atopic dermatitis (SCORAD) index. Swabs were taken from their skin and *S. aureus* was isolated, then the *mec*A, SCC*mec* types and agr genes besides exotoxins with super antigen properties, *Hla* and *TSST* genes were evaluated by performing polymerase chain reaction (PCR).

Results: *Staphylococcus aureus* was isolated in 38 (73.07%) out of 52 AD patients. The SCORAD index and AD severity were strongly correlated with *S. aureus* colonization (P=0.00). The staphylococcal alpha-hemolysin (*Hla*) was the predominant toxin gene found in AD patients, *Hla* was produced in 22 patients (57.9%). The toxic shock syndrome toxin-1 (*TSST-1*) gene was found in 12 (31.6%) isolates and, in 11 patients both *TSST-1* and *Hla* toxin gene were detected. There was no significant relationship between the presence of TSST1 and *Hla* gene and the severity of the disease *Hla* (P=0.11 and P=0.08, respectively).

Conclusion:
AD severity based on the SCORAD index was strongly correlated with *S.aureus* colonization, and the most frequent super antigen gene present in *S. aureous* isolates was that coding for (*Hla*).

Key Words: Atopic Dermatitis, Colonization, Staphylococcus aureus, Toxin gene.

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1- INTRODUCTION

Atopic dermatitis (AD) is the most prevalent chronic and relapsing skin condition in pediatrics, which affects between 10 and 20 percent of infants and children worldwide (1). Patients with AD are highly susceptible to bacterial, viral, and fungal skin infections. Different bacteria are involved in these infections, among which is staphylococcus aureus (*S. aureus*), especially the methicillin-resistant species that has a crucial role. This colonization and infection might lead to re-inflammation or chronicity of dermatitis. Studies have shown that 80 to 100 percent of patients with atopic dermatitis are colonized with *S. aureus* on the skin or in the nose, whereas this rate is between 5 and 30 percent among healthy people. Research has established a link between the severity of dermatitis lesions and *S. aureus* colonization, demonstrating that colonization with this bacterium aggravates atopic dermatitis lesions (2, 3).

Given the structure and action mechanism of toxins with super-antigenic property of *S. aureus* in induction of severe inflammation and their role in the development of resistance to steroid therapy of atopic dermatitis, the authors chose toxic shock syndrome toxin (TSST) as the major super-antigenic toxin of *S. aureus*, and evaluating its impact on the severity of lesions as the purpose of the present study. Furthermore, other factors affecting the severity of atopic dermatitis lesions are cellular damage resulting in the release of various cytokines and the aggravation of lesions symptoms. One of the powerful cytotoxins of *S.* aureus is Alpha-hemolysin (Hla) that destroys host cells, resulting in the release of various cellular substances into the environment and the occurrence of many other effects. Considering the role of *S. aureous* in induction or exacerbation of AD lesions and lack of a comprehensive study of this subject in Iran, this study was conducted to study *TSST1* and *Hla* as two major super antigens of *S.aureous* in order to determine their effects on the severity of atopic dermatitis lesions.

2- MATERIALS AND METHODS

2-1. Study design and population

The present descriptive and analytical cross-sectional study was performed on 52 children with atopic dermatitis, who referred to the dermatology clinics of Qaem, Emam Reza and Sheikh University hospitals, Mashhad, Iran, from October 2016 to November 2017.

2-2. Inclusion and exclusion criteria

Children with atopic dermatitis from infancy to 13 years old were selected based on diagnostic criteria. AD was diagnosed according to the major and minor diagnostic criteria (4). The following children were excluded from the study: those under treatment with topical antibiotics recently in last 2 weeks or with systemic antibiotics in last 4 weeks, concomitant presence of another severe systemic infection, patients under treatment with systemic corticosteroids or immune-suppressive agents in the last 4 weeks or with a severe fungal infection or other skin diseases that might disturb the diagnosis.

2-3. Measuring tool

After the diagnosis and determination of the disease severity by a dermatologist, the results were recorded using severity scoring of atopic dermatitis (SCORAD), which is one of the best and most reliable systems in determining the severity of atopic dermatitis (5). This scoring system combines extent, severity, and subjective symptoms of AD. To measure the extent of AD, the rule of nines is applied on a front/back drawing of the patient's inflammatory lesions. The extent can be graded 0-100. The intensity part of the SCORAD index consists of six items:

erythema, oedema/papulation, excoriations, lichenification, oozing/crusts and dryness. Each item can be graded on a scale 0-3. The subjective items include daily pruritus and sleeplessness. Both subjective items can be graded on a 10-cm visual analogue scale. The maximum subjective score is 20. All items should be filled out in the SCORAD evaluation form. The SCORAD index formula is: A/5 + 7B/2 + C. In this formula A is defined as the extent (0-100), B is defined as the intensity (0-18), and C is defined as the subjective symptoms (0-20). The maximum SCORAD score is 103. For interpretation of scores, SCORAD ≤ 25 is considered mild, SCORAD 25-50 is moderate and SCORAD ≥ 50 is considered high severity (5).

2-4. Intervention

Swabs were taken from skin lesions of patients, placed in transport media, and sent to the microbiology laboratory of Qaem Hospital, Mashhad, Iran for the isolation of *S. aureus*. Once the isolates were detected, phenotypic tests were performed for their confirmation. Then, antibiotics susceptibility tests were performed by the disk diffusion method for the following antibiotics: oxacillin, vancomycin, minocycline, levofloxacin, ciprofloxacin, tetracycline, co-trimoxazole, gentamicin, clindamycin, and rifampin.

2-5. PCR for superantigen genes testing

Molecular tests were conducted by extracting the DNA of *S. aureus* using QIAGEN kit, (Germany). After determining the quality of the extracted DNA, the presence of *mec*A, *Hla* and *TSST* genes were evaluated using Polymerase Chain Reaction (PCR) testing. Also, to identify the five main known SCC*mec* types and detecting accessory gene regulator PCR was used. It is noteworthy that Staphylococcal cassette chromosome *mec* (SCC*mec*) is a mobile genetic element of *Staphylococcus* genus that carries the *mecA* or *mecC* gene and plays a core role in the antimicrobial resistance characteristics and evolution of MRSA, also accessory gene regulator (agr) of *S. aureus* is a global regulator of the staphylococcal virulon, which includes secreted virulence factors and surface proteins.

2-6. Ethical consideration

Ethical approval to conduct the study was given by Mashhad University Hospital and consent was obtained from all patients' guardians before inclusion in the study.

2-7. Data and Statistical analysis

A simple non-random sampling method was used with a maximum sample size of 52 as derived through the formula $n = \frac{z^2 p(1-p)}{d^2}$ (i.e., the result/outcome of one ratio to the population), with the confidence coefficient of 95%, p = 0.75, and d = 0.12. Collected data were analyzed by the SPSS software (version 13.0; SPSS, Chicago, IL, USA), and the statistical differences of efficacy were calculated using the Chi-square test and the significance level was set at $p<0.05$.

3- RESULTS

A total of 52 samples were collected from atopic dermatitis lesions with different intensities. 31 (59.6%) patients were female. The age of the study group ranged from 3 months to 13 years old, with a mean age of 4.9 years. The mean duration of the disease was 8.4 months and the mean SCORAD of the patients was 48.32. Most samples isolated from the lesions (53.85%) had severe score (SCORAD ≥ 50), 36.54% moderate (SCORAD: 25-50), and 9.61% (SCORAD ≥ 50). **Figure.1** compares the severity of lesions, based on SCORAD system, in different groups of samples with *S. aureus* and samples without this bacterium. According to the above figure and the

statistical analysis using the Chi-square test, there was a significant relationship between colonization with *S. aureus* and the severity of lesions (p=0.000). Disk diffusion method was used for evaluation of antibiotic sensitivity on all samples isolated from lesions.

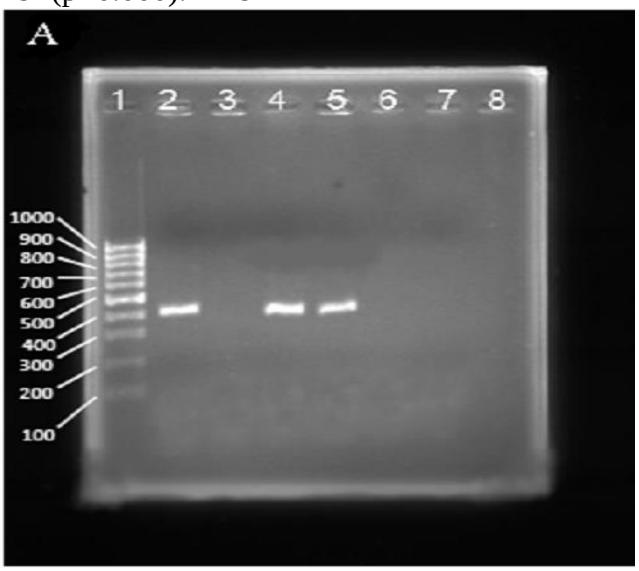

Fig.1: Electrophoresis of PCR product for different toxins of TSST1 (A.) and Hla (B.). It can be observed that Ladder100bp in lane 1, lanes 3, 6, 7, and 8 belong to negative sample and lanes 2, 4, and 5 show PCR product of positive clinical isolates for TSST1 (size: 398bp). On the other hand, in B. lane 1 demonstrates Ladder100bp, lane 8 presents negative sample and lanes 2-7 illustrate PCR product of positive clinical isolates for Hla (size: bp744).

As shown in **Figure.2**, the highest and the lowest resistance rates exist against oxacillin and ciprofloxacin, respectively. It should be noted that all of the isolates in this study were susceptible to vancomycin. **Figure.3** compares the resistance of isolates taken from different lesions. Using the Kruskal-Wallis test, we observed a statistically significant difference in terms of resistance to oxacillin, minocycline, ciprofloxacin, co-trimoxazole, and clindamycin, compared to the rest of the other antibiotics ($p<0.05$). Polymerase chain reaction (PCR) was performed on all specimens in the case of the mecA gene; the mecA gene characterized 65.8% of isolates, and 34.2% displayed an adverse reaction.

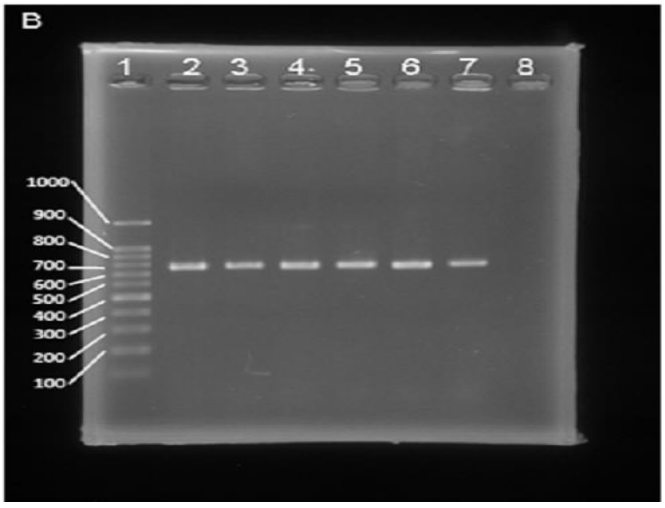

Fig.2: Comparing the severity of lesions, based on SCORAD system, in different groups of samples with *S. aureus* and samples without this bacterium.

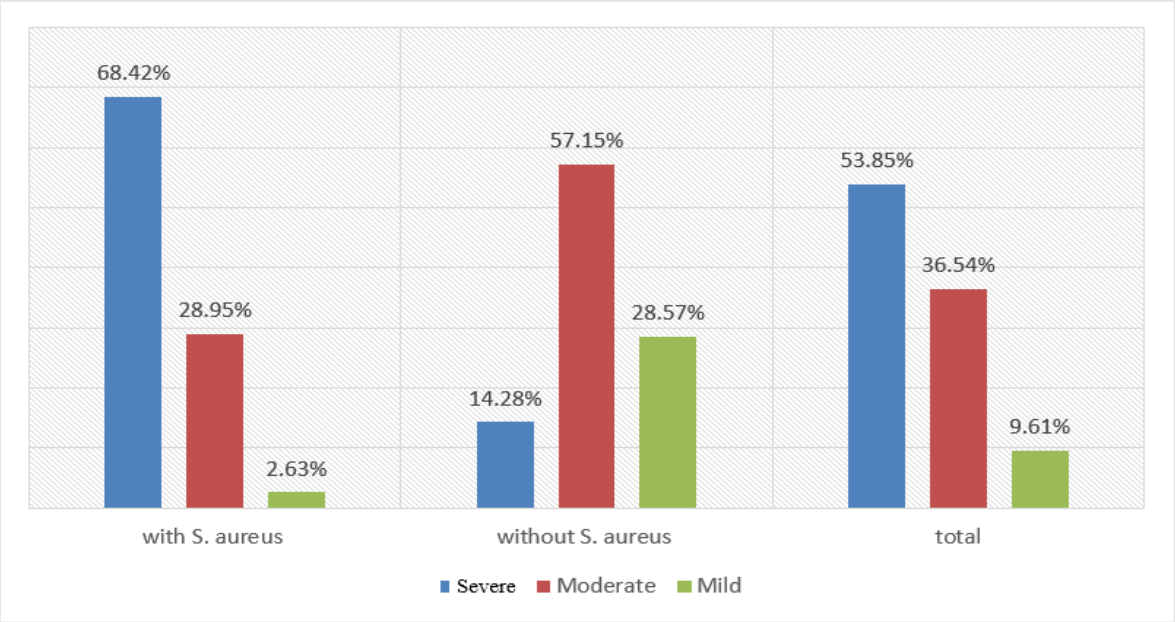

Fig.3: Comparing the severity of lesions, based on SCORAD system, in different groups of samples with *S. aureus* and samples without this bacterium.

Figure.4 compares the severity of lesions based on SCORAD in cases colonized with methicillin-resistant versus sensitive strains. In statistical analysis using the Chi-square test, there was no significant relationship between the severity of lesions and the number carrying the *mec*A gene (p=0.448).

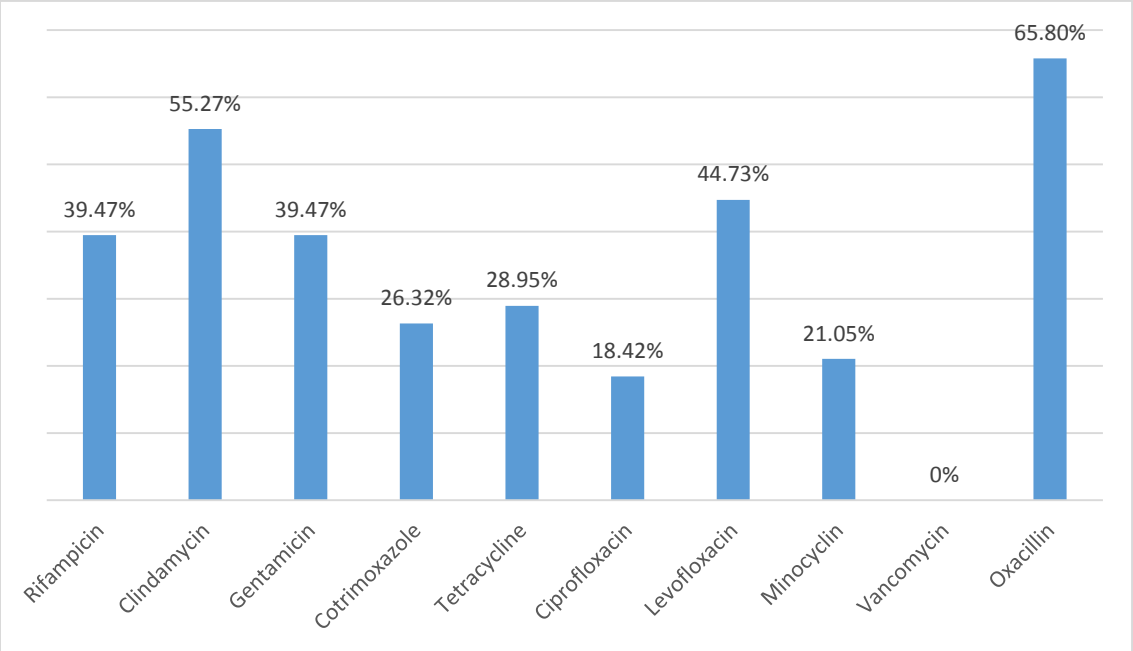

Fig.4: Resistance rates of samples with *S. aureus* against different antibiotics.

After that, PCR was done to determine different types of SCCmec, the results of which are represented in **Table.1**. As can be seen, the highest percentage of samples belonged to group III (55%) followed by group II (21.5%), and then to groups I, IV

and V (10%) each. Comparing SCORAD of lesions in cases colonized with methicillin-resistant strains belonging to different types of SCCmec did not suggest a statistically significant relationship (p=0.926). PCR results for identifying *agr* groups have been shown in **Table.2**. As it shows the highest percentage of samples belonged to agr group I (73%), and then III (18.43%), and II (7.89%). None of the isolates belonged to group IV.

Table-1: PCR results to determine different SCC*mec* type I–V. SCCmec*:* Staphylococcal chromosomal cassettes.

SCCmec type	I	II	III	IV	V
Number	2	3	11	2	2
Percentage	10	15	55	10	10

Table-2: PCR results for identifying agr groups.

agr group	I	II	III	IV
Number	28	3	7	0
Percentage	73.68	7.89	18.43	0

agr: Accessory gene regulatory.

Figure.1 shows PCR results for investigating *TSST1*and *Hla*. After evaluating the presence of genes through PCR method, the authors analyzed the relationship between *TSST1*and *Hla* (**Figures 5-10**). Based on our results, there was no statistically significant relationship between the presence of *TSST1* gene *and Hla* gene and the severity of lesions. (p=0.11, p=0.089, respectively). Comparing the cases of *Hla-/TSST1+* and *Hla+/TSST1-*, using the Chi-square test did not reveal a significant relationship (p=0.0*21*). Similarly, comparing the cases of *Hla-/TSST1-* and *Hla+/TSST*1+ despite the great difference, no statistically significant relationship was observed (p=0.06). According to the analyses discussed above, among all of the study variables, only the severity of lesions had a statistically significant relationship with colonization by *S. aureus* (p<*0.05*).

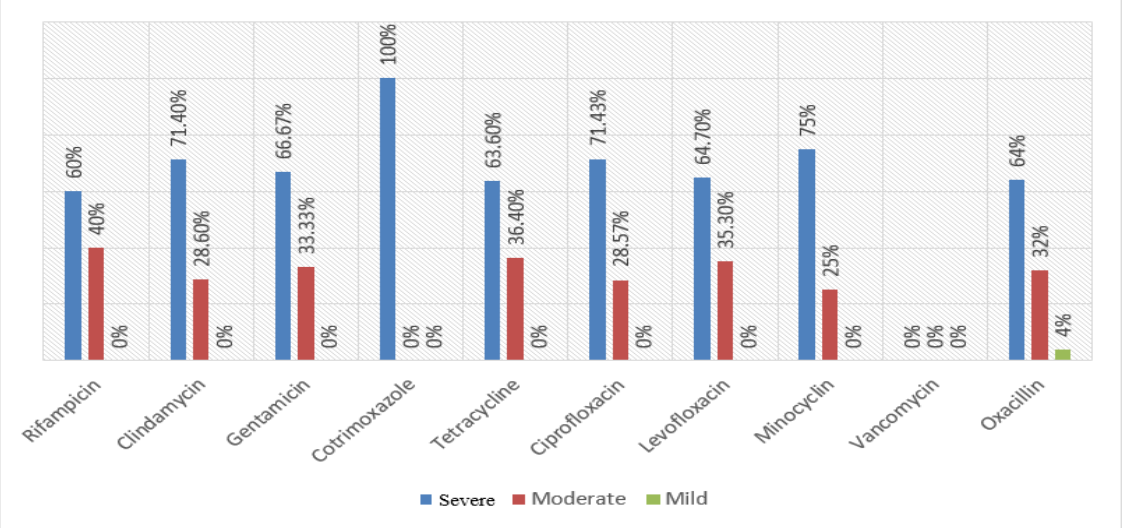

Fig.5: Comparison of the resistance of isolates taken from different lesions.

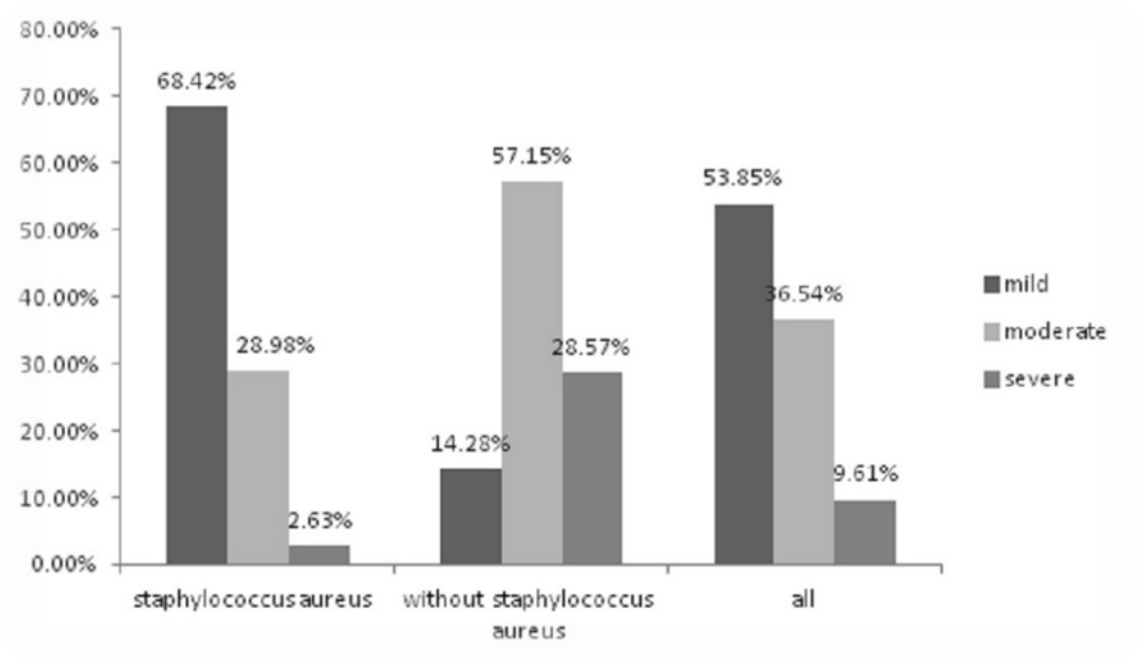

Fig.6: Resistance rates of samples with *S. aureus* against different antibiotics.

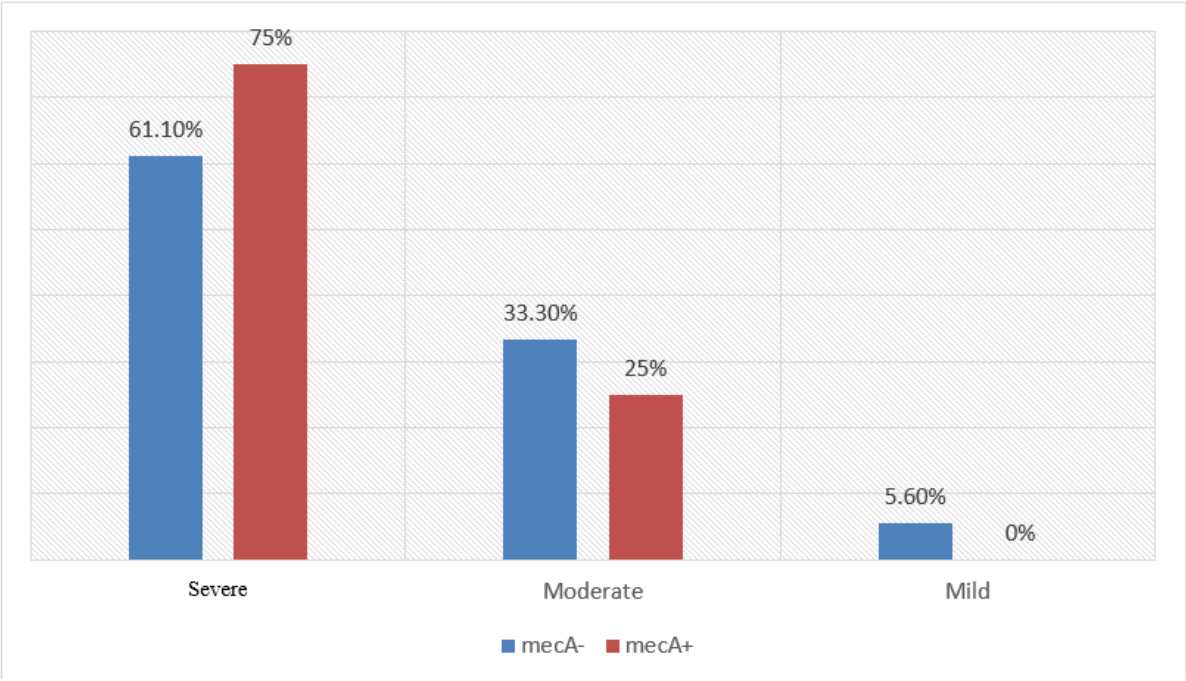

Fig.7: Comparison of lesions' severity based on SCORAD in cases colonized with methicillin-resistant versus sensitive strains.

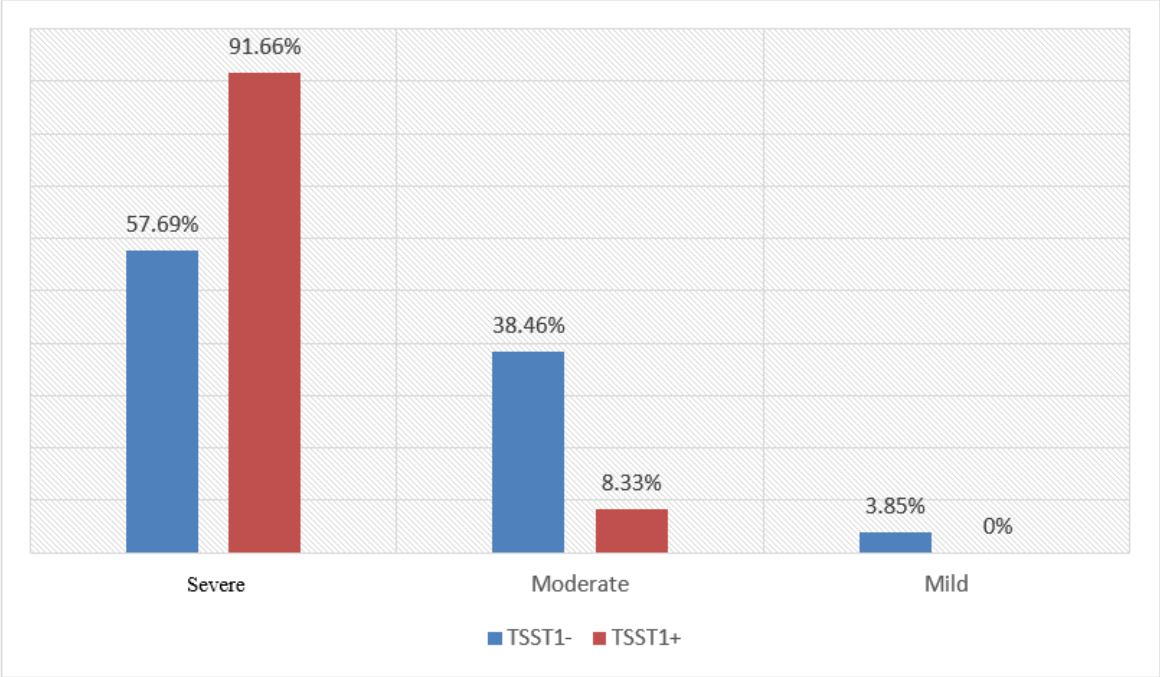

Fig.8: Comparing SCORAD of lesions in cases of positive versus negative *TSST1*.

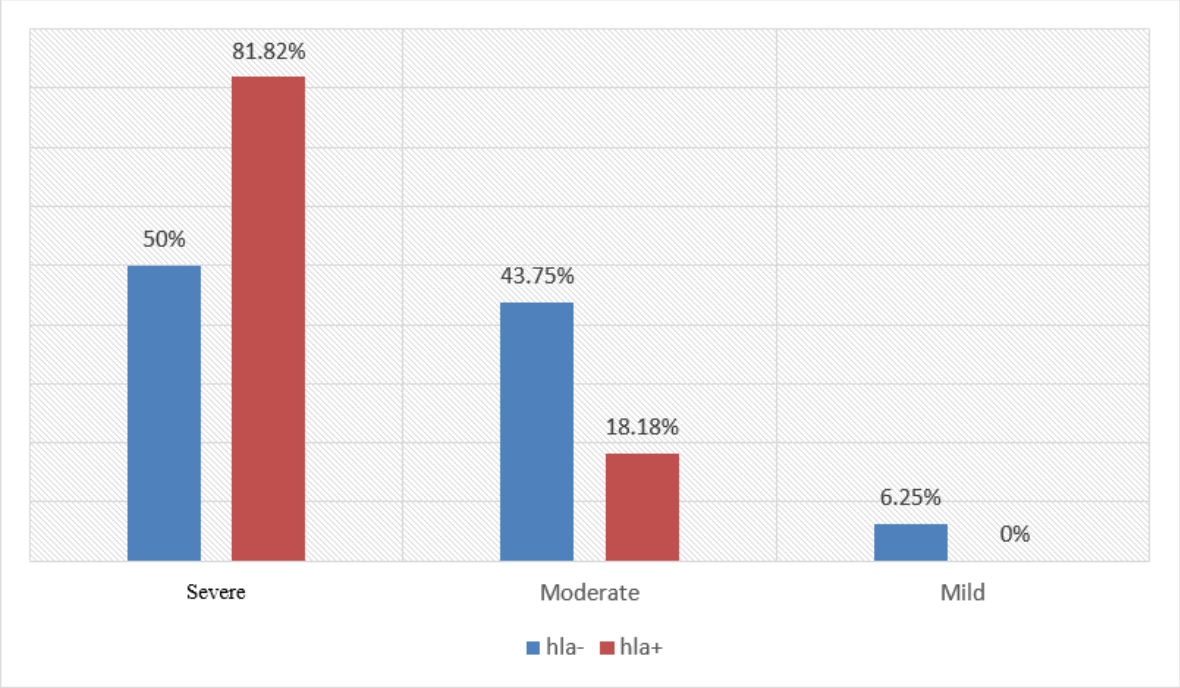

Fig.9: Comparing SCORAD severity of lesions in cases with and without *Hla.*

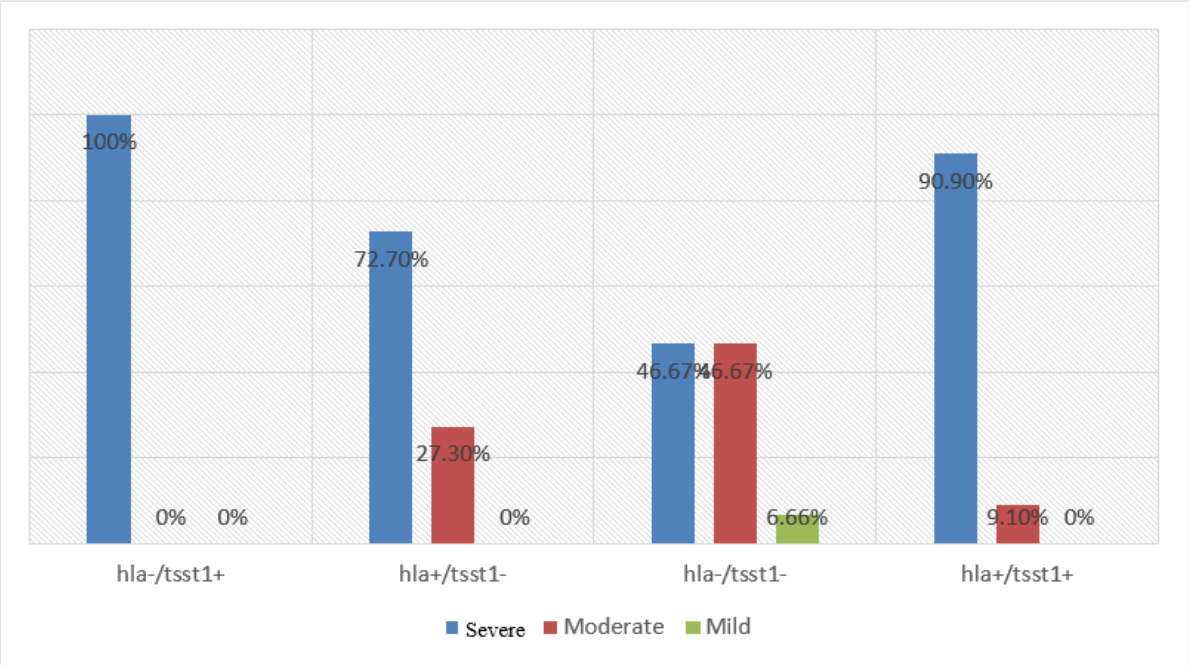

Fig.10: Comparing SCORAD severity of lesions in different instances of toxin patterns.

4- DISCUSSION

Atopic dermatitis is a common chronic inflammatory and immune-mediated skin disease. Complex interactions among susceptibility genes encode skin barrier molecules, environmental factors, inflammatory response and infectious agents especially *S. aureus* that lead to dysbiosis of the microbial community resident in AD skin. Rates of *S. aureus* carriage in AD skin reach 30–100% in comparison to 20-30% of healthy subjects (6). In this study, the number of colonization of atopic dermatitis lesions with *S. aureus* was initially examined, and 73.07% of cases revealed this association. Other studies carried out in several parts of the world, including Iran, have reported different rates of colonization of atopic dermatitis lesions by *S. aureus*. Soares et al., conducted a study about diversity profile from the Staph community on AD patients. The staphylococcal microflora was dominated by *S. aureus* (69 isolates, 35_6%) followed by *S. epidermidis* (59 isolates, 30_4%), and *S. hominis* (54 isolates, 27_8%) species (7). Pezeshkpour et al., observed that 42.5% of patients with atopic dermatitis lesions were colonized by *S. aureus*, which is a high rate compared to their control group (7.5%) (8); another research performed by Tang et al. (2011) noted that the rate of colonization of AD lesions by *S. aureus* was 46.4% (9). Hill et al. reported *S. aureus* colonization rate of 68% in AD cases (10). Lower rates of colonization with *S. aureus* in the mentioned studies have been reported in comparison to our results; however, other reports suggest closer rates to our research or even greater ones. For instance, Bell et al. (2012) stated a colonization of 89% (11), and Petry et al. found that 73.6% of patients were colonized by this bacterium (12). Considering those and similar studies, it might be concluded that the amount of colonization of AD lesions with *S. aureus* has increased over time. The notable finding of the current study is the existence of a statistically significant relationship between the severity of lesions and colonization by *S. aureus* (p=0.000). The majority of severe cases were

colonized by *S. aureus*, whereas the mild ones mostly lacked such colonization (Figure.1). In our study the antibiotic resistance of each isolate was as follows: oxacillin (65.8%), vancomycin (0%), minocycline (21.05%), levofloxacin (44.73%), ciprofloxacin (18.42%), tetracycline (28.95%), co-trimoxazole (26.32%), gentamicin (39.47%), clindamycin (55.27%), and rifampin (39.47%). Niebuhr et al., studied antibiotic resistance of *S. aureus* isolates taken from atopic dermatitis lesions (13). Their study noted the following resistance rates: oxacillin, co-amoxiclav, cephalexin, and cefuroxime (3%); tetracycline (17%); gentamicin and rifampicin (16%); erythromycin and clindamycin (21%); co-trimoxazole and levofloxacin (23%); fusidic acid (25%); and phosphomycin (12%). As it is clear, the rates of resistance in almost all these cases are lower than those of our present study.

In another study, Hoeger revealed that the rate of colonization of AD lesions with *S. aureus* was 87%, and the rates of antibiotic resistance were as: erythromycin (18%), roxithromycin (19%), fusidic acid (6%), amoxicillin (13%), and clindamycin (1%). Further, all isolates were susceptible to cefadroxil, oxacillin, co-amoxiclav, and cefuroxime (14). As it is evident in the mentioned research, the rates of antibiotic resistance were markedly lower than our results; nevertheless, their reported general amount of lesions'colonization with *S. aureus* was high. Figure.2 compares the rates of antibiotic resistance in lesions of various severity. Considering this figure, one can see that the general rate of resistance to antibiotics used in the isolates taken from severe and moderate lesions is higher than that of bacteria isolated from mild lesions. The highest amount of resistance was observed in strains isolated from severe lesions. The assessment of isolates taken from various lesions implied that resistance to oxacillin, minocycline, ciprofloxacin, co-trimoxazole, and clindamycin was significantly different from other antibiotics ($p<0.05$). These differences could be due to the diverse range of patients studied in this research. Generally, if the lesions are colonized by organisms of low pathogenicity, it is anticipated that antibiotic resistance would be low; in contrast, if organisms are highly pathogenic, it is possible that a high antibiotic resistance could occur; this is due to high pathogenic strains that usually go through high antibiotic pressure conditions whereby strains with high resistance are selected. Since the strains isolated in this study showed high antibiotic resistance, it could be expected that they have undergone selective antibiotic pressure, which preserves resistant strains of each clone that later colonize one's skin. Another point which might be inferred is that of considering the time frame of this research as well as those of other studies mentioned above, where the rate of antibiotic resistance of *S. aureus* strains might have escalated over time.

This could be due to many reasons, including selective antibiotic pressure and the acquisition of mobile genetic elements carrying antibiotic resistance genes such as transposons or plasmids. One of the critical points concerning the antibiotic resistance of *S. aureus* is the methicillin resistance. Strains of methicillin-resistance gene (mecA) are resistant to all β-lactams. In this study, methicillin-resistance was evaluated using phenotypic and genotypic methods and was identified as 65.8%. The results of various studies in this field which were also conducted in Iran are consistent with our present study, suggesting that the prevalence of methicillin-resistant *S. aureus* (MRSA) isolates has increased in our country over time (15). This finding has also been corroborated by many studies in other parts of the world. For instance, the prevalence of MRSA in Malaysia was

34.5% in 1990-1991 (13). In India, the prevalence rates of MRSA have been 6.9% in 1988, 12% in 1992, 24 to 32% in 1994, and about 80% in 1999 (17). Another study in Spain indicated that MRSA prevalence has increased from 39.9% in 2002 to 46.4% in 2006 (18). It was observed that the prevalence of MRSA isolates has varied across different regions in the United States, between 10 to 49 percent from 2004 to 2005 (19). Finally, the study by Kim et al. (2006) in South Korea reported a 68.3 percent prevalence of MRSA isolates (20). Accordingly, it could be argued that the prevalence of MRSA has been on the rise. Complete *S. aureus* eradication may have an improvement role in AD symptoms. Indeed, it has recently been suggested that antibiotic therapy might be necessary to successfully target skin colonizing *S. aureus* (21). It also has to be considered that vaccines against *S. aureus* represent a possible novel approach to manipulating the AD skin microbiome.

In AD, *S. aureus* a-hemolysin is capable of activating important immune complex in keratinocytes, and inflammasome activation is greatly compromised due to the reduced expression of its components, driven by the action of T helper 2 (TH2) cytokines. Moreover, *Hla* has a role in disruption of the skin barrier generating pores in the cell membrane that culminate in keratinocyte lysis. AD patients show a higher susceptibility to *Hla* action (6). In the current study, TSST1 and *Hla* have been examined using PCR method. It was found that out of 38 cases of *S. aureus* isolated from lesions, 22 (57.9%), and 12 (31.6%) specimens carried *Hla* and *TSST1* genes, respectively. Besides, 11 isolated strains had both *Hla* and *TSST1*, and 15 others showed none of these genes. Analyzing Table.1 clarifies that overall, *Hla* has the highest frequency among the isolates obtained from all samples. *Hla* is one of the cytolytic toxins of *S. aureus* that can affect a wide range of eukaryotic cells and lead to inflammation. Recent studies proposed that an increase in the production of *Hla* due to *S. aureus* is correlated with increased severity of atopic dermatitis lesions. Moreover, *Hla* production may destroy keratinocytes and impair epidermal hyperplasia (22). Since in the present study most of the isolates characterized by *Hla* have been taken from severe lesions, it is possible to infer the existence of a relationship between this toxin and the severity of atopic dermatitis lesions; however, it has to be noted that this relationship was not statistically significant ($p > 0.05$). In this research, the prevalence of *TSST1* in the studied isolates was lower than that of *Hla*. Additionally, although the isolated strains have been obtained from severe lesions, in most cases, no significant relationship was statistically observed between the presence of this gene and the severity of lesions ($p> 0.05$). Furthermore, comparing the cases of *Hla*-/*TSST*1- and *Hla* +/*TSST1*+ notwithstanding the vast difference did not reveal a statistically significant relationship ($p=0.06$). Analyzing the variables of this study, the authors noted that the only statistically significant relationship exists between colonization by *S. aureus* and the severity of lesions ($p<0.05$). Based on the results, it seems that by increasing the number of study samples, the existence of a statistically significant relationship between toxins and severity of lesions could probably be observed.

5- CONCLUSION

This research proposes that *S. aureus* has a high prevalence in the infection and colonization of atopic dermatitis skin lesions. The one crucial and alarming finding of this study is the great prevalence of antibiotic especially methicillin resistance in such isolates. SCC*mec* type III was by far the most common SCC*mec* type among MRSA isolates.

Finally, the high prevalence of toxins in these isolates is noteworthy as well.

6- CONFLICT OF INTEREST: None.

7- ACKNOWLEDGMENTS

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