Microbial Colonization in Atopic Dermatitis and its Associated Risk Factors in Children from Aseer Region, Saudi Arabia

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ABSTRACT

Background: Atopic dermatitis (AD) is the most common chronic relapsing multifactorial inflammatory skin disease. Colonization of bacteria, mainly Staphylococcus aureus (S. aureus) has been increased and raised antibiotic resistance to different antibiotics.

Objectives: The objectives of this study are to investigate the microbial colonization in atopic dermatitis patients and its associated risk factors in children from Aseer region, Saudi Arabia.

Methods and materials: Skin swabs were collected from AD patients (n = 78) and from healthy controls (n = 44) from Aseer regional hospital. AD patients were examined, bacteria were isolated and identified, and the severity of the disease was determined using a standardized scale: Scoring Atopic Dermatitis (SCORAD).

Results: S. aureus was the most prevalent, followed by S. epidermidis, and S. lentus whereas S. haemolyticus, Pantoea and S. hominis were prevalent in healthy controls. The severity of AD was linked to staphylococcal colonization. The occurrence of AD in age group 2 to 12 years were at risk more than others.

Conclusion: Staphylococcus spp. were positively correlated with AD incidence and its severity. The study concluded that S. aureus is the leading pathogenic determinant in the occurrence and outcome of AD in children.

Keywords: Atopic dermatitis, SCORAD, Staphylococcal species

INTRODUCTION

Atopic dermatitis (AD) is the most common relapsing inflammatory skin disease which has a high incidence during the past few decades. It is associated with significant morbidity and quality of life impairment worldwide. AD affects the quality of life of both the patient and their family[1]. It is often associated with time of school, work, and high economic costs. In Canada, one study estimated the annual cost to be more than a billion dollars[2]. In the UK, the annual cost was estimated to be around £465 million in the 1990s, and in 2002, the prescribing cost for corticosteroids alone to treat AD was £11.6 million[3].

The prevalence of AD is about 25% in children and up to 7% in adults[4]. Fifty percent of children with severe AD go on to develop asthma and allergic rhinitis[5]. Management of AD focuses primarily on measures to supplement skin barrier function (moisturizers and cotton or silk garments), but in more severe diseases also involves suppressing cutaneous inflammation (topical corticosteroids and calcineurin inhibitors) and eradicating secondary skin infections (antibiotics and antiseptics).

AD is a multifactorial inflammatory skin disease characterized by disturbances in the epidermal barrier with clinical features, pruritus, redness and dry skin. The pathogenesis of AD is still poorly understood, although it is a result of the interaction between genetic predisposition and environmental factors such as immunologic responses, a shift in skin micro-biome, nutritional, and psychological factors[6-7]. The initial signs of AD appear between 6 months of birth and 5 years in 45% and 80-90% of the children, respectively[5-10]. Different genetic factors such as filaggrin gene defect which has a proven as a risk factor for AD and

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may lead to lesions in the epidermal barrier[8]. Children present with defect skin barrier thought to have a reduced production of ceramides in the epidermal layer and high level of γ-linolenic and arachidic acid in blood serum. All these indications cause high water loss through the skin and causes dryness of the skin. All these interactions thought to be responsible for cutaneous hypersensitivity to a number of environmental factors[6,7].

The available data propose a relation between the disturbed skin microbiome and the pathogenesis of the disease[8]. AD is associated with the expression of Th2-type cytokines, increased levels of IgE and eosinophilic infiltration in the skin[9]. AD patients overexpress Th2 cytokines such as IL-4, IL-5, IL-13, and other cytokines promoting Th2 response such as TSLP, IL-25 and IL-33. No data were published on microbial colonization and related cytokines in AD patients in the south region of Saudi Arabia.

In addition, colonization with staphylococcal species was found to correlate with skin disease severity as seen previously in vernal keratoconjunctivitis in our region[10,11]. Another study showed high severity of AD and increased levels of type 2 immune biomarkers in S. aureus carriers and noncarrier AD patients[12]. Interestingly, it has been suggested that shifts in skin microbial composition may appear before the start of AD[13]. In AD patients, it has been reported that the percentage of *Clostridium difficile*, *Escherichia coli* and *S. aureus* was higher in the gut more than that in healthy controls, while *Bifidobacteria* and Bacteroides species are lower in the ratio[14-16].

Besides accumulating evidence on the influence of dysbiosis and S. aureus in AD, the clinical ability of antibacterial efficiency in AD remains controversial[17]. Furthermore, the using of antibiotics treatment as the first line of choice may have systemic effects on the skin biofilm and lead to the emergence of antibiotic-resistant bacteria. High-throughput antimicrobial screening of coagulase-negative staphylococcal (CoNS) species against S. aureus has revealed new antimicrobial peptides that inhibit S. aureus growth. Importantly, these antimicrobial peptide-producing CoNS strains are less frequent in atopic individuals, and reintroduction of CoNS decreases S. aureus colonization[18].

In Saudi Arabia, it has been reported that 65% of children with AD are colonized with S. aureus in their skin lesions. Also, the severity of the disease was linked to the age and colonization with this bacterium[19]. In another study, showed that the most abundant bacteria colonizing Saudi children was Streptococcal and Gram-negative bacteria as well as Enterococcus and Corynebacterium species[20].

During the increase in the prevalence and severity of AD in the recent few decades as well as the chronicity and absence of effective treatments that can control AD, more research is required to understand the pathogenesis of this disease. In addition, patients with AD show defects in innate and acquired immune responses resulting in a high susceptibility to bacterial, fungal, and viral infections. Furthermore, AD has a high impact on the social lives of the patients and their care and high significant cost for the family and health care systems. However, the management of AD is problematic because diagnosis is often difficult. This study aimed to investigate the microbial colonization in atopic dermatitis patients and its associated risk factors in children from Aseer region, Saudi Arabia.

MATERIALS AND METHODS

Sample Collection: The study was conducted in June 2019 to June 2020 in Aseer children hospitals. Swabs were collected from skin lesions of AD patients and healthy individuals; the latter were used as a control. All skin swabs were inoculated into Tryptone soya broth (Cat. No.: LQ508, HIMEDIA, India), which served as a transport medium to preserve microbiological specimens and to prevent drying and dying of bacteria.

Isolation and Identification of Bacterial Species: A swab from each specimen was streaked onto a blood agar plate and MacConkey agar plate. Inoculated plates were incubated aerobically and anaerobically at 37°C for 24 h. The bacterial species were initially identified using selected phenotypic characteristics including Gram stain and culture[21].

Identification of the isolated bacteria was then confirmed using Vitek 2 automated identification system following the procedures described by the manufacturer (BioMérieux SA, Marcy, France). When a test

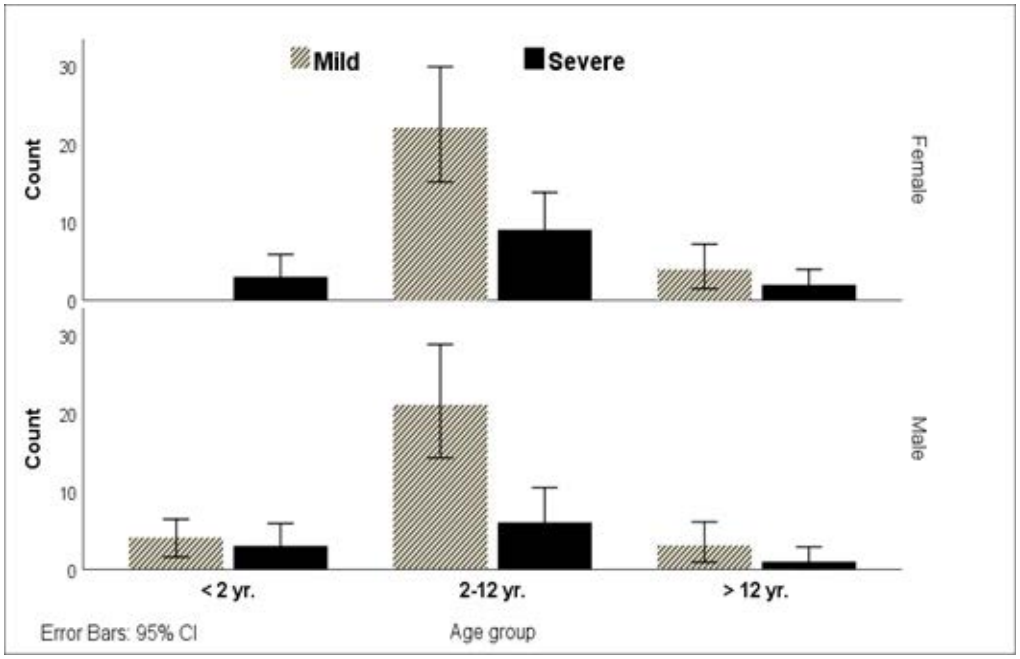

Figure 1: The occurrence of AD and it severity in males and females among different age groups among children in Aseer region, Saudi Arabia

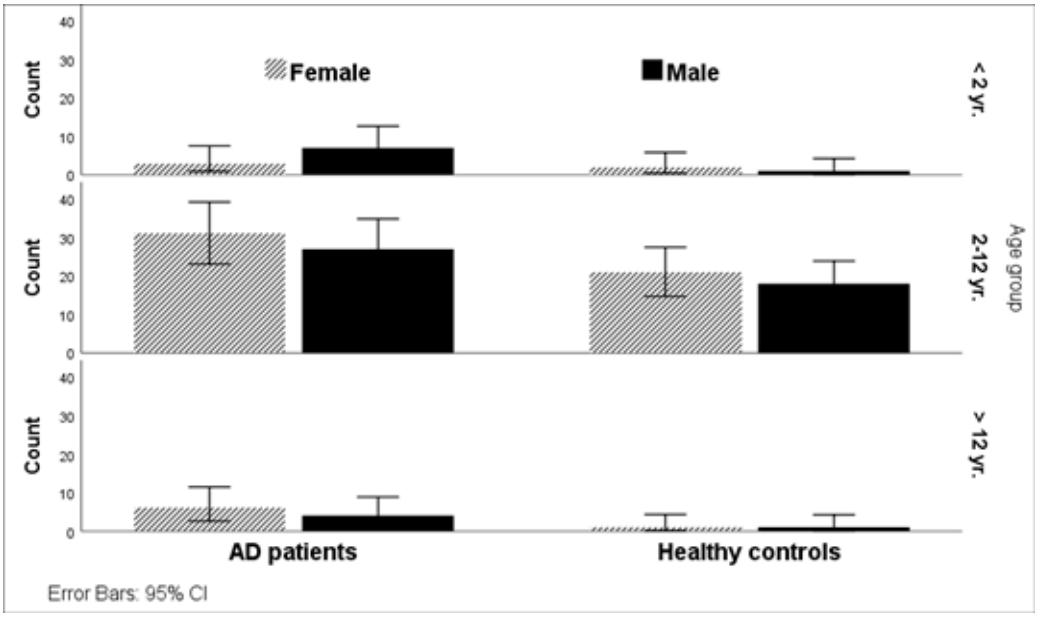

Figure 2: Quantifiable level of colonization with different bacteria in AD patients compared to healthy controls in males and females among child age groups in the study population in Aseer region, Saudi Arabia

outcome is documented as ''low discrimination'', this indicates that the result is doubtful. In these cases, the morphological and biochemical tests were repeated to come to a decision with such uncertain results. Each resulting profile is decoded according to a precise algorithm. The acquired results were compared to the ID-GP (identification of Gram-negative bacteria) database. In the majority of the cases, the recognized Gram-negative bacteria are identified with high percentages of certainty.

Statistical Analysis: Data (patient records, clinical, and culture results) were analyzed using SPSS software (SPSS version 16.0.). Descriptive statistics were reported as mean with standard deviation or percentages where appropriate. Data were analyzed using ANOVA and p-value ≤ 0.05 was taken as statistically significant.

RESULTS

The occurrence of AD in males and females is the same (p = 0.872) although females indicated a slight increase (48.7%) than male patients (43.6%). The age group who are at risk of AD in this study was between 2 and 12 years old (66.7%) and the disease was found mainly as mild form in 69.2% compared to 30.8% as severe form (p < 0.05) (Figure 1).

Patients with AD revealed 92.3% positive colonization with different bacteria compared to 90.9% positive colonization in healthy controls. Quantitatively, the result of colonization showed no significant variation between AD patients and healthy controls (p = 0.789). Similarly, colonization did not differ between genders (p = 0.7315) or among the age groups (p = 0.102) (Figure 2).

The most prevalent colonization in healthy controls was S. haemolyticus (18.2%), followed by S. hominis (13.6%) and Pantoea sp. (13.6%). AD was correlated with Staphylococcus colonization in contrast to colonization with other bacteria (p = 0.007). Among these, S. aureus is the most prevalent (30.8%) followed by S. epidermidis (20.5%), and S. lentus (10.3%). Analysis of colonization according to AD severity, S. epidermidis (30.6%), S. aureus (25.5%), S. lentus (15.3%), S. hominis (15.3%), and Kocuria rosea (10.2%) were the dominant species isolated from the mild cases. Whereas, S. aureus (58.3%), S. epidermidis (16.7%), S. lentus (8.3%), and S. capitis (8.3%) were the dominant species in the severe form of AD (Table 1).

Table 1: Bacteria isolates from AD patients and distribution between mild and severely affected patients and healthy controls in the study population in Aseer region, Saudi Arabia

Species	AD patients						Healthy controls	
	Mild		Severe		Total			
	No.	%	No.	%	No.	%	No.	%
Acinetobacter baumannii	0	0.0	0	0.0	0	0.0	2	4.5
Aerococcus viridans	2	3.7	0	0.0	2	2.6	0	0.0
Enterococcus gallinarum	2	3.7	0	0.0	2	2.6	0	0.0
Kocuria rosea	4	7.4	0	0.0	4	5.1	0	0.0
Lactococcus garvieae	0	0.0	0	0.0	0	0.0	2	4.5
Leuconostoc mesenteroides	2	3.7	0	0.0	2	2.6	2	4.5
Pantoea sp.	0	0.0	0	0.0	0	0.0	6	13.6
Staphylococcus aureus	10	18.5	14	58.3	24	30.8	0	0.0
Staphylococcus auricularis	2	3.7	0	0.0	2	2.6	2	4.5
Staphylococcus capitis	0	0.0	2	8.3	2	2.6	0	0.0
Staphylococcus epidermidis	12	22.2	4	16.7	16	20.5	4	9.1
Staphylococcus haemolyticus	0	0.0	0	0.0	0	0.0	8	18.2
Staphylococcus hominis	6	11.1	0	0.0	6	7.7	6	13.6
Staphylococcus lentus	6	11.1	2	8.3	8	10.3	2	4.5
Staphylococcus simulans	2	3.7	0	0.0	2	2.6	0	0.0

Staphylococcus warneri	0	0.0	0	0.0	0	0.0	2	4.5
Streptococcus facials	2	3.7	0	0.0	2	2.6	0	0.0
Streptococcus pseudoporcinus	0	0.0	0	0.0	0	0.0	2	4.5
Streptococcus salivarius	0	0.0	0	0.0	0	0.0	2	4.5
No growth	4	7.4	2	8.3	6	7.7	4	9.1
Total	54	100.0	24	100.0	78	100.0	44	100.0

The severity of AD was correlated with Staphylococcus species colonization (p = 0.045; CI: 045 - 1.028). On the other hand, other bacteria or no growth are linked to mild cases of AD Figure 3.

DISCUSSION

Colonization of atopic patients with S. aureus has been well reported, and some early studies suggested colonization rates over 90%[21-23]. In some studies, the rate of colonization was found to be varied between 30 to 100% depending on different factors such as the sample size, nature of the patients, severity of the disease, using of antibiotics, patient age, and the methods used for detection and analysis of the results[24-25]. In healthy controls or non-lesion skin of AD, the colonization with S. aureus was found to be around 39%[26-27]. Two studies have been carried out before in the Qassim region, Saudi Arabia, these studies showed that the most prevalent skin disorder was a viral infection[28-29]. Another study showed that the most common pathogen associated with AD patients was S. aureus with high resistance to different antibiotics including streptomycin, benzyl penicillin, ampicillin, oxacillin, trimethoprim/sulfamethoxazole, tigecycline and vancomycin[26]. In another study in Arar in the Northern region of Saudi Arabia, 65% of Saudi AD skin lesions were found to be colonized with S. aureus[19]. these are in agreement with the findings of this study (Figure 3).

In this study, 78 skin swabs were collected from the AD skin lesions of children from hospitals in the southern region of Saudi Arabia. 92.3% of the AD patients were found to be colonized with different Gram-positive bacteria, but no Gram-negative has been found (Figure 2). Around 76.9% of the isolated bacteria were Staphylococcus species with S. aureus being the dominant (30.8%). In healthy controls, 90.9% of the skin swabs were positive culture with 54.5% were Staphylococcus spp., but remarkably no S. aureus had been detected in this group (Table 1).

According to gender in the present study, AD prevalence showed a minor increase in female than male patients but with no significance difference. Many studies showed no significant differences were observed in the prevalence of AD between the genders[28,30]. Age group between 2-12 years is the age at risk noticed in our study (79.5%). In contrast, only 7.7% of the healthy controls were positive cultures among the 2-12 years' age group. It is clear that the prevalence tended to decrease with age. This is in agreement with many previous studies which showed the same tendency in the reduction of the prevalence of colonized AD with older age than others[31,32].

The severity of AD was graded as mild and severe in our current analysis, and this was modified from the Scoring Atopic Dermatitis (SCORAD) criteria. The modification was that mild and moderate were merged in the "mild form" to allow a clearer binary analysis outcome. Consequently, 69.2% of colonized AD were classified as being mild and 30.8% were severe. AD cases revealed 92.3% positive cultures and 7.7% were negative which indicate the important of colonization in AD pathogenesis, Nonetheless, 7.7% were negative for colonization. This could be attributed to a type of skin sanitation or the use of antibiotics. Potential other reasons affecting AD and colonization include environmental factors such as physical or psychogenic stress and reactions to allergens or microorganisms[33]. Staphylococcal colonization eradication is thought to occur over a prolonged period of time by a nonirritant method[34]. In negative culture AD patients, 5.1% were classified as mild and 2.6% were severe, which indicated the negative correlation between positive culture and the severity of the AD disease. Previous studies suggested that about 30% of AD patients would be classified as moderate to severe grading[4,35]. It has been observed in many studies that bacterial colonization and infection with S. aureus have been increased in AD[36]. It has been suggested that the microbial community in the skin of AD patients was changed compared to the skin biofilm of healthy individuals[37]. Previous studies showed that colonization with S. aureus has a strong association with the severity of AD[36]. This experience has been reinforced by our current findings (Figure 3).

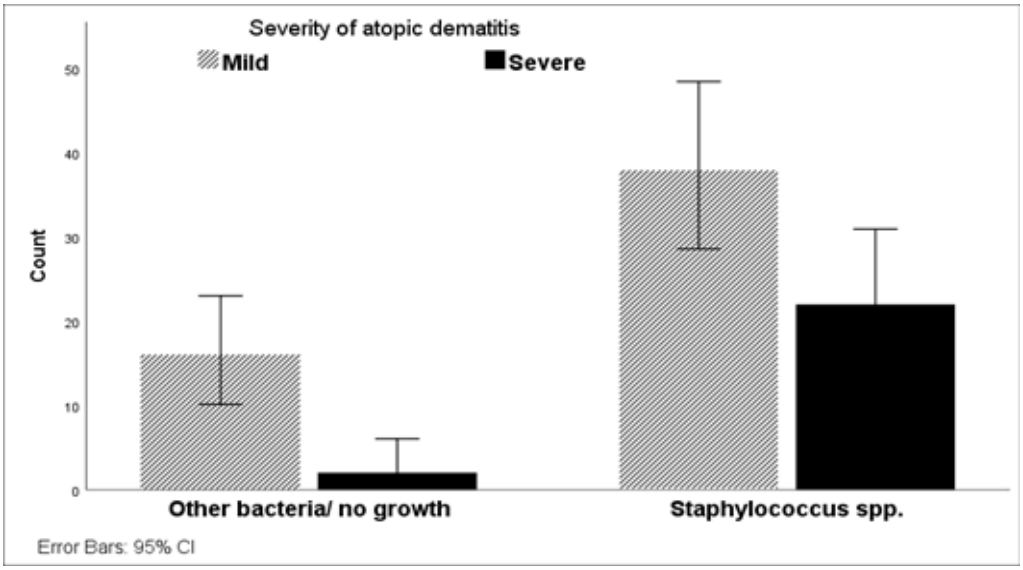

Figure 3: Correlation of Staphylococcus species colonization with the severity of AD disease in the study population in Aseer region, Saudi Arabia

The most prevalent colonization in healthy controls was S. haemolyticus (18.2%), followed by S. hominis (13.6%) and Pantoea sp. (13.6%). AD was correlated with Staphylococcus colonization in contrast to colonization with other bacteria ($p = 0.007$). Among these, S. aureus is the most prevalent (30.8%) followed by S. epidermidis (20.5%), and S. lentus (10.3%). Analysis of colonization according to AD severity. S. epidermidis (30.6%), S. aureus (25.5%), S. lentus (15.3%), S. hominis (15.3%), and Kocuria rosea (10.2%) were the dominant species isolated from the mild cases. Whereas, S. aureus (58.3%), S. epidermidis (16.7%), S. lentus (8.3%) and S. capitis (8.3%) were the dominant species in the severe form of AD (Table 1).

Our study indicated that bacterial colonization, especially S. aureus, in AD patients is correlated with the severity of the disease. S. aureus is followed by S. epidermidis, S. lentus and S. capitis. While the mild conditions were mainly associated with S. epidermidis followed by S. aureus, S. lentus, S. hominis, and Kocuria rosea. It seems that S. aureus is the leading pathogenic determinant in the occurrence and outcome of AD and the leading in the severe cases. In contrast, S. haemolyticus, followed by S. hominis and Pantoea sp. (13.6%) have been detected in the skin of the healthy controls who participated in this study. The most common bacteria found to colonize the skin of young children were Streptococcus, Rothia, Gemella, Granulicatella and Haemophilus; while Cutibacterium, Lactobacillus, Anaerococcus, Finegoldia and Corynebacterium spp. were common in adult[22]. It has been found that the AD patient's skin carries more than 20 genera. Many studies showed the diversity of the skin microbiota is site and age-specific[23]. S. hominis, S. aureus, S. warneri, Escherichia coli, Micrococcus luteus, Enterococcus faecalis, Coryneform spp., S. capitis, Actinomyces spp., S. haemolyticus Actinomyces neuii, Micrococcus spp., S. lugdunensis and S. epidermidis are found to be the most commonly isolated bacteria from AD patients. These latter studies agreed to some extent with our findings but disagreed with some other. This is expected since the skin is reported to have a large number of normal flora life on the surface and inside the skin tissue like sweat glands and hair follicles. The skin microbiota is well known to have high diversity between young and adult healthy individuals[22].

In conclusion, this study presents and updates the epidemiological characteristics of microbial colonization in AD patients. The prevalence of microbial colonization of AD is still significant and changed over time, which will provide a suggestion for the prevention and control of skin infections. Potential factors lead to increased risk of skin infections in AD such as age, sex and bacterial colonization. Besides, bacterial virulence in some strains such as methicillin-resistant S. aureus (MRSA) produces significantly a number of pathogenic factors which may increase their chance to cause infection and severe skin inflammation in AD patients. In addition, more recent studies suggest that skin microbiomes such as S. epidermidis may have a role in the controlling of S. aureus skin infections in AD.

The study concluded that S. aureus is the leading pathogenic determinant in the occurrence and the outcome of AD in children. AD occurrence showed no gender difference, but children between 2 and 12 years old are at higher risk of infection. Quantitatively, the colonization indicated no variation between AD patients and healthy control AD. The severity of AD was correlated with staphylococcal colonization rather than with other bacterial colonization. Although it is well known that S. aureus colonizes the skin and causes inflammation in AD, many questions related to this complex relationship remain unanswered. Further research is needed for a better definition of features that distinguish infection from colonization. Future work is in need to provide regulation regarding the use of antimicrobial therapy in AD. Improving our understanding of S. aureus and other skin flora virulence mechanisms and downstream host immune mediators of S. aureus driven inflammatory pathways may help to identify novel therapeutic targets for infection in AD.

Author Contributions: AM Alkahtani, EAA designed the study and obtained the ethical approval for the study. KAB, MKA, MYA, MEEE, AM Al Hakami participated in clinical and laboratory data acquisition. IMA, EP analyzed and interpreted the data. AMA, AM Alkahtani wrote the drat paper. All authors revised the manuscript and agreed to the final version for publication.

Potential Conflict of Interest: None

Competing Interest: None

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration obtained from the Research Ethics Committee of the College of Medicine, King Khalid University (#09-06-2018). During the data collection stage, the information was anonymous, and the confidentiality of the data was assured.

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