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Distribution of Super Antigensgene of *Staphylococcus aureus* in Allergic Rhinitis Patients

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Summary *Staphylococcus aureus is a representative constituted pathogen related with the major community and hospital acquired sickness and has been taken into consideration for a long time as a major problem of Public Health,Several virulence factors engaged in the pathogenesis of S. aureus strains Perhaps the most notable virulence factors associated with this microorganism are the heat-stable enterotoxins that cause the sporadic food-poisoning syndrome or foodborne outbreaks (Martin et al.,2003).*

Hypothesis: *The pathogenesis of Allergic rhinitis(AR) has been a hot subject , recent studies had suggested that Staphylococcus aureus excretes exotoxins that may act as superantigens and can influence the activity of both immune modulatory and pro inflammatory effector cell type and therefore, may have a potentially important role in the pathogenesis of chronic inflammatory disease or lead to exacerbation of upper airway disease*

Methodology A case *–control study has been conducted to determine the prevalence of enterotoxin producing Staphylococcus aureus in the nasal cavities of patients with allergic rhinitis during the period from March 2014 to November 2014, 100 patients with Allergic rhinitis and 100 control subjects, the patients attended Al-Sadder Medical City, outpatient clinic of ENT in Najaf city. For isolation of S. aureus, a nasal swab was taken from each of the 100 patients and 100 controls by using a sterile cotton swab. Isolates were identified by a conventional test and then confirmed by Biomerieux Vitek 2 Compact Automated Microbial Identification. The super antigen genes (SEA, SEB,SEC,SED,SEE,and TSST) were detected by using conventional PCR on the isolate then an enzyme immune assay RIDASCREEN ® SET A, B,C,D,Epreformed on broth culture of these isolates in order to identify the enterotoxins production or libration.*

Result: *Nasal S. aureus carriage was significantly more frequent in patients with allergic rhinitis than in non-allergic controls (P < 0.001),(Odds=3.4306 CI=1.8-6.26).The distribution of toxigenic S. aureus that recover from allergic rhinitis patients by PCR test was (63.16%) and (26.32%) from control groups and the difference was non-significant P= 0.434.The most frequently detected enterotoxin was staphylococcal enterotoxin B (SEB).ELISA test seem to be more sensitive for (SED, SEC, and SEE) and less sensitive for (SEA and SEB), however the specificity of ELISA is better in (SEB, SEC, SED, SEE than SEA*

Conclusion: *We have demonstrated that the rate of nasal carriage of Staphylococcus aureus in allergic rhinitis patients was significantly higher than that of control subject,-Staphylococcus aureus isolated from allergic rhinitis patients have a probably a relationship with the disease and their superantigen have been a role in triggering and exacerbation of the disease*

Keyword; *Allergic rhinitis,Staphylococcus aureus, Superantigens*

Allergic Rhinitis

Allergic rhinitis (AR) is a disease that is a chronic airway inflammatorycondition with the eosinophilic cell as a responseto aninhalant allergic material in geneticallyliablepatients (Liu *et al*., 2014).

The following are the predominant symptom of the AR patients: sneezing, nasal itching, congestion,of the nasal mucosa and running nose (Al-Abri *et al* .,2014) another organ of the body (nearby organs) are also involved which are the eyes, ears, sinuses, and throat, the smell sense can also be affected if the disease is for a prolong period (Guilemany,2009).

Epidemiology

Allergic Rhinitis is a global health problem that affects 20%-40% of the population in developed countries and whose incidence is rising. It can be induced by different mechanisms and involves several etiological agents (Rondon *et al*.,2007).

Staphylococcus aureus

Staphylococci are sphericalin shape and gram-positivebacteria which are non motile and do not form spores (Murray *et al.,* 2003).

Staphylococcus aureus isan important human pathogensespecially in hospital-acquired infections (Guidey *et al*, 2014) and also in community-acquired infections, with methicillin-resistant *S. aureus* (MRSA) having a considerable public health threat.(Liu *et al*.,2009) *S. aureus* have the property to colonize asymptomatically healthy individuals.The carriers are at higher risk of infection, and they are considered as an important origin of the *S. aureus* strains that spread among other individual (Chambers &DeLeo.,2009).

Some are considered to be part of the normal flora (normal inhabitant) lives within the body, colonizing the skin and mucous membranes in 10 to 20% of healthy adults of humans and also in the nose of a healthy human (Lowy, 1998).

They can be a causative agent of different superficial and deep infections, where pus was formed in many times in human. Occasionally, *S. aureus* is regarded as an opportunistic pathogens as in case of infections of the urinary tract, respiratory tract, and gastrointestinal tract.The most commonly affected area of the bodybecause of S. *aureus* infection is the skin (Daum, 2007). However, nasal colonization increases the risk of infection byfour-fold (Safdar & Bradley, 2008).

Superantigens: enterotoxins and toxic shock syndrome toxin

Two types of staphylococcal toxins are having superantigenactivity,They are secreted *by S. aureus*enterotoxins, six antigenic types have been found in this catigory (named SE-A, B, C, D, E and G), and toxic shock syndrome toxin (TSST-1) (Schlievert *et al.,* 2000). These enterotoxins after ingested with food induce diarrhea and vomiting and are responsible for staphylococcal food poisoning.TSST-1 has a systemic effect and is the main cause of toxic shock syndrome (TSS); in addition , enterotoxins also can induce toxic shock syndrome.However, nasal colonization increases the risk of infection byfour-fold (Safdar & Bradley, 2008).

Forroutine detection of superantigens, commercially produced kits, such as reverse passive latex agglutination assays and enzyme-linked immunosorbent assays, were most commonly used. However, these methods were to date designed only to detect limited types of superantigens. As an alternative to these more traditional methods, the PCR approach can provide detection of toxin genes and is presently designed to detect the majority of (SAg) (McLauchlin *et al.,* 2001).However, a gene's presence does not establish its enterotoxigenic properties of a strain therefore, the expression of the gene should also be evaluated (Fooladi*et al* , 2010)

Material and Method

The study was included two groups:

The first group (patients group): This group included 100 patients with Allergic rhinitis. These patients attended Al-Sadder Medical City, an outpatient clinic of ENT in Najaf city during the period from March 2014 to November 2014.

Exclusion criteria;

1. Patients who received antibiotics or oral corticosteroid therapy or had upper respiratory infections during the four weeks before enrollment.
2. Patients who already started or completed immunotherapy.

The control groups consist of 100 non allergic healthy volunteers not suffering from respiratory symptoms for isolation of *S.aureus*, a nasal swab was taken from each of the 100 patients

Isolation and Identification of *S. aureus*

Specimens were taken via the insertion of a sterile moistened swab in both nostrils to a depth of approximately 1 cm into the nostril and rotated five times. After collection, specimens were immediately transported to the lab for inoculation on the culture medium. Samples were directly inoculated onto mannitol salt agar, Chromoagar plates and incubated at 37°C for 24 hours. *S. aureus* isolate identification was based on morphology, Gram's stain property, coagulase test, catalase test and mannitol salt agar fermentation.

Biomerieux Vitek 2 Compact Automated Microbial Identification

Automated VITEK® 2 ID cards this technique isused in order to provide reliable, accurate results for isolated bacteria.

DNA Extraction

According to Mini gDNA Bacteria Kit protocol (Geneaid Biotech Ltd)

Polymerase Chain Reaction Protocols

Programs for PCR thermocycling conditions for detection of virulencegenes

MONOPLEX GENE	TEMPERATURE (^{O}C)/ TIME					CYCLE NUMBER
	Initial denaturation	Cycling condition			Final extension	
		Denaturation	Annealing	Extension		
Sea	94°/5 min	94°/2 min	57°/2min	72°/1min	72°/7 min	35
Seb	94°/5 min	94°/2 min	57°/2min	72°/1min	72°/7 min	35
Sec	94°/5 min	94°/2 min	57°/2min	72°/1min	72°/7 min	35
Sed	94°/5 min	94°/2 min	57°/2min	72°/1min	72°/7 min	35
See	94°/5 min	94°/2 min	57°/2min	72°/1min	72°/7 min	35
Tsst	94°/5 min	94°/2 min	57°/2min	72°/1min	72°/7 min	35

Preparing the Primers Suspension

The DNA primers were suspendedby addition the lyophilized product after spinning down briefly with TE buffer molecular grad depending on manufacturer instruction as stock suspension. Working primer tube was prepared by diluted with TE buffer molecular grad. The final picomoles depended on the procedure of each primer

Enzyme immunoassay for identifying of Staphylococcus enterotoxins A,B,C,D and E in cultures

Is a sandwich enzyme immunoassay for identification of Staphylococcus enterotoxins,all *Staphylococcus aureus* bacteria were examined in each allergic patient and each control subject. after preliminary growth on the agar plates, *Staphylococcus aureus* cultured were transferred to brain heart infusion BHI broth and cultured aerobically at 37°C ,centrifuged supernatants of microbiological fluid cultures 5 min/at a minimum of 3500g /10°C sterile filtration of the supernatant is strongly advisable as any precipitated or re-suspended cells may influence the test reaction ,

the ability of bacteria to produced toxin was measured by enzyme immune assay RIDASCREEN ® SET A, B,C,D,E.

Primers forMonoplex PCR

TYPE	PRIMER NAME	OLIGO SEQUENCE (3'-5')	PRODUCT SIZE (BP)	REFERENCE	ORIGIN
Superantigens	*sea*	F: GGTTATCAATGTGCGGGTGG R: CGGCACTTTTTTCTCTTCGG	102	Mehrotra *et al.*,2000	Korea
	seb	F:GTATGGTGGTGTAACTGAGC R:CCAAATAGTGACGAGTTAGG	164	Mehrotra *et al.*,2000	Korea
	sec	F:AGATGAAGTAGTTGATGTGTATGG R:CACACTTTTAGAATCAACCG	451	Mehrotra *et al.*,2000	Korea
	sed	F:CCAATAATAGGAGAAAATAAAAG R:ATTGGTATTTTTTTTCGTTC	278	Mehrotra *et a.*,2000	Korea
	see	F:AGGTTTTTTCACAGGTCATCC R:CTTTTTTTTCTTCGGTCAATC	209	Mehrotra *et al.*,2000	Korea
	tst	F:ACCCCTGTTCCCTTATCATC R:TTTTCAGTATTTGTAACGCC	326	Mehrotra *et al.*,2000	Korea

Result

Table (1) Total studied patients with allergic rhinitis and control included in the study

Study groups	NO	Positive for *S.aureus*	%	Negative for *S.aureus*	%	P value
Allergic rhinitis patients	100	52	52%	48	48.0%	<0.001
control	100	24	24.0%	76	76%	Odds=3.4306 CI=1.8-6.26
Total	200	76	38%	124	62%	

Table (2) show the distribution of toxigenic and non- toxigenic of *S. aureus* isolates recovered from allergic rhinitis patients and control by PCR

	S.AUREUS ISOLATED FROM PATIENTS GROUP	%	*S.AUREUS* ISOLATED FROM CONTROL GROUP	%	TOTAL	%
toxigenic	48	63.16%	20	26.32%	68	89.48%
Non toxigenic	4	5.26%	4	5.26%	8	10.52%
Total	52	68.42%	24	31.58%	76	100%

P-value=0.434

Table (3) show the distribution of superantigens among (76 *S. aureus*) isolates recovered from allergic rhinitis patients and control by both PCR and Elisa test

ENTEROTOXIN	PCR	100%	ELISA	100%	SENSITIVITY	SPECIFICITY
SEA	52	68.42	16	21.052	23.1%	83.3%
SEB	64	84.21	8	10.53	12.5%	100%
SEE	16	21.05	12	15.79	100%	100%
SEC	8	10.53	8	10.53	100%	100%
SED	8	10.53	8	10.53	100%	100%
TSST	0	0	0	0	0	0

Table (4) distribution of superantiens genes among toxigenic *S aureus* in AR patients and control groups

	S AUREUS	ONE TYPE OF TOXIN	100%	TWOTYPE OF TOXIN	100%	THREE TYPE OF TOXIN	100%	FOUR TYPE OF TOXIN	100%
Patients	48	8	16.67%	32	66.66%	8	16.67%	4	8.33%
Control	20	8	40%	8	40%	4	20%	0	0
Total	68	16	56.67%	40	106.66%	12	36.67%	4	8.33%

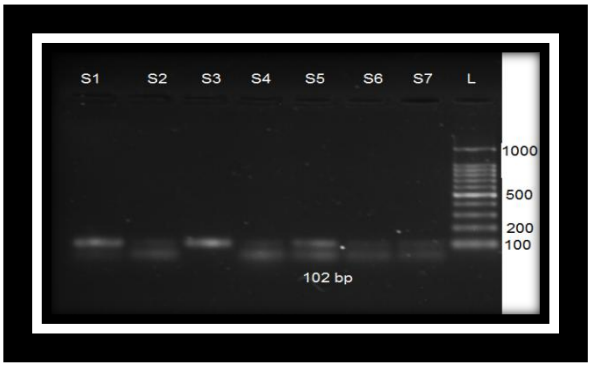

Figure (1.): Ethidium bromide-stained agarose gel of PCR, amplified products from extracted DNA of *S. aureus*isolates and amplified with forward and reverse primer *sea*. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (1, 2, 3, 4, 5 ,6 and 7) shows positive results with *sea* gene (102 bp).

Figure (2.): Ethidium bromide-stained agarose gel of PCR, amplified products from extracted DNA of *S. aureus*isolates and amplified with forward and reverse primer *sea*. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (8,9,12 and 14) shows positive results with *sea* gene (102 bp) Lanes (S10, 11, and 13) show negative results with *sea* gene.

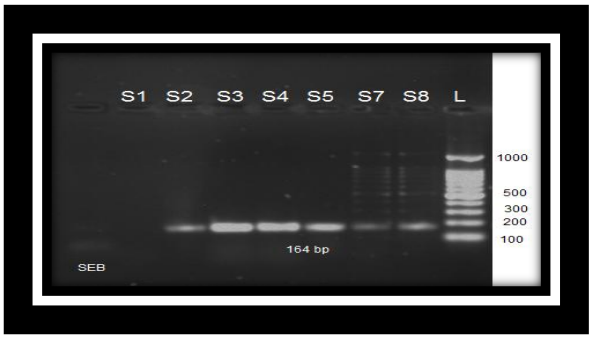

Figure (3): Ethidium bromide-stained agarose gel of PCR, amplified products from extracted DNA of *S. aureus*isolates and amplified with forward and reverse primer *seb*. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (S2,3,4,5,7 and 8) shows positive results with *seb* gene (164 bp), Lanes (1) show negative result with *seb* gene

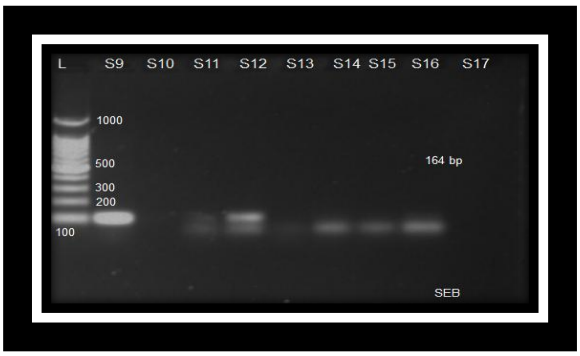

Figure (4): Ethidium bromide-stained agarose gel of PCR, amplified products from extracted DNA of *S. aureus*isolates and amplified with forward and reverse primer *seb*. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (S9,11,12,14, and 16) shows positive results with *seb* gene (164 bp), Lanes (10,13 and 17) show negative result with *seb* gene.

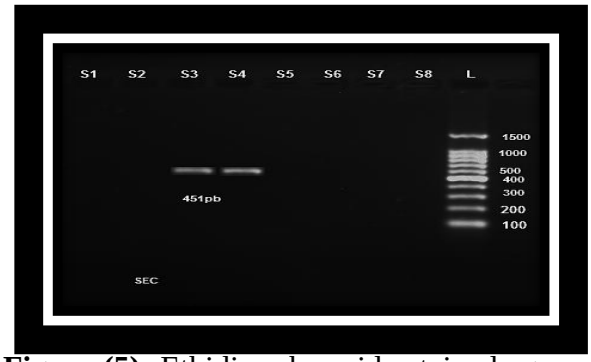

Figure (5): Ethidium bromide-stained agarose gel of PC R, amplifiedproducts from extracted DNA of *S. aureus*isolates and amplified with forward and reverse primer *sec*. The electrophoresis was performe d at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (S 3 and 4) shows positive results with *sec* gene (451 bp), Lanes (1,2,5,6,7 and 8) show negative results with *sec* gene.

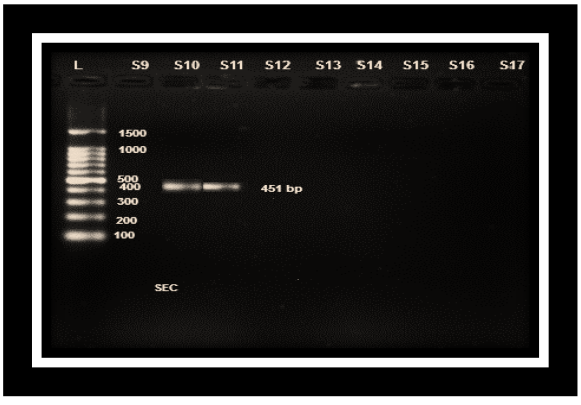

Figure (6): Ethidium bromide-stained agarose gel of PCR, amplified products from extracted DNA of *S. aureus*isolates and amplified with forward and reverse primer *sec*. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (S 10and 11) shows positive results with *sec* gene (451 bp), Lanes (9,2,13,14,15,16, and 17) show negative results with *sec* gene.

Figure (7): Ethidium bromide-stained agarose gel of PCR, amplified products from extracted DNA of *S. aureus*isolates and amplified with forward and reverse primer *sed*. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (S 11and 13) shows positive results with *sed* gene (278 bp), Lanes (S10,12,14,15,16 and 17) show negative results with *sed*gene

Figure (8): Ethidium bromide-stained agarose gel of P CR, amplified products from extracted DNA of *S. aureus* isolates and amplified with forward and reverse primer sed. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (S

18) shows positive results with sed gene (278 bp), Lanes (16,17,19,20,21,22, and 23) show negative results with sed gene

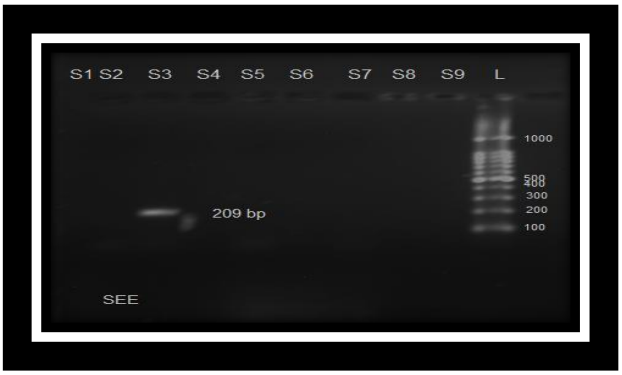

Figure (9): Ethidium bromide-stained agarose gel of PCR, amplified products from extracted DNA of *S. aureus* isolates and amplified with forward and reverse primer see. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (S 3) shows positive results with see gene (209 bp), Lanes(1,2,4,5,6,7,8 and 9) show negative results with see gene

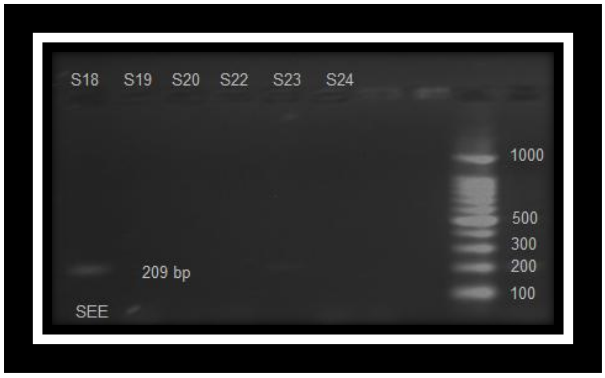

Figure (10): Ethidium bromide-stained agarose gel of PCR, amplified products from extracted DNA of *S. aureus* isolates and amplified with forward and reverse primer see. The electrophoresis was performed at 70 volt for 1.5hr. Lane (L) DNA molecular size marker (1000 bp ladder), Lanes (S 18) shows positive results with see gene (209 bp), Lanes (19,20,22,23 and 24) show negative results with see gene

Discussion

The carriage rate of *S aureus* in nasal cavity:

It was focused in table (1) regarded the rate of isolation of *S. aureus* from the nasal cavity of patients with allergic rhinitis (AR) in comparison to that of nasal cavity of control subjects (not - allergic subject) it was seen from the table that 52(52%) of the nasal swab from patients with allergic rhinitis (AR) showed growth of *S. aureus* in comparison to 24(24%) of the swab that was taken from the nasal cavity of control subject, the difference between the two results was shown to be significant P<0.001,Odds 3.43306.

The patients with allergic rhinitis showed to be higher carriers of *S. aureus* in their noses.The result of this table is in agreement with (Shiomori *et al*.,2000)

Superantigenic exotoxins produced by *S aureus*

Staphylococcus aureus (SA) is one of the most common human bacterial pathogens and produces enterotoxins that act as toxins and superantigens. Staphylococcal enterotoxins are a family of structurally related proteins comprised of different serological types: *Staphylococcus* enterotoxins A (SEA), B (SEB), C, D, E (up to U), and toxic shock syndrome toxin-1 (TSST-1). (Bachert *et al*.,2007) the stimulatory role of superantigens in the development of inflammation in chronic rhinosinusitis and nasal polyposis has been documented. It has been postulated that SA and its products are related to the pathogenesis of allergic inflammatory diseases, including rhinitis and atopic dermatitis (Bachert *et al*., 2010; Ikezawa *et al*., 2010; Liu *et al*., 2014).There has recently been much interest in the role of bacterial superantigens in allergic inflammatory reactions. Numerous studies have demonstrated that bacterial superantigens regulate the activity of immuno-modulatory (T lymphocytes) and pro-inflammatory cell types (dendritic cells, eosinophils and epithelial cells etc), and play an important role on allergic disease.

Mechanistic studies have shown that superantigens stimulate the T cells by cross-linking the variable part on the beta chain of the T-cell receptor (TCR) with MHC class II

molecules outside the peptide-binding groove area. This leads to stimulation of up to 30% of the naive T-cell population in a nonspecific way, compared with stimulation of only about 0.1% of the T cell population via the conventional allergen-specific MHC-restricted route utilizing both TCR-Va and b chains. Thus, direct binding of SEB to an MHC class II molecule loaded with antigen-derived peptides might enhance the antigenicity of the allergen and the development of allergic disease. In addition, SEB may act as allergens. SEB can induce antigen-specific T cells that are able to promote the generation of antigen-specific IgE antibodies, which subsequently play a role in'conventional'allergen-mediated reactions. Humans are natural carriers for staphylococcus aureus, the nasal passage and skin being the most common site for staphylococcus aureus colonization.(Jusufagic*et al* .,2006;Tang., 2012)

The destination of this study was to evaluate the frequency of genes that code for superantigens including enterotoxins through A, B, CD,E and toxic shock syndrome toxin (TSST) in *S. aureus* isolates recovered from allergic rhinitis patients, all (76)*S. aureus*isolates diagnostic positive were applied for toxin gene distribution analysis. Sequences specific for staphylococcal toxin genes were detected by PCR.

In the present study, monoplex PCR assay was designed to detect toxin genes employed here

Table (2) shows that, (63.16%) of *S.aureus* isolated strains from AR patients were producing one or more enterotoxins, whereas (26.32%) of *S.aureus* from the control subjects produced detectable enterotoxins the difference non-significant (p=0.434), and *S. aureus* non toxigenic isolated in equal number in AR patients and control group (5.26%), Our finding were in agreement with those of (Azzazy *et al.*,2015) who reported thatthe frequency of nasal carriage of *S. aureus*in the patients group was 25/45 (55.56%), while in control group was 6/45 (13.33%) with highly significant difference (χ^2 = 17.8 and *P*_ 0.001).

The results of molecular investigations for the finding of genes ncoding the toxins; SEA, SEB, SEC, SED, SEE and TSST.

In table no (3) show the distribution of superantigens among (76 *S. aureus*) isolates recovered from allergic rhinitis patients and control by both PCR and Elisa test *S. Aureus* containing enterotoxins were (84.21%) for SEB, (68.42%)for SEA,(21.05%) for SEE,(10.53%) for SED, (10.53%)for SEC and (0%) for TSST in patients by PCR method As noticed, the most frequently detected enterotoxin was staphylococcal enterotoxin B (SEB).

While by ELISA method we found that the main type of toxin was SEA (21.052%), SEB (10.526%), SEC (10.526%), SED (10.526%), TSST (0) and SEE (15.789%).

While in case of PCR results for gene detection the present results was also not much different from that seen by (Shiomori *et al.*,2000) who found that the rates of culture supernatants containing the individual superantigenic exotoxins were 13% for SEA, 54% for SEB, and the rate of SEB was the highest among the enterotoxins, the result was similar with little difference especially in TSST, which was 20% in case of Shiomori study and 0% in the present study ,but it was the same as in case of (Azzazy *et al* .,2015) who found that TSST toxin gene was 0% both in case of PCR ,and also when ELISA detection was performed ,he also found that SEB toxin was higher than other enterotoxin a result which was slightly different from the present study in which SEA was found to be the highest. The most frequent enterotoxin type was type B(PALA *et al.*, 2010).

ELISA test seem to be more sensitive for (SED, SEC, and SEE) and less sensitive for (SEA and SEB), however the specificity of ELISA is better in (SEB, SEC, SED, SEE than SEA.

However, a possible limitation of this procedure is that the molecular methods are only able to demonstrate the existence of the genes encoding for SEs in bacteria but cannot prove that production of SEs protein occurs unless RT-PCR is carried out (Morandi *et al.*, 2007). High

percentages of *S. aureus*with enterotoxin genes, especially SEA, were confirmed phenotypically and the results of PCR showed a clear relationship with immune assay results, this was in agreement with other previous study as (Anvari *et al.,* 2008). Nevertheless, with regard to SEB, more differences are observed between genotypical and phenotypical methods (Fooladi *et al.*, 2010).

Distribution of enterotoxin genes in toxigenic *S aureus* in patients and control groups

Table (4) show the distribution of enterotoxin genes in toxigenic *S aureus* in patients and control groups toxigenic *S.aureus* that produced one type of toxin isolated from patients and control group from 16 strains (56.67%), *S.aureus* that produced two type of toxin isolated from 40 isolated strain (106.66%)*S.aureus* that produced three type of toxin isolated from 12isolated strain (36.67%) and *S.aureus* that produced fore type of toxin isolated from 4isolated strain (8.33%), In fact when a revision of the results of this table was done, we could found that the bacterial isolates which excreted 2 type of enterotoxin was higher than that which excreted either one type or 3,4 type of the enterotoxins, at the same time there were a scanty of studies which explained or demonstrated the combined existence of enterotoxigenic both in case of molecular studies or in ELISA studies.

Conclusion we have demonstrated that the rate of nasal carriage of *Staphylococcus aureu*s in allergic rhinitis patients was significantly higher than that of control subject,-*Staphylococcus aureu*s isolated from allergic rhinitis patients have a probably a relationship with the disease and their superantigen have been arole in triggering and exacerbation of the disease

References

1. Liu ,Jing., Nan, Young-Min., Ye, Dong-Ho., Nahmand, Hae-Sim. Park 2014 The Prevalence of Serum Specific IgE to Superantigens in Asthma and Allergic Rhinitis Patients y Asthma Immunol Res. 6(3):263-266.
2. Al-Abri, R,.Bharghava, D,.Kurien ,.M, Chaly, V,. Al-Badaai ,Y,. Bharghava ,K.(2014) Allergic rhinitis and associated comorbidities: prevalence in oman with knowledge gaps in literature.Oman Med J.;29 (6):414-8.
3. Guilemany, J.M., 2009. Persistent allergic rhinitis has a moderate impact on the sense of smell, depending on both nasal congestion and inflammation .Laryngoscope, 119(2): p. 233-8.
4. Rondón,C., Romero, JJ., Lopez, S., Antúnez, C., Martín-Casañez E, Torres, MJ., ayorga ,C., R-Pena, R., Blanca, M.(2007)Local IgEproductionand positive nasal provocation test in patients with persistent nonallergic rhinitis. J Allergy Clin Immunol;119:899-905.
5. Murray, P., Baron, E., Jorgensen, J., Pfaller, M. and Yole, R. (2003).Manual of clinical Microbiology. 8th ed., American Society for Clinical Microbiology, Washington, DC., 1: 57-61.
6. Liu ,Z,. Albanese ,E., Li, S., Huang ,Y., Ferri, CP., Yan ,F., et al. (2009) Chronic disease prevalence and care among the elderly in urban and rural Beijing, China - a 10/66 Dementia Research Group cross-sectional survey. BMC Public Health;9:394
7. Chambers ,H, F and F, R, DeLeo. (2009). Waves of resistance: *Staphylococcus aureus*in the antibiotic era, Nature Reviews Microbiology, vol. 7, no. 9, pp. 629–641
8. Lowy, FD. (1998 *) Staphylococcus aureus* infections ,The New England Journal of Medicine, vol. 339, pp. 520–532.
9. Daum, R.S. (2007). Skin and soft tissue infections caused by methicilin – resist and *Staphylococcus aureus* .New England Journal of medicine 357:380-390.
10. Safdar,N ., and Bradley,E,A.(2008). The risk of infection after nasal col- onization with *Staphylococcus aureus.Am.J.Med.* 121, 310–315.

11. Schlievert,P.,M.,Jablonski,L.M., Roggiani, M., Sadler,I.,Callantine, S., Mitchell ,D.T., Ohlendorf, D. H, and Bohach,G.A. (2000). Pyrogenic toxin superantigensite specificity in toxic shock syndrome and food poisoning inanimals.*Infect.Immun.* 68, 3630–3634.
12. Shiomori ,Teruo., MD,A Shin-ichi., Yoshida, MD., Hiroshi, Miyamoto, MD.(2000) Relationship of nasal carriage of *Staphylococcus aureus*to thogenesis of perennial allergic rhinitis j allergy clinimmunol .450-454
13. Bachert,C, P., Gevaert, P., Van, Cauwenberge .*Staphylococcus aureus* enterotoxins.(2002): a key in airway disease? Allergy: 57: 480–487.
14. Bachert, C.,Zhang,N.,Holtappels, G., DeLobel, L., Van Cauwenberge, P., Liu,S.,Lin ,P., Bousquet,J., and VanSteen ,K. (2010).Presenceof IL-5proteinand IgE antibodies to *staphylococcal* enterotoxins in nasal polyps is associated with comorbid asthma. *J. Allergy Clin. I mmunol.*126, 962– 968,968e961 –966.
15. Ikezawa, Z., Komori ,J., Ikezawa, Y., Inoue, Y., Kirino, M., Katsuyama ,M., Aihara, M.(2010) A role of Staphyococcus aureus, interleukin-18, nerve growth factor and semaphorin 3A, an axon guidance molecule, in pathogenesis and treatment of atopic dermatitis. Allergy Asthma Immunol Res.;2:235–246.
16. Liu ,Jing., Nan, Young-Min., Ye, Dong-Ho., Nahmand, Hae-Sim,Park .(2014) The Prevalence of Serum Specific IgE to Superantigens in Asthma and Allergic Rhinitis Patients y Asthma Immunol Res. 6(3):263-266
17. Tang, X.Y. 2012 Effects Of Staphylococcal Enterotoxin B In The Pathogenesis Of Allergic Rhinitis Dissertation
18. Bachert C, Gevaert P, Zhang N, van Zele T, Perez-Novo C.(2007) Role of staphylo-coccal superantigens in airway disease. ChemImmunol Allergy;93:214–236.
19. Azzazy , Ensaf ,A., Hossam, A., Aziz, Maha K. Gohar, Reham H. Anis 2015 Nasal Carriage of Superantigen Producing Staphylococcus Aureus and Its Role in Pathogenesis of Allergic Rhinitis and Bronchial Asthma Egyptian Journal of Medical Microbiology Vol 24 /No. 4 / Oct.
20. Mclauchlan, A.; Ogbonnaya, F.C.; Hollingsworth, B.; CarTer, M.; Gale, K.R.; Henry, R.J.; Holton, T.A.; Morell, M.K.; Rampling, L.R.; Sharp, P.J.; Shariflou, M.R.; Jones, M.G.K. And Appels, R. (, 2001)Development of robust PCR-based DNA markers for each homoeo-allele of granule-bound starch synthase and their application in wheat breeding programs. Australian Journal ofAgriculture Research, vol. 52, no. 11-12, p. 1409-1416.
21. Fooladi,Imani., A, Tavakoli., HR2, Naderi. (2010) Detection of enterotoxigenic *Staphylococcus aureus*isolates in domestic dairy products Iranian jornal of microbiology *Vol 2 No 3 (Sept*) 135-140.
22. PALA , Kayıhan., Cüneyt, ÖZAKIN., Nalan ,AKIŞ., Melda ,Sinirtaş., Suna, gedikoğlu., hamdi, aytekin.(2010) Asymptomatic carriage of bacteria in food workers in Nilüfer district, Bursa, Turkey Turk J Med Sci 2010; 40 (1): 133-139
23. Morandi L, de Biase D, Visani M, Cesari V, De Maglio G, Pizzolitto S, Pession A, Tallini G.(2012) Allele Specific Locked Nucleic Acid Quantitative PCR (ASLNAqPCR): an accurate and cost-effective assay to diagnose and quantify KRAS and BRAF mutation. PLoS One.;7:e36084. doi: 10.1371/ journal. pone.0036084
24. Anvari SH, Sattari M, Forozandehe Moghadam M, (2008).NajarPeerayeh SH,Imanee Fouladi AA. Detection of *Staphylococcus aureus* Enterotoxins A to E from clinical samples by PCR. Res J of BioloScie; 3 (8): 826-829.

25. Morandi S, Brasca M, Lodi R, Cremonesi P, Castiglioni B.(2007) Detection of classical enterotoxins and identification of enterotoxin genes in *Staphylococcus aureus*from milk and dairy products. *VeterMicrobiol*; 124: 66-72.
26. Mehrotra ,M., Wang,G., Johnson ,WM. 2000Multiplex PCR for detection of genes for Staphylococcus aureus enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance.J ClinMicrobiol. Mar;38(3):1032-5.