Prevalence of Staphylococcus aureus in Atopic Dermatitis (Eczema) cases in Al- Najaf City/Iraq

مدى انتشار المكورات العنقوديه الذهبية في حالات الاكزيما الاستشرائيه في مدينة النجف/العراق

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الخلاصة:
الهدف: هو تقييم مدى انتشار الإصابة المتسببة عن المكورات العنقودية في حالات الاكزيما الاستشرائية في مدينة النجف.
المنهجية: تضمنت هذه الدراسة جمع 100 مسحة مأخوذة من حالات الاصابة بالأكزيما الاستشرائية, والذين ارتادوا مدينة الصدر الطبية للعلاج, تتراوح اعمارهم بين 1 شهر الى 50 سنة , وقد تم جمع هذه العينات للفترة من خلال الفترة من ايار 2014- تشرين الاول 2014. كما تضمنت الدراسة جمع 50 عينة من غير المصابين بالتهاب الجلد التأتبي كمجموعة سيطرة. وتم اخذ هذه المسحات من منطقة الجلد ومن ثم شخصت البكتريا بواسطة الفحوص التشخيصية والفحوصات الكيموحيوية النوعية لتأكيد تشخيص عزلات البكتريا العنقودية الذهبية. وقد تم تقييم مدى انتشار الحالات المترافقة مع وجود البكتريا العنقودية الذهبية وحسب العمر, الجنس, فترة تواجد الأعراض شدة المرض وكذلك الوراثة لمرض الاكزيما داخل العائلة كما تم دراسة علاقة وجود الحالات الموجبة للفحص البكتيري حسب شدة المرض Disease severity. وقد تم اعتماد الفحص الاحصائي SPSS 18.0 , الوسط الحسابي ± الانحراف المعياري,ومربع كاي واعتماد مستوى المعنوية P <0.05.
النتائج: أظهرت نتائج هذه الدراسة أن البكتريا العنقودية الذهبية كانت مترافقة مع الاصابة بالاكزيما الاستشرائية في 54 حالة وبنسبة مئوية قدرها 54% من العدد الكلي للعينات قيد الدراسة.كما تم تشخيص هذه العزلات الموجبة في 10 عينات (20%) من مجموعة السيطرة. كما أظهرت هذه الدراسة ان اكثر الحالات الموجبة تعود الى الحالات المعتدلة وبنسبة 55,56% ,وهي أعلى مما سجل للحالات غير الحادة او الشديدة الضراوة والتي شكلت نسبة 20,37% و 24,07% على التوالي. وقد سجلت هذه الدراسة علاقة موجبة لحالات الاكزيما الاستشرائية مع عامل الوراثة داخل العائلة وبنسبة 68,52% وبفارق معنوي احصائيا,(P< 0.05) .وبنسبة ذكور : اناث قدرها , 1:1,25 وقد تم تشخيص سبعة عشر حالة (31,5%) كحالات حادة , في حين سجلت 37 حالة (68,5%) كحالات مزمنة.
الاستنتاج: ان المكورات العنقودية الذهبية ذات علاقة موجبة مع شدة الاصابة بالاكزيما الاستشرائية, كما ان عامل الوراثة لهذا المرض مهم جدا في مدى انتشار هذه الحالات .
التوصيات: نوصي باجراء دراسات وراثية تتضمن استخدام جينات خاصة للكشف عن انواع عوامل الضراوة المتعلقة بالمكورات العنقودية الذهبية ذات العلاقة بحالات الاكزيما الاستشرائية.

ABSTRACT

Objectives: This study was planned to evaluate the prevalence of Staphylococcus aureus in cases of atopic dermatitis (AD) in Najaf city.
A total of 100 skin swabs were obtained from the effected skin areas of patients who were attending to AL-Sader Teaching Hospital in AL-Najaf city during the period from May to October 2014.with (AD), along with 50 skin swabs that were obtained from a comparable skin area of 50 persons who were regarded as a control group and comparable in ages and genders with the patient group. All the skin swabs (patients and control) were then immediately streaked on the surface of selective media for isolation and identification of S. aureus preliminary. Then the suspected isolates were confirmed by specific biochemical and enzymatic confirmative tests. The incidence of Staphylococcal isolates was detected according to the age, gender, duration, severity and also according to the family history.
Results: From the 100 swab samples of patients, there were 54/100 (54%) showed positive isolation of S. aureus, while only 10/50 (20%) swab samples of the control group showed positive S. aureus isolation. The difference between the two groups was statistically significant (P< 0.05). The prevalence of moderate AD cases was higher than that of mild and severe (55.56%> 20.37%< 24.07%) respectively. Thirty seven patients (68.52%) were categorized with family history inheritance of AD (Results were statistically significant (P< 0.05), with male to female ratio was 1.25:1. Seventeen patients (31.5%) were categorized as acute AD, while 37 patients (68.5%) were diagnosed as chronic.
Conclusions: Staphylococcus aureus infection is positively correlated with AD cases, and genetic factors may play an important role in increasing the frequency of AD cases.
Recommendations: We recommended to use genetic studies for the monitoring of Staphylococcus aureus virulence factors correlated with AD.
Key words: Staphylococcus aureus, atopic dermatitis, SCORAD index.

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INTRODUCTION

The role of S. aureus virulence factors in the pathophysiology of (AD) is an area of active research. Staphylococcus aureus is an extraordinarily versatile pathogen, and it can cause a large spectrum of infections, from mild to severe and fatal. It is important in humans and also economically important when infecting animals, able to cause superficial lesions and systemic infections, S. aureus is responsible for toxin-mediated diseases, such as the Toxic Shock Syndrome (TSS), Kawasaki's Syndrome and staphylococcal food poisoning [(1)].

Staphylococcal enterotoxins are members of a family of more than 20 different staphylococcal and streptococcal exotoxins that are functionally related and may share sequence homology. These proteins are known to be pyrogenic and are connected to significant human diseases that include food poisoning and toxic shock syndrome [(2)].

Atopic dermatitis (AD) is a chronic inflammatory disease causing intense pruritus, and with typical clinical features, eczematous dermatitis affecting 10-20% of children and regarded as a major cause for morbidity, since patients with AD have a higher susceptibility for microbial colonization and an increased risk of skin infections [(3)]. Staphylococcus aureus infection was also found with prevalence of about 1-3% in adults, present in 80-100% of skin from atopic patients and is related to worsening of the disease by the action of enterotoxins [(4)]. In addition, eradication or reduction of S. aureus colonization on AD patients has demonstrated a positive correlation with decreased severity of atopic eczema [(5)] .Our study was planned for the detection of the prevalence of S. aureus from skin lesions of AD patients who were admitted to Al-Sader Teaching Hospital in Al-Najaf city.

OBJECTIVES:

This study was planned to evaluate the prevalence of S. aureus in cases of AD as compared with control group in Najaf city and according to different parameters including age, gender, family history and distribution of these cases clinically according to SCORAD index.

METHODODLOGY

Collection of Samples and case definition: One hundred samples from the dermal lesions related to persons from Al Najaf city who were suffering from acute and chronic lesions of AD signs were collected, their ages ranging from 1 month to 50 years. The data were obtained for each patient according to a questionnaires statement, including information about gender, age, duration of the infection, family history, being treated or not, and the issue date of case admission. The clinical diagnosis of AD patients was confirmed by a dermatologist in the dermatologic clinic referred to above. All cases included in this study were selected according to criteria mentioned by Kumar [(6)]. These cases did not use topical or systemic antimicrobial drugs, or any corticosteroids antihistamines drugs for at least 2 weeks before investigation. Control group contains

50 persons of the same age range with no symptoms or family history of eczema or other allergies or skin diseases.

Isolation and Identification of S. aureus from dermal lesion: The collection of skin lesion specimens was by the end of wetting sterilized cotton swab with the lesion or discharge of fluid from blisters scrape tissue, then placed in a sterile test tube containing sterilized 2 ml of transport media. All isolates were primarily examined by gram stain and by biochemical examinations [(7)]**.** All skin swabs were inoculated on 5% human blood agar, nutrient agar and mannitol salt agar plates. After incubation at 37Co for 18-24 hr, cultural and morphological features of the colonies were evaluated. All mannitol-fermenting isolates subcultured on mannitol salt agar and incubated at 37ºC for 24 hr**.** These S. aureus isolates were further investigated by growth on chromagar specific for the identification and isolation of S. aureus as shown in figure 1.

Tube Coagulase Test: To test coagulase enzyme as bound or free coagulase, suspension 0.5ml of bacteria colony is added to 0.5ml plasma of human have been prepared and incubated in human 37C , then the plasma inoculated periodically examine the composition of fibrin formation of coagulase clot within four hours, and this is interpreted as a positive result indicates on S. aureus strains. The absence of a blood clot after 24 hours incubation is the negative result.

Catalase test: This test was used for the detection of catalase enzyme, to differentiate Streptococci (Catalase -ve) from Staphylococci (Catalase +ve). The exposed to hydrogen peroxide, bacteria catalase positive conversion of peroxide to water and oxygen gas, this test was performed by a capillary tube containing hydrogen peroxide solution was carefully dipped into a single colony, and when catalase was represented, oxygen gas was released and the bubbles observed in the capillary tube .

Preservation and Maintenance of Bacterial Isolates: The maintained bacterial isolates on nutrient agar slant in 4C. The isolates were maintained monthly by re-cultivation of the new nutrient agar medium (short-term maintenance). The use of nutrient broth supplemented with 15% glycerol was used for long preservation and isolates were maintained frozen strains in -70ºC (deep freeze) for several months[(8)].

Subculture of Preserved and Frozen Stock Cultures: Preserved and frozen stock cultures were sub-cultured on fresh blood agar plates, and then incubated in aerobic condition at 37°C for 24 hr. [(8)]**.**

Statistical Analysis: Statistical analysis using SPSS 18.0 for Windows was conducted. . Inc. Data expressed as mean ± SD, Chi-square test. In all tests, P <0.05 was considered statistically significant.

RESULTS

Table(1): Staphylococcus aureus positivity in AD cases as compared with control group.

Cases	S. aureus positive cases	S. aureus negative	Total cases	P value
AD patients	54 (54%)	46(46%)	100(100%)	7.2E-05
Control group	10 (20%)	40 (80%)	50(100%)	
Total cases	64 (42.7%)	86 (57.3%)	150(100%)	

Subjects and Grouping

Atopic dermatitis and Staphylococcus aureus incidence

Our results are based on a total of 100 cases with AD (the clinical diagnosis of AD patients is confirmed by dermatologists and specific AD criteria. Table (1) showed that from the total of AD patients investigated, S. aureus positive cases was correlated with 54 cases (54%), and 10 cases (20%) from the total control group, with significant statistical differences ($P< 0.05$). All isolates were investigated and the detection was confirmed as S. aureus, Gram positive cocci, with mannitol fermentation, Catalase and Coagulase positive.

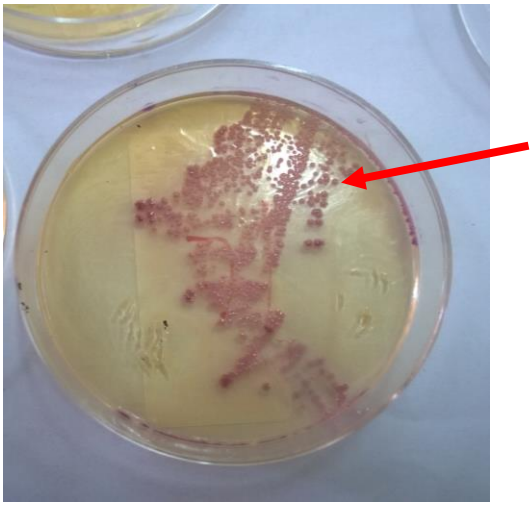

Figure-1: Staphylococcus aureus (mauve colonies) on Chrmoagar ™

These S. aureus isolates were further investigated by growth on Chrmoagar™ specific for the identification and isolation of S. aureus as shown in figure (1). Sensitivity, specificity and diagnostic accuracy of cultural results on Chrmoagar™ were also 100%, 95.2% and 97.6% respectively.

SCORAD index for severity of disease

Table(2): Staphylococcus aureus positivity in AD cases according to SCORAD index

AD patients	Mild	Moderate	Severe	Total cases	P value
Males	7(23.3 %)	15(50 %)	8(26.7 %)	30(55.56%)	
Females	4(16.7%)	15(62.5 %)	5(20.83 %)	24(44.44%)	0.652343
Total cases	11 (20.37%)	30(55.56%)	13(24.07%)	54(100%)	
Mean ± SD	24.17±4.72	56.25±8.84	19.6±4.1		

As shown in table (2), patients were divided into 3 groups; Mild, Moderate and severe according to the severity of dermatitis using SCORAD index,. The prevalence of moderate

cases was higher than that of Mild and severe (55.56% > 20.37%<24.07%) with mean ± SD of 56.25±8.84, 24.17±4.72 and 19.6±4.1 respectively. However, these results were statistically not significant (P> 0.05).

Atopic dermatitis according to the duration period

Table(3): Distribution of S. aureus isolates in AD patients according to duration period.

AD patients	Acute	Chronic	Total cases	P value
Males	9(30%)	21(70%)	30(55.56%)	0.793268
Females	8 (33.3%)	16(66.7%)	24(44.44%)	
Total cases	17 (31.5%)	37(68.5%)	54(100%)	

Table (3) showed that from the total of 54 AD patients included, 17 patients (31.5%) were categorized as acute AD, while 37 AD patients (68.5%) were diagnosed as chronic AD. Results were statistically not significant (P> 0.05).

Atopic dermatitis according to the family history

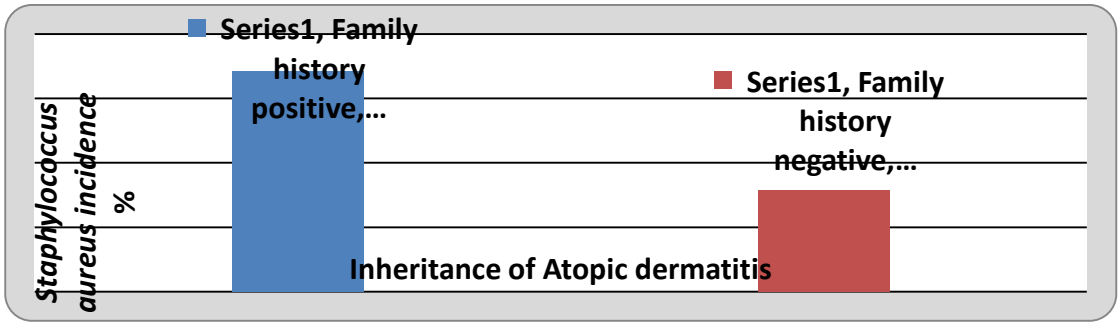

Figure-2: Distribution of S. aureus isolates according to family history of AD.

Table(4): Distribution of S. aureus isolates according to family history of AD.

AD patients	Family history Positive	Family history negative	Total	P value
Males	25(83.3%)	5(16.7%)	30(55.56%)	0.008775
Females	12(50%)	12(50%)	24(44.44%)	
Total Number	37(68.52%)	17(31.48%)	54(100%)	

From the total of 54 AD patients included, 37 patients (68.5%) were categorized with family history inheritance of AD, while 17 patients (31.5%) were diagnosed as negative to family history inheritance of AD. Results were statistically significant (P< 0.05), (Figure-2) and (Table 4).

Atopic dermatitis according to the age groups and gender

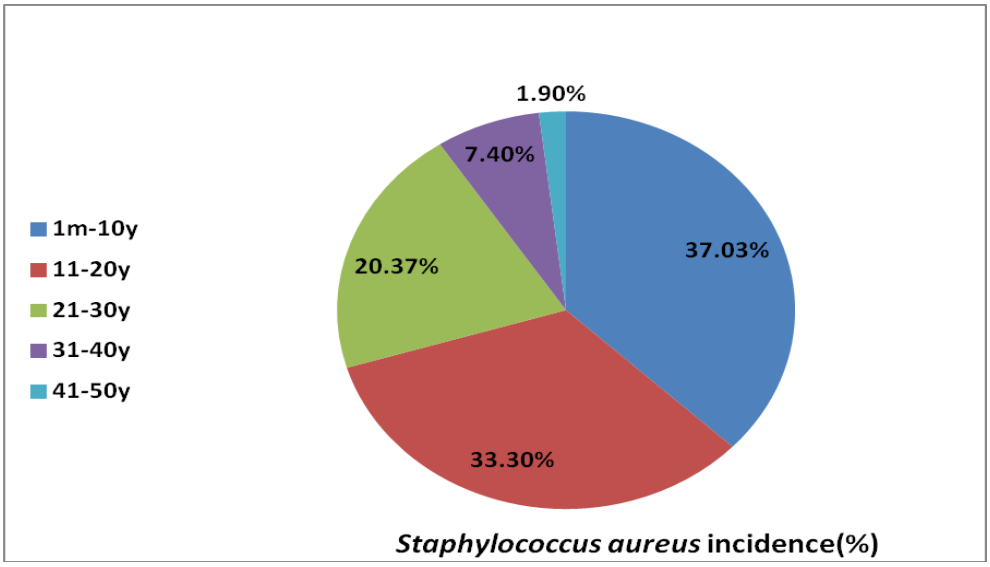

Figure-3: Distribution of S. aureus isolates according to age groups of AD cases.

Table(5): Distribution of S. aureus isolates according to family history and age groups.

AD patients	**1m-10y**	**11-20y**	**21-30y**	**31-40y**	**41-50y**	**Total**
*F.H positive	14(37.8%)	10(27.0%)	9(24.3%)	4(10.8%)	0(0%)	37
F.H negative	6(35.3%)	8(47.05%)	2(11.76%)	0(0%)	1(5.9%)	17
Total	20(37.03%)	18(33.3%)	11(20.37)	4(7.4%)	1(1.9%)	54

The P-Value is 0.175229. *=Family history of AD

From the total of 54 AD patients included, 49:54 patients (90.7%) of positive S. aureus positive AD cases were diagnosed during the first 30 years of life (Figure-3), however, results were statistically not significant (P> 0.05), (Table-5). According to the gender distribution, there is a variation between Atopic males and females in the frequency of S. aureus. Males were more affected by the disease than females. The relative frequency of males in total AD is 30(55.56%), whereas for the females is 24(44.44%); male: female ratio of 1,25:1.

DISCUSSION

Disease severity was evaluated through the Scoring AD (SCORAD) tool [(2).] Similar study was done by Nada,et al [(5)] who were included SCORAD index for the evaluation of AD severity and showed that S. aureus positive cases was correlated with 54% of the total cases studied. Staphylococcus aureus is a member of normal resident colonizing microorganisms of the skin, a fraction from carriage in the perineal and nasal areas. However, about 14×10^6 organisms/cm^2 in the skin area present in eczematous lesions of

patients with AD. Colonization of S. aureus by the majority of patients with AD does not necessarily indicate that it acts as a pathogen [9].

The binding of S. aureus to the skin is enhanced significantly by AD skin inflammation, scratching may enhance S. aureus attachment by alteration the skin barrier. Bacterial colonization may be facilitated by the cracking and dryness of AD skin, which is caused by epidermal water loss, resulted from altered lipid content. Increased activity of ceramidase of isolated S. aureus aggravated the skin barrier dysfunction [10].

Chrmoagar™ S. aureus media is a new chromogenic medium propagate a purified mauve colonies of S. aureus after 24 h of incubation. Other studies also revealed that Chrmoagar™ S. aureus result in the most accurate identification as compared with other biochemical tests. This result is in agreement with the findings of Samra [10]. Many advantages from using chromogenic media such as high sensitivity, rapid detection, needless to further biochemical tests, and highly specific identification of S. aureus [11]. These techniques based on specific enzymatic reaction, the produced color aid in the microorganism identification easily [12].

The relative frequency of males in total AD is 30(55.56%), whereas for the females is 24(44.44%); male: female ratio of 1,25:1. These data are similar to that mentioned by Pucci et al [13] study in Italy (58.7% for males, 41.3% for females). This result was in disagreement with other studies [13] with an overall female/male ratio of 1.66:1.0, as well as for a local study by (Al-Dosaki, 2006) [14]. This difference may be related to the age of individuals involved in the study , as they referred to ages of both genders involved, this leads to a conclusion that female increasing percentage can be gained ongoing age due to intrinsic factors like hormonal changes during puberty and menstrual cycle and extrinsic factors such as contact with a lot of substances in her life that are prone to irritation and inflammation such as industrial chemicals, cosmetics, soups, house dust mite s during cleaning and detergents [15]. Other investigators [16] observed that S. aureus growth was more prevalent among males than females. Sex hormones and skin physiology may influence the persistency and chroncity of S. aureus on the skin of AD patients, as estrogen protect against S. aureus infections and inhibit Staphylococcal toxin synthesis. In addition, sex hormones balance may regulate the composition of skin lipid which could explain the increased S. aureus persistency [17]. Atopic dermatitis cases can be sub-grouped as mention previously, depending on various causes, such as immune stimuli specific including food, aeroallergens and deduction of AD by infection of the skin with various microorganisms, as well as constant exposure to irritants combined effect for a specific set of genes cytokine that is believed to Immune mechanisms are important in the pathogenesis of AD [18] .

The majority of AD patients included, 49:54 patients (90.7%) of positive S. aureus in AD cases were diagnosed during the first 30 years of life, however, results were statistically not significant (P>0.05). Staphylococcus aureus have shown to release exotoxins with superantigenic characteristics in about 30-60% of AD patients [19]. Atopic dermatitis is a chronic disorder illness with relapsing skin inflammation. The onset before 4 months of age has been regarded as an infantile and childhood risk factor for air borne allergens sensitization and the eczema severity that positively associated with the risk of asthma [20]. Staphylococcus aureus might play a role in the severity and chroncity of AD illness through its secretion of superantigenic exotoxins [19]. These exotoxins may directly damage the skin barrier, beside their immunologic effects, provoke the exacerbation or maintaining skin inflammation in AD, this process is mediated by the stimulation of T-cells with a

significant percentage leading to polyclonal activation and induce the generation of exotoxin-specific T-cells able to enhance the production of specific immune response mediated by IgE antibodies [21] . A number of studies have found the risk of inheritance of AD is higher if the mother rather than the father has the disease. However, if the affected parent has moderate to severe AD persisting into adulthood, their risk of transmitting AD to their off spring is about (50.0%) [22].

The majority of AD patients (70.0%) have family members history of one or more major atopic diseases such as asthma and eczema [6],a result that is in agreement with the current study, as it has been demonstrated that (68.77%) had familial history of atopic disease.

Similar results were obtained by other investigators Edan et al. [17]. AD could occur at any age, In this study the percentage of total AD patients of age less than 3 years was (38.4%) which show the highest percentage ,while the lowest occurs in the age group 8 – 12 years of AD patients with a percentage of (24.6%). These results show a marked variation to age in the proportion of AD , which is confirmed by other studies, that show about (50%) of AD patients develops the illness during the 1st years of age, and about (30%) during the ages between 3 and 5 years, with increasing persistency after puberty [13] .

The tendency to develop AD is inherited. In epidemiologic studies, the child is at increased risk to develop atopy, if either parent is affected. More than one-fourth of offspring of atopic mothers develop AD in the first 3 months of life. If one parent is atopic, more than half the children develop allergic symptoms by age of 2; this rate rises to (79.0%) if both parents are atopic [23].

CONCLUSIONS

Atopic dermatitis is a disease affecting different ages and sexes, and correlated positively with the existence of S. aureus. Chromagar™ was specific and necessary for the identification and isolation of S. aureus from skin lesions of AD patients.

The incidence of S. aureus infections was variable in frequency among AD patients who were classified according to SCORAD index, but with slightly elevation among moderate more than severe and mild AD cases, and the majority seemed to be from moderate and chronic AD.

The incidence of S. aureus infections was more frequent among patients with Family history of atopic diseases with significant statistical analysis.

RECOMMENDATIONS:

We recommended using genetic studies for the monitoring of S. aureus virulence factors.

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