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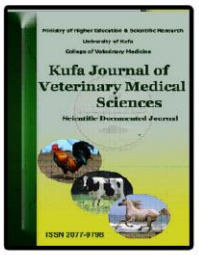

The Role of Staphylococcus aureus Enterotoxins genes in The occurrence of Allergic Rhinitis

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Abstract

at the period from May to November 2013 a total of fifty Staphylococcus aureus isolates were collected from the anterior nares of patients suffering from allergic rhinitis and the same number were also obtained from control subjects at Al-Sader Teaching Hospital in Annajaf city. DNA was extracted from all isolates and by PCR technique a search was done for detection of genes that are responsible for excretion of enterotoxins ,Then a comparison was performed between the existence of these genes in both groups and it was found that the presence of these genes was higher in the patients group than that in the control group.

الخلاصة:

للفترة من أيار الى تشرين الثاني من عام 2013 تم جمع 50 عزلة من بكتريا المكورات العنقودية الذهبية من أنوف مرضى مصابين بحساسية الأنف و خمسين عزلة أخرى من أناس أصحاء ثم جرى استخلاص الحامض النووي للبكتريا المعزولة من كلا الفريقين ومن ثم التحري عن وجود الجينات المسؤولة عن افراز الذيفانات المعوية في كل عزلة بواسطة تفاعل انزيم سلسلة البلمرة وبعد ذلك تمت مقارنة نسبة عزل تلك الجينات في الأشخاص المرضى مقارنة بمجموعة الأصحاء وقد لوحظ أن العزلات من المرضى كانت أكثر نسبة حاملة للجينات المفرزة للذيفانات المعوية من قريناتها المعزولة من الأشخاص الأصحاء.

Introduction

Allergic rhinitis (AR) is an inflammatory disease of the nasal mucosa characterized by the infiltration of inflammatory cells and the release of inflammatory mediators.(1,2)

Many previous studies have shown that environmental factors such as temperature, dry air, air pollution, sex hormones, stress and alcohol can modify the pathogenesis of AR.(1)

After a series of papers has been published on the role of bacteria in the pathogenesis of atopic diseases, including allergic rhinitis and bronchial asthma, it is generally known that bacterial colonization is associated with AR (.3,4)

Some of the previous studies have focused on the role of Staphylococcus aureus enterotoxin in the development of AR. According to one of them, the frequency of Staphylococcus aureus nasal carriage in patients with AR is higher than that in the normal subject .(5)

In addition, S. aureus carrier positive groups in allergic patients revealed significantly severe symptoms than S. aureus negative groups(.6)

Another study has demonstrated that allergic patients sensitive to staphylococcal enterotoxin have higher serum levels of total IgE and allergen-specific IgE antibodies.(5.6)

Some studies also showed that among patients with persistent rhinitis, those with IgE antibodies against staphylococcal enterotoxins have higher ECP levels which are considered as a useful parameter of eosinophil activation than those without specific IgE against staphylococcal enterotoxins. In addition, it has been suggested that microbial superantigens may induce resistance to steroids.(7,8)

However, the role of staphylococcal enterotoxin in the pathogenesis of AR has not yet been completely elucidated.

Aim of The Study:

In this study, we aimed to investigate the relationship between certain staphylococcal enterotoxins genes and the development of allergic rhinitis in the allergic patients in comparison to control group who were colonized by S. aureus .

Materials and Methods:

Samples collection:

The study was included two groups:

First group (patients group): This group included 258 patients with allergic rhinitis. These patients attended Al-Sadder Medical City, outpatient clinic of otolaryngology in Najaf city during the period from May 2013 to October 2013. The study included patients who have not received any treatment for their allergic rhinitis condition whether topical or systemic for at least 3 weeks before taking the swab, have no other nasal diseases which may interfere with the results (such as superficial bacterial infections , boil or folliculitis) and recent exacerbation of their disease.

Healthy or control group consisted also 463 persons who were attended the same hospital , at the same period, for other different medical or surgical problems but they were free from allergic rhinitis or any other nasal problem.

For isolation of Staphylococcus aureus, nasal swab was taken from anterior nares of all patients and control groups by using a sterile cotton swab.

Isolation and identification of Staphylococcus aureus were done according to the standard recent bacteriological methods(15).

Preservation and Maintenance of Bacterial Isolates

The bacterial isolates were preserved on nutrient agar slant at 4˚C. The isolates were maintained monthly by re-culturing on new nutrient agar medium (short-term maintenance). Nutrient broth supplemented with 15% glycerol was used for long preservation and the isolates were maintained frozen at -70°C (deep freeze) for several months (.9).

Subculture of Preserved and Frozen Stock Cultures

Preserved and frozen stock cultures sub-cultured on fresh blood agar plates, and then incubated in aerobic condition at 37°C for 24 hr.(9**)**

Bacterial DNA Extraction:

Extraction of DNA materials from all staphylococcal isolates was done following the standard methods for DNA extraction and kept for PCR assay for the enterotoxins governing genes(16).

Polymerase Chain Reaction

PCR assay was performed for confirmative detection of Staphylococcus aureus based on amplification of 16S rRNA gene as well as detection enterotoxin gene (sea, seb, sec, see, and tst gene) in Staphylococcus aureus isolates, this assay was carried out according to McLauchlin *et al*.2000.(10)

Results:

Table (1): Total number of allergic rhinitis patients and control that were included in the study:

Groups	Positive staph. (%)	P value
Patients (n=258)	50(19.37)	0.001
Controls (n=463)	50(10.7)	

It was seen from table (1) That from 258 patients with allergic rhinitis we could obtained 50 members who showed positive Staphylococcus aureus nasal isolates which represented 19.37% of the total number of the patients studied. While in case of control subjects we noticed that from
463 studied subjects we could obtained 50 persons who were positive for Staphylococcus aureus isolates in their nasal swabs, this represented only 10.7%, and we can see that the patients group was significantly higher than the control group regarding the rate of isolation of isolation of Staphylococcus aureus from their anterior nares P=0.001.

Table(2): Comparison between enterotoxin A gene in patients and control Staphylococcal nasal isolates as detected by PCR method.

		Groups		P value
		Control	Patient	
Enterotoxin A	Negative	38	26	0.012
		76.0%	52.0%	
	Positive	12	24	
		24.0%	48.0%	
Total		50	50	
		100.0%	100.0%	

When the enterotoxin A is taken into consideration, it was shown from table 2 that among the patients isolates of Staphylococcus aureus there were 24 isolates that were positive for excretion of that enterotoxin as detected by PCR genomic study while 26 isolates were negative in that respect.
In case of the control isolates, there were only 12 isolates that showed enterotoxin A excretion ability and 38 isolates were negative in that respect. When the two groups, the patients and the control were compared , it was seen that the Staphylococcus aureus that strains that were isolated from the patients group were significantly higher

than those isolated from the control group in the ability of enterotoxin excretion(p=0.012).

Table(3): Comparison between enterotoxin B gene in patients and control staphylococcal nasal isolates as detected by PCR study.

		Groups		P value
		Control	Patient	
Enterotoxin B	Negative	40	35	0.248
		80.0%	70.0%	
	Positive	10	15	
		20.0%	30.0%	
Total		50	50	
		100.0%	100.0%	

In case of enterotoxin B excretion by Staphylococcus aureus nasal isolates(table3), it was shown that in case of patients with allergic rhinitis nasal isolates there were 15 out of 50(70%) isolates showed positive results while in case of control there were 10 out of 50(80%) isolates that showed positive results the comparison is not significant spastically(p=0.248

Table(4): Comparison between enterotoxin C gene in patients and control group staphylococcal isolates as detected by PCR method.

		Groups		P value
		Control	Patient	
Enterotoxin C	Negative	46	32	0.001
		92.0%	64.0%	
	Positive	4	18	
		8.0%	36.0%	
Total		50	50	
		100.0%	100.0%	

Regarding enterotoxin C (table 4) , it was shown that this enterotoxin gene was significantly higher in case of patients nasal isolates (18 out of 50 ,36%) when compared with that of control nasal isolates of Staphylococcus aureus bacteria(4 out of 50, 8%).

Table(5): Comparison between enterotoxin E gene in Staphylococcus aureus isolates from patients with allergic rhinitis and control group as detected by PCR.

		Groups		P value
		Control	Patient	
Enterotoxin E	Negative	47	32	<0.001
		94.0%	64.0%	

	Positive	3	18	
		6.0%	36.0%	
Total		50	50	
		100.0%	100.0%	

In case of enterotoxin E the story was, more or less, the same as in case of enterotoxin C where the patients Staphylococcus aureus nasal isolates showed a higher rate of enterotoxin E excretion gene than those of the control group nasal isolates(15 out of 50,36%, versus 3 out of 50,6%,respectively, the difference was statically highly significant(P value is less than 0.001 ,table 5).

Table(6): Comparison between toxic shock syndrome toxin(TSST) e gene in staphylococcal isolates from patients and control group.

		Groups		**P value**
		Control	Patient	
Toxic shock syndrome toxin(TSST)	Negative	44	31	0.003
		88.0%	62.0%	
	Positive	6	19	
		12.0%	38.0%	
Total		50	50	
		100.0%	100.0%	

Toxic shock syndrome toxin excretion was studied in allergic rhinitis patients in comparison to control group Staphylococcus aureus nasal isolates(table 3.9), it was shown that there were 19 out of 50 (38%) isolates in which the gene of this enterotoxin was detected by PCR in patient group versus 6 out of 50(12%) in control group. The difference is stastically significant(P=0.003).

Discussion:

Regarding tables (2, 3 , 4 , 5 , and 6) which studied the differences in enterotoxin excretion between patients and control group Staphylococcus aureus nasal isolates , it was found that that the nasal isolates of this bacteria from patients with allergic rhinitis excreting these enterotoxins significantly more than those which were isolated from anterior nares of the healthy control group. These results are of critical importance as it sheds a light on the role of Staphylococcus aureus in the epidemiology and the pathophysiology of allergic rhinitis.

These results are in consistency with many other studies done in different parts of the word which , more or less , demonstrated the same results. Many other human studies that were done in differents locations of the word has indicated that the mean carriage rate of *Staphylococcus aureus* in allergic rhinitis patients is higher than that in the normal population, and those who are sensitized to staphylococcal enterotoxins found to suffer from more severe allergic diseases .(11)

Another study, the serum levels of eosinophil cationic protein (ECP) and specific IgE towards a variety of staphylococcal enterotoxins (A, B, C, D) and TSST-1 were measured in patients suffering from rhinitis and/or asthma due to allergy.

It was concluded that patients sensitive to staphylococcal enterotoxins have higher serum level

of ECP, which can be a reliable marker for clinical severity of allergic disease. In another human study with allergic rhinitis patients, it has been suggested that *Staphylococcus aureus*-positive groups have significantly higher nasal symptom scores than *Staphylococcus aureus*-negative groups. (12)

Other studies support the role of Staphylococcus aureus in secreting a range of enterotoxins such as *Staphylococcus aureus* enterotoxin A (SEA), *Staphylococcus aureus* enterotoxin B (SEB), and Toxic shock syndrome toxin-1 (TSST-1) Ig to these toxins can be found in several allergic disease, such as atopic eczema/dermatitis syndrome (AEDS)25, allergic rhinitis (AR) (13,140 . Levels of Staphylococcus aureus enterotoxin – specific IgE correlate with markers of eosinophil activation and recruitment.

Conclusion: It is concluded from the results of this study that Staphylococcus aureus enterotoxin genes were present in the allergic rhinitis patients isolates significantly more than in the same isolates from control subjects.

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