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ORIGINAL ARTICLE

The association between allergic rhinitis and Polymorphism of Toll like receptors 2 & 4 genes

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ABSTRACT

Background: *Allergic rhinitis is atopic disorder, 10% to 25% of the population worldwide are suffering from it, The prevalence is increasing during the last 10 years.* ***Objectives:*** *To study the relationship among polymorphism of single nucleotide in TLR2 and TLR4 genes and the risk of allergic rhinitis disease.* ***Methodology:*** *This study was done on 60 patients suffering from allergic rhinitis and 30 healthy subjects as a control group from April 2019 to March 2020. The patients were collected from Otorhinolaryngology Department of Benha University Hospital. Test of Skin prick (SPT) was done to assess atopic state. Blood samples were collected to detect TLR gene polymorphism by Polymerase chain reaction-Restriction Fragment Length Polymorphism (PCR-RFLP).* ***Results:*** *The genotypic frequencies of TLR2 Arg753Gln showed increased frequency of the homozygous (GG) genotype among the controls (80%) more than the allergic rhinitis patients (30%). The heterozygous (AG) genotype was increased among the allergic rhinitis patients (62.5%) more than in the healthy group (15%) with OR =9.4, 95% CI (2.4-37.7) and significant P-value. Also, the homozygous mutant (AA) genotype has more trend in the patients (7.5%) than in the control subjects (5%), with OR = 0. 6, 95% CI (0.1-6.7) and non-significant P-value. The genotypic frequencies Statistical data in TLR4 Asp299Gly revealed that the homozygous (AA) genotype has more frequency in the controls (70%) than the allergic rhinitis patients (20%). The heterozygous (AG) genotype was more prevalent among the allergic rhinitis patients (65%) than the controls (30%) with OR =4.3, 95% CI (1.4-13.8) and significant P-value.* ***Conclusion****: GG genotype of TLR2 and AA genotype of TLR4 are least affected by allergic rhinitis disease and the major allele in both gene is protective against the disease.*

INTRODUCTION

Allergic rhinitis is a great problem affecting 10% to 25% of the people all over the world, the incidence is increasing during the last 10 years. Allergic rhinitis represent asocioeconomical problem to the patient , that includes the costs of disease treatment, absence from work, and treatment of the related problems such as sinusitis, nasal polyps, asthma and respiratory tract infection[1].

The interactions between genetic elements and environmental elements represent the main aetiolgies of this disease. More than 100 genes have been associated with atopic disease[2].

The role of genetic factors on AR pathogenesis is currently an area of intense investigation. Various loci and candidate genes have been suggested to affect allergy. One of these chromosomal regions is toll like receptor gene. Some of the genes in these loci may regulate T-helper 2 responses and IgE production. Other genes may have tissue specific influences[2].

Toll like receptors (TLRs) are trans membrane proteins involved in innate immunity and pathogen recognition. TLRs play a mjor role in specific and non specific immunity .TLR2 responds mainly to components from gram-positive bacteria cell wall, like peptidoglycan, and TLR4 mainly recognizes gram-negative microbial membrane components, like lipopolysaccharide (LPS)[3].

Identification of pathogen-associated molecular patterns by TLRs activates signaling events that intiate activation of transcription factors and expression of effector molecules, like cytokines, controlling specific immune response. TLRs also affect T-cell polarization and development; both of them help in initiation of allergy. Single nucleotide Polymorphisms (SNPs) affecting the genes of TLRs is responsible for change in susceptibility to the disease[4].

There is great role of TLRs pathway in the overall immune reaction, so variation in TLRs genes are responsible for altered immune response which may be related to allergic diseases[5].

Our study aims to show the relation between the allergic rhinitis risk and TLR2 and TLR4 single nucleotide polymorphism.

METHODOLOGY

Patients:

The study was done on 60 patients complaining from allergic rhinitis and 30 healthy persons as a control group during the period from March 2019 to April 2020. The control healthy subjects were chosen to be similar with the patients regarding the age and sex.Approval of the work was obtained from the Local Ethics Committee of the departments of Otorhinolaryngiology, Benha faculty of medicine, Benha University. A consent was obtained to perform skin prick test (SPT) and to take the blood sample. The test was performed at the Otorhinolaryngiology Department of Benha University Hospital. Patients stopped antihistaminic treatment 72 hours before the test and corticosteroids 3 weeks. The test was done by intradermal injection of standard panels of common airborne allergens. SPT was performed on the volar side of the forearm with saline buffer as negative and histamine chloride (10 mg/ml) as positive controls. The appearance of redness , swelling and awheal reaction diameter of ≥3 mm was considered a positive [6]. It reaches a maximum size in15-20 minutes and they disappear after few hours[6]. Atopy was defined when there is a positive reaction of the skin to either one of tested allergens . AR diagnosis was confirmed by the appearance of two or more AR symptoms (nasal congestion, nasal itching, rhinorrhea, sneezing) lasting for four or more days per week during the last year and prescence of atopic status[7] . The non-allergic controls were having no atopy neither typical AR symptoms.

Polymerase chain reaction -Restriction Fragment Length Polymorphism:-

The reaction was done at Medical Microbiology and Immunology Department, Benha Faculty of Medicine, Benha University.

Blood samples:

2 ml whole venous blood sample was collected under complete aseptic condition into an EDTA tube. The samples were stored at -80°C until further processing.

- DNA extraction:

It was done as described by the manufacture (Thermo Scientific).

Principle:

Blood specimens in the supplied Lysis Solution are digested by Proteinase K. Ethanol is added to the resulted material and putted into the purification column, where the DNA attaches to the silica membrane. Wash Buffers is used to remove impurities from the column . DNA is then eluted using the Elution Buffer.

Procedure:

Blood samples were thawed; 20 µL of Proteinase K and 400 µL of Lysis Solution were added to 200 µL of samples, after mixing by vortex, they were incubated at 56°C for 10 minutes. 200 µL of ethanol (96-100%) was added then mixed by repeated pipetting ,centrifuged at 8000 rpm; washing buffer 1 then 2 were added to tubes, respectively. After centrifuge, 200 µL elution buffer was added and centrifuged at 8000 rpm, finally, Eluted genomic DNA stored at -20°C for for further analysis

***- DNA amplification*:**

Forward primer was used to amplify exon I of TLR2 gene (5'd CATTCCCCAGCGCTTCTGCAAGCTCC 3') Reverse primer: (5'. d GGAACCTAGGACTTTATCGCAGCTC -3') and exon I of TLR4gene was amplified using PCR with Forward primer (5' dAGCATACTTAGACTACTACCTCCAT 3') Reverse primer: (5'-d GAGAGATTTGAGTTTCAATGTGGG-3'), the amplification was performed in thermal cycler (Biometra, Germany). The following materials were placed to each 50µl reaction tube: 25µl Maxima Hot Start PCR Master Mix (2X), 2.5 µl forward primer, 2.5 ml reverse primer, 5 ml template DNA, and 15 ml nuclease free water reaction was performed as follow . PCR results were separated by agarose gel electrophoresis then identified by ethedium bromide staining. The amplified product of TLR2gene was 129bp and of TLR4 gene was 188bp[8].

- Restriction endonuclease digestion:

The resulting PCR products of TLR2 gene were treated with Msp1restriction enzyme and The resulting PCR products of TLR4 gene were treated with Nco11restriction enzyme[9]. The following components were added: 17 µL Water, nuclease-free,2 µL 10X FastDigest , 10 µL DNA and 1 µLFastDigest enzyme. The mixture was mixed gently and spinned down, then incubated at 37°C for 15 min.

- Detection of DNA.

After digestion, the GG genotype of TL2 gene was defined by two bands at 104 bp and 25 bp, while AA genotype was indicated by an uncut band at129 bp. The heterozygote AG genotype was indicated by bands at 129,104 bp and 25 bp. the wild-type AA genotype of TL4 gene was indicated by uncut band at 188 bp, while the mutant GG genotype was indicated by bands at168 and 20 bp. The heterozygote genotype was indicated by bands at 188,168 bp and 20 bp.

RESULTS

Study population characteristics:

In our study, TLR2 (Arg753Gln) and TLR4 (Asp299Gly) polymorphisms were studied in 90 subjects, including 30 healthy controls and 60 patients

complaining of allergic rhinitis. Many variables were examined such as gender, age, atopic status, smoke exposure and family history. The mean age for patients and for healthy controls was 44 years and 65% of the patients had a positive family history and were atopic.

The results of TLR2 Arg753Gln(Fig 1) showed that the homozygous (GG) genotype had more incidence in the control group (80%) than the allergic rhinitis patients (30%) as shown in table (1).The (AA) genotype was heigher in the allergic rhinitis (7.5%) while 5% in the healthy group. Also,the heterozygous (AG) genotype was more prevalent among the allergic rhinitis patients (62.5%) than the controls (15%) with OR =9.4, 95% CI (2.4-37.7) and significant P-value. Also, the GG versus AG+AA combinations are found to have OR=0.11, 95% CI (0.03-0.4) and P-value significant.

Table 1: Frequency of different genotypes of TLR2 in allergic rhinitis patients and control subjects:-

	allergic rhinitis patients		Control		OR (95% CI)	P-value
	No.	%	No.	%		
GG genotype	18	30.0%	24	80.0%	0.11 (0.03-0.4)	<0.001
AA genotype	5	7.5%	2	5.0%	0.6 (0.1-6.7)	>0.05
AG genotype	37	62.5%	4	15.0%	9.4 (2.4-37.7)	<0.001
Total	60	100.0%	30	100.0%		

Odds ratio (OR) which shows the relation between genotypes and risk of allergic rhinitis.
-95% confidence interval (95% CI). - P value <0.001 (highly significant) , >0.05(non-significant)

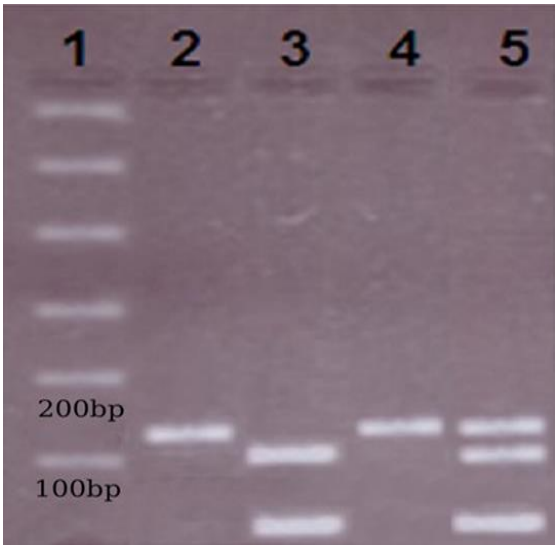

Fig. 1: The Msp1restriction profiles of the 753A/G polymorphic sites of TLR2 gene of blood samples of allergic rhinitis patients and control subjects:- Lane 1: DNA ladder (100-1000bp), Lane 2:PCR product at 129bp, Lane 3: GG genotype (homogenous- normal) at 104 and 25bp Lane 4:AA genotype (homogenous - polymorphic) at 129bp and Lane 5:GG genotype (heterozygous – polymorphic) at 129,104 and 25bp.

The results for the genotypic frequencies in TLR4 Asp299Gly showed that (AA) genotype is more frequent in healthy group (70%) than the allergic rhinitis (20%) as shown in table (2). The (AG) genotype was more increased among the allergic rhinitis patients (65%) than the normal group (30%). Also, the AA versus AG+GG combinations and AG vs AA & GG combinations are found to have a significant P-value with OR =0.11, 95% CI (0.03-0.4) and 9.4, 95% CI (2.4-37.7) respectively. The Nco1restriction profile of the TLR4 gene at 299 position was shown in figure (2).

Table 2: Frequency of different genotypes of TLR4 in allergic rhinitis patients and control subjects:-

	allergic rhinitis patients		Control		OR (95% CI)	p-value
	No.	%	No.	%		
AA genotype	12	20.0%	21	70.0%	0.11 (0.03-0.4)	<0.001 (Hs)
GG genotype	9	15.0%	0	0.0%	---	>0.05 (Ns)
AG genotype	39	65.0%	9	30.0%	4.3 (1.4-13.8)	<0.05 (s)
Total	60	100.0%	30	100.0%		

-Odds ratio (OR) which shows the relation between genotypes and risk of allergic rhinitis.
-95% confidence interval (95% CI). - P value <0.001 (highly significant), > 0.05(non-significant)

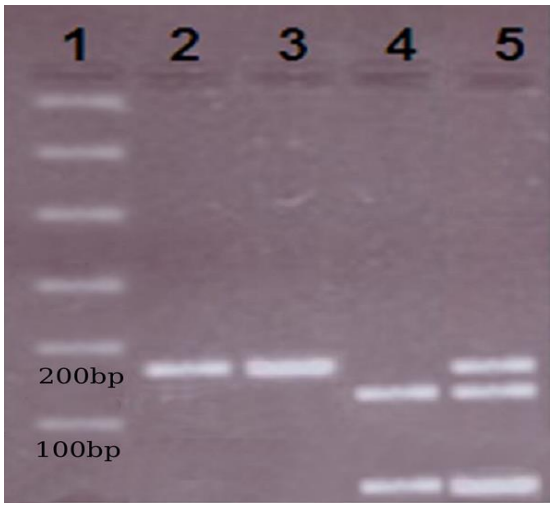

Fig. 2: The Nco1restriction profiles of the 299A/G polymorphic sites of TLR4 gene of blood samples of allergic rhinitis and control subjects::- Lane 1: DNA ladder (100-1000bp), Lane 2:PCR product at 188bp, Lane 3: AA genotype (homogenous- normal) at 188 bp, Lane 4:GG genotype (homogenous - polymorphic) at 168 and 20 bp and Lane 5:AG genotype (heterozygous – polymorphic) at 188,168 and 20 bp.

DISCUSSION

Allergic rhinitis is an immune system reaction to the air allergens. It is caused by environmental allergens such as pollen, pet hair, dust, or mold. Inherited genetics and environmental exposures share in the development of allergies. [10]. This work aims to show the relation between TLR2 and TLR4 genes polymorphism and the allergic rhinitis.

Our study found that for TLR2 Arg753Gln , the homozygote genotype (GG) and major G allele was significantly related to decreased allergic rhinitis risk (p<0.001). This matches with results of Eder et al.[11] in Europe, Smit et al.[12] in France and Klassen et al[13] in Caucasian children. They demonstrated that a SNP in TLR2 was related to allergy. Smit et al.[12] found that the homozygous (GG) genotype showed an increase in frequency among the controls when compared with the patients. Also, the GG versus AG+AA combination in patients complaining of allergic rhinitis have significant P-value. A large cross-sectional study of 3099 subjects from Germany detected that the minor allele was related significantly with atopy determined by skin test (OR 1.53, 95% CI 1.06–2.19, P = 0.023) and specific serum inhalative allergens (OR 1.57, 95% CI 1.12–2.20, P = 0.009).[14] Niebuhr et al[15] found that the production of cytokines by monocytes from atopic dermatitis patients carrying minor allele was higher than those carrying wild-type.

This is not coincide with results of Bahrami et al.[16] in Iran who found that there is minor relation between polymorphisms occurring in Toll Like Receptor-2 Gene and atopy in atopic patients. They found that homozygous genotype was 92.9% among atopic patients and 94.2% among controls and AG+AA combination was 7.1% among atopic patients and 5.8% among controls.

Our study showed that for *TLR4 Asp299Gly*, The decreased allergic rhinitis risk occurred in AA homozygous patients in significant rate. (p<0.001). These results determine that the reference allele in *TLR* polymorphisms might be related to decreasing the prevelance of allergic rhinitis. This agrees with results of Bottcher et al.[17], Kerkhof et al.[18] and Voronko et al.[19]. These Studies were conducted on Sweddish, Danish and Russian populations and revealed a good relation between TLR4 and atopic allergic rhinitis. This is not coincide with results of Sinha et.al.[20] in a study

conducted on population of North India with atpoy. They reported non statistical association between TLR4 polymorphism and atopy while (AA) genotype in TLR4 (Asp299Gly) increased significantly among the atopic patients (81.1%) in comparison to the control persons (78.9%). Hussein et al.[21] in Egypt showed non significant relation between the allele frequencies and genotype of the Arg753Gln (TLR2) and Asp299Gly (TLR4) polymorphisms and between the rate of the disease in children in comparison to controls. Although, their study reported that in moderate and severe groups, the mutant allele of both TLR2 or TLR4 was present in significant rate in comparison to the mild group of allergy ($p > 0.001$ for each). So they demonstrated a lack of association of TLR2 and TLR4 polymorphisms with asthma and allergic rhinitis but suggested a strong relation between these genetic variants and the disease severity.

CONCLUSION

We can conclude that GG genotype of TLR2 and AA genotype of TLR4 are least affected by allergic rhinitis disease and the major allele in both gene is protective against the disease. So we need more studies on the relation between gene polymorphism in TLR2 and TLR4 and allergic rhinitis risk and to do more researches to detect other responsible genes for allergic rhinitis disease.

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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