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ORIGINAL ARTICLE

The Role of *Staphylococcus aureus* Superantigens in Pediatric Atopic Dermatitis

[1]Amal A. Wafy*, [1]Wageih S. Elnaghy, [2]Nashwa El-Far, [3]Sara A. Hamam, [4]Mohammed El-Gamasy[4]

Departments of [1]Microbiology & Immunology, [2]Dermatology, [3]Clinical Pathology and [4]Paediatric, Faculty of Medicine, Tanta University, Tanta, Egypt

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Corresponding Author:
Amal A. Wafy
Department of Microbiology & Immunology, Faculty of Medicine, Tanta University, Tanta, Egypt;
Tel.: 01110102232
amlwafy@gmail.com

ABSTRACT

Background: *Atopic dermatitis (AD) is a chronic inflammatory skin disease prevalent in children with a rate of 60–90% of superantigen producing staphylococcal colonisation that may play a role in its pathogenesis.* ***Objective:*** *This study aims to investigate the role of superantigen in the pathogenesis of atopic dermatitis by identifying enterotoxins producing S. aureus on the skin and nose of the patients using reverse passive latex agglutination test.* ***Methodology:*** *This study comprised 44 cases with atopic dermatitis attending the Outpatient Clinic of Dermatology & Paediatric Departments of Tanta University Hospital from December 2016 to the end of November 2017. Twenty healthy control volunteers were included.* ***Results:*** *Bacteriological skin cultures of patients revealed colonization with staph. aureus in(72.2)%, whereas, only 6 normal subjects (30%) showed positive cultures. This difference was statistically significant. Culture of nasal swabs revealed colonization with S. aureus in 21 atopic patients (47.2%) and only 8 subjects of control group (40%) with a statistically non significant difference. Wet lesions (in 23 patients) showed highly statistically significant colonization with S. aureus in comparison to dry lesions (in 6 patients). Out of the (32) positive staph. cultures from the skin of patients,(18) isolates were enterotoxin producers;(3) isolates produced enterotoxin A,(4) produced enterotoxin B,(9) produced enterotoxin C and (2) produced enterotoxin D. In control group, only (2) positive skin cultures were enterotoxins producers; (1) produced enterotoxin B and (1) produced enterotoxin C., the statistical difference was significant. Out of (21) nasal positive Staphylococcal cultures, 6 isolates were enterotoxins producers. Four of them produced enterotoxin B and two produced enterotoxin C. In control group, only 2 positive nasal cultures were enterotoxins producers; one produced enterotoxin B and one produced enterotoxin C. After treatment, skin lesions were clinically improved and the number of positive cultures were reduced from 18 to 8 (6 of them were still superantigen producing). This difference was significant.* ***Conclusion****: It is concluded that daily skin care, use of topical steroids and systemic anti-Staphylococcal antibiotics, are necessary to reduce skin inflammation.*

INTRODUCTION

Atopic dermatitis (AD) is a common, chronic itchy skin disease with prevalence of 7 - 17%. Both hereditary and environmental factors play a role in the pathogenesis of AD. Four chromosomal regions of linkage to the disease, which differ from atopy-associated loci, have been identified in genome scans of children with AD[1,2], deficient epidermal barrier defenses, impaired innate immunity and abnormal insufficient acquired immunity resulting in defective skin barrier and inflammation [3, 4].

The main environmental factor to be implicated in the pathogenesis of AD is the reduction in the exposure to infections during early childhood, which may result from the increased use of antibiotics, a reduction in family size and an increase in the hygiene hypothesis [5,6].

Microorganisms play an effective role in the pathogenesis of atopic dermatitis, 60–90% of children with atopic dermatitis have very high rates of superantigen secreting staphylococci colonisation of their skin, which may play a role in the pathogenesis and inflammatory responses in atopic dermatitis [7]. This is augmented by the clinical improvement of atopic skin lesions after antistaphylococcal treatment [8-10].

Staphylococcal superantigens (SAG) are potent toxins produced by particular strains of *Staphylococcus aureus*[11,12]. These superantigens, by their ability to bind multiple T cell receptors in an antigen non-specific manner, are capable of inducing massive T cell activation[6]. Stimulation of T cells by SAG is considered to trigger diseases such as staphylococcal toxic shock syndrome and staphylococcal food poisoning [13] and has been implicated in other disorders such as Kawasaki disease and atopic dermatitis [14,15].

The major effects of staphylococcal superantigens in AD are likely to be mediated through the polyclonal activation of superantigen-specific TCR Vβ families of T cells[16]. In the peripheral blood of AD patients whose skin is colonized by superantigen-secreting S. aureus, a relevant skewing of superantigen-reactive Vβ families was observed in CD4+ and CD8+ T cells coexpressing cutaneous lymphocyte antigen (CLA), a skin homing receptor [17]. Superantigens up-regulate CLA expression by T cells via stimulation of IL-12 production, thus promoting their homing to the skin [18]. IL-31 is able to induce itching in AD patients and is mainly upregulated by SsAGS [19].

The main consequence of increased colonization of AD skin by *S. aureus* is exacerbation of the inflammatory immune response, which is largely mediated by the release of staphylococcal enterotoxins (SE), such as SEA, SEB and toxic shock syndrome toxin 1 (TSST-1) [20]. These enterotoxins when applied to intact normal skin or the non-lesional skin of patients with AD can induce erythema and dermatitis, [16]. These findings suggest that superantigens can initiate, exacerbate and maintain inflammation associated with AD.

Unlike the classic antigen, the superantigen does not need intracellular processing within the antigen presenting cells or previous sensitization of the host cells [21]. It binds to MHC class II molecule outside the antigen binding groove [22] and activates T cells with specific T cell receptor, (Vβ chain).[23]

The growing knowledge of the rule of s. aureus in fulminating AD contribute to an important rationale for the use of antibiotics in treatment of AD [24,25]. Topical steroids are widely used for treatment of AD. Meanwhile, recent studies demonstrated that local steroid treatment, even without antibiotics, can reduce S. aureus colonization[26,27] and improve cases with AD[27]. The aim of the present study was to investigate the possible role of superantigens in the pathogenesis of atopic dermatitis by identifying enterotoxins A, B, C and D producing *S. aureus* on the skin and nose of patients with atopic dermatitis using reverse passive latex agglutination (RPLA) test. Also, to study the effect of treatment with corticosteroids on colonization of patients' skin with superantigens producing *S. aureus* and on clinical improvement.

METHODOLOGY

The present study was done on 44 patients with typical picture of atopic dermatitis attending the Outpatient Clinic of Dermatology & Pediatric Departments of Tanta University Hospital. Twenty healthy age and sex matched volunteers with age ranged between 6 months and 14 years. The mean age was 4.2±3 years. were included in the study and served as a control group. The patients were subjected to thorough / history taking with stress on the age, duration and severity of itching and possible precipitating factors. None of patients or controls had received systemic or topical therapy 2 weeks before the study. Study participants were also subjected to general and dermatological examination regarding the type of the lesions and distributions

Bacteriological culture

Swabs were taken from patients and controls using sterile cotton tipped swabs from the skin lesions and nasal mucosa and cultured on blood agar plates. The cultures were grown overnight (37°C in 5% CO2). Isolates were identified as *S. aureus* according to their colonial morphology, morphology by Gram stain and positive results in coagulase and latex agglutination tests [28].

Production of enterotoxins A, B, C and D was determined by reverse passive latex agglutination (RPLA), using commercial kit tests (SET -RPLA, , Oxoid), according to the manufacturer's instructions [29]. Briefly, isolates were incubated on the nutrient agar plates and incubated for 24 h at 37°C. One loopful of the growth was transferred to 5 ml tubes of sterile distilled saline solution to obtain McFarland turbidity value of (300.000.000 organism per ml) was inoculated into 10 ml Trypton Soya Broth (Oxoid CM 129) and incubated at 37° C for 18 - 24 h with shaking. After growth, centrifugation at 3000 rpm for 20 minutes at 4°C was made and the supernatant was retained for assay of toxin content. Into each well of five rows of a V-bottomed microtitration plate, 25 (μl of phosphate-buffered saline containing 0.5% bovine serum albumin (W/V) were dispensed and 25 μl of the test culture supernatant were then dispensed into the first well of each row and doubly diluted; the dilutions were not extended into the last well of each row. A volume of 25 μl of latex sensitized with rabbit anti-staphylococcal enterotoxin A IgG antibody was added to each well of the first row. Similarly, 25 μl of anti-enterotoxin -B-sensitized latex, anti-enterotoxin -C - sensitized latex, anti-enterotoxin-D - sensitized latex and control latex suspension coated with non-immune rabbit globulin were added to each of rows 2, 3, 4 and 5, respectively. The contents were mixed by agitation by hand, covered and left undisturbed on a vibration-free surface at room temperature for 20-24 h. Each of the test wells (rows 1-4) was then examined for agglutination which indicate

the presence of the respective enterotoxin (A, B,' C or D). The absence of agglutination in row 5 and in the last well of rows 1- 5 (negative controls) served to exclude non-specific agglutination and auto agglutination, respectively.

Eighteen cases with positive staphylococcal skin culture (8 of them were enterotoxin producers) had received treatment in the form of topical corticosteroid alone for 7days and another swab is taken after treatment.

RESULTS

Bacteriological culture of skin swabs of atopic patients revealed colonization with S. *aureus* in 32 out of 44 cases (72.2%). Whereas, only 6 cases from the control group (30%) showed positive culture for *S. aureus.* This difference is statistically significant ($p<0.001$). Culture of nasal swabs revealed colonization with *S. aureus* in 21 atopic patients (47.2%) and only 8 subjects of control group (40%) with a statistically non-significant difference (p=0.88) (table l).

Table 1: The numbers of *Staphylococcus aureus* cultures from atopic dermatitis (AD) and control subjects.

Sample	Cases (n=44)		Control (n=20)		X^2	P
	No	%	No	%		
Skin						
Positive	32	72.2	6	30	9.55	0.001**
Negative	12	28.6	14	70		
Nose						
Positive	21	47.2	8	40	0.2	0.88
Negative	23	54.8	12	60		

** = highly significant

As regards staphylococcal colonization based on the type of lesions, wet lesions (in 23 patients) showed highly statistically significant colonization with *S. aureus* in comparison to dry lesions (in 6 patients) (p=0.0006). The remaining three patients had mixed lesions (wet and dry) and not included in the comparison (Table 2)

Table 2. Staphylococcal colonization of the skin according to the type of the lesions

	Wet		Dry		P
	No	%	No	%	
+ve culture	23	71.5	6	18.8	0.0006**

*** = very highly significant

Out of the thirty two positive staphylococcal cultures from the skin of atopic patients, 18 isolates were enterotoxin producers (40.9% of the total cases & 56.3% of the positive cultures). 3 isolates produced enterotoxin A, 4 isolates produced enterotoxin B, 9 isolates produced enterotoxin C and two isolates produced enterotoxin D. In control group, only two positive skin cultures were enterotoxins producers (10% of the total cases & 33.3 % of the positive culture); one produced enterotoxin B and the other produced enterotoxin C. The difference between cases and control groups regarding enterotoxin production by *S. aureus* is statistically significant (P< 0.047) (Table 3).

Table 3: Enterotoxin production by staphylococci isolated from skin in atopic dermatitis patients and control groups

Enterotoxin	Cases	(n=44) -	Control (n=20)		Test significance Fisher's Exact	P
	No.	%	No	%		
A	3	6	0	0	X^2=3.92	0.7
B	4	9	1	5		0.53
C	9	2.6	1	5		0.1
D	2	4.5	0	0		0.65
Total	18	40.9	2	10		0.047*

* = significant

For nasal colonization, out of the 21 positive staphylococcal cultures, *6* isolates were enterotoxins producers (13.6% of the total cases & 28.6 % of the positive cultures). Four of them produced enterotoxin B and two produced enterotoxin C. In control group, only 2 positive nasal cultures were enterotoxins producers; one produced enterotoxin B and the other one produced enterotoxin C. (Table 4).

Table 4: Enterotoxins production by staphylococci isolated from nasal mucosal of atopic dermatitis patients and control groups

Enterotoxin	Cases (n=44)		Control (n=20)		Test of significance Fisher's Exact
	No.	%	No.	%	
A	0	0	0	0	-
B	4	7.9	1	5	P= 0.38
C	2	4.5	1	5	P= 0.54
D	0	0	0	0	-
Total	6	13.6	2	10	P=0.63

After treatment, skin lesions were clinically improved. Positive cultures were reduced from 18 to 8 (6 of them were still superantigen producing); this difference is statistically significant (Table 5).

Table 5. *Staphylococcal* colonization and enterotoxin production pre and post treatment

	Before		After		Test of significance "Fisher's Exact"
	No.	%	No.	%	
Staph, aureus					
+ ve culture	18	100	*8*	*44.4*	P= 0.0001***
- ve culture	-	-	10	55.5	
Enterotoxins					
Producer	8	100	6	75%	P = 0.003**
Non-producer	-	-	2	25%	

*** = very highly significant ** = highly significant

DISCUSSION

The skin lesions of atopic dermatitis are frequently colonized with superantigen producing strains of *S. aureus*. These superantigens have the capacity to activate T cells expressing specific T cell receptor Vβ chain gene segment increasing their skin homing capacity and enhancing the chronic cutaneous inflammation of AD [30].

In the present study, we investigated a group of infants and children with AD for the presence of *S. aureus* on the skin lesions as well as for colonization of the vestibulum nasi. Colonies of *S. aureus* were subcultured on sheep blood agar plates for identification by testing coagulase activity. *S. aureus* strains were assayed for production of staphylococcal enterotoxins (SEA, SEB, SEC and SED). The incidence of *S. aureus* colonization in AD patients in the current study, was found to be 72.2% (32 out of 44 cases), while the incidence of *S. aureus* colonization of skin in the control group was 30%. The difference is statistically significant (P=0.001**). These results were slightly higher than that of Dhar et al. [31] and Jappe et al. [32] who found that the incidence of positive *S. aureus* colonization in AD patients were 50% and 62.9%; respectively. Higher results, however, have been reported by Masenga et al. (14), Goodyear et al.[33] and Hoeger et al. [34] who reported a prevalence rate of 80%, 81.9% and 93%, respectively. The high incidence of *S. aureus* colonization in skin esion of atopic dermatitis could be attributed to skin lipid abnormalities in atopic patients. Skin surface lipid from human stratum corneum has *anti-Staphylococcal* activity *in vitro* and *in vivo* [35]. *S. aureus* colonization differed in relation to the type of skin lesions; in wet lesions colonization is heavier than in dry lesions that could reflects its role in exacerbating the inflammatory response In our study, staphylococcal nasal carriers were detected in 47.2% in patient with AD. This ratio is less than that reported by Jappe et al.[32] who found that nasal carriers were present in 57.1% of cases. Moreover, in our study we found no significant difference in nasal colonization as well as enterotoxins production between AD patients (45.2%) and control groups (40%). As regards

superantigen production, only 18 of 32 strains (56.3%) of *S. aureus* are capable of producing enterotoxins predominantly SEC (in 9 cases) followed by SEB (in 4 cases), SEA (in 3 cases), and the least one is SED (in 2 cases). These fndings agreed with the finding that only 10 of 22 strains (45%) of *S. aureus* isolated from AD patients are capable of producing superantigens especially SEB, SEC and TSST-1[30]. The predominance of SEC and SEB in our study is in contrast to other authors who reported that SEA (10) or TSST-1[36] are considered to be the most commonly identified toxins.

In the current study, treatment of 18 patients with positive *S. aureus* culture with topical steroids for 7 days without associated antibiotic therapy, significantly reduce colonization by 55.5% and superantigen production by 25%. These results are in accordance with those reported by Nilsson et al.[37] and Stalder et al.[38] who concluded that topical steroids of sufficient potency reduce the density of *S. aureus* in atopic skin. Reduction of inflammation and decreasing the adhesion of these bacteria to coraeocytes are the probable mechanism of this action.[38]

CONCLUSION

In conclusion, skin lesions of AD are frequently colonized with superantigens producing strains of *s. aureus.* These superantigens could act as triggering factors in atopic skin inflammation. Topical steroid therapy induce clinical improvement and reduce colonization of superantigens producing *S. aureus* in skin lesions of atopic dermatitis.

For patients who are resistant to therapy, alternative therapies should be considered because long-term treatment with oral or high-potency topical corticosteroids can lead to significant adverse events.

Recommendations:

Moreover, we implicate factors that must be considered and eliminated in treatment of AD including irritants, food, inhalant allergens and emotional stress. Maintainance of daily skin hydration and optimum use of topical steroids to reduce skin inflammation is critical. Systemic antimicrobial therapy, particularly anti-staphylococcal antibiotics, may be necessary.

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