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Investigation of IL-4 (rs2243250) Gene Polymorphism in Sample of Iraqi Allergic Patients

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Abstract: Allergy is the reaction by your immune-system. Substances that cause reactions include food and pollen. The study was aimed to reveal circulating T-lymphocyte-subsets and related cytokines during asthmatic-attacks is still unclear. This study included 89 subjects total; 31 controls, 23 Allergy Food and 35 Allergic Rhinitis individuals. The aim of this study is to estimate the Total IgE levels in allergic patients and healthy control subjects. Also, investigates about single nucleotide polymorphisms (rs2243250) of IL-4 genes in allergic diseases such as, allergic rhinitis and allergic food. Patients' serum IgE levels were noticeably greater than those of the controls (AF: 234.4 ± 35.8, AR: 253.4± 29.1, control: 48.0 ± 5.7 IU/ml, P<0.001). The CC genotype in the IL-4 gene was not related with AR patients. Additionally, the polymorphism (rs2243250 = C-590T) in the IL-4 gene had no correlation with AR in the sample being examined due to the sample size of the subject was small. It was concluded the sample being examined and IgE is a unique immunoglobulin that plays a central role in the pathophysiology of allergic disorders.

Key words: Immunoglobulin-E, AR, SNP, rs2243250

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Introduction

Atopic diseases such as asthma, rhinitis, conjunctivitis, and dermatitis and allergic diseases, allergic reactions to foreign allergens such as anaphylaxis, urticaria, angioedema, food and drug allergies are examples of type I hypersensitivities. Allergens that cause type I hypersensitivity can range from less dangerous, like mites, pollen, food, and medications to more dangerous, like insect venoms (1). One of the most widespread allergens and one of the most prevalent chronic diseases in both children and adults is allergic rhinitis (2). Asthma and/or allergic rhinitis can develop later in life as a result of early sensitization to food and aeroallergens, as well as the presence of atopic dermatitis, which is marked by an immunoglobulin E (IgE)-mediated inflammation. This process is known as the "atopic march"(3). Immunoglobulin-E that is allergen-specific is essential to the pathophysiology of allergy diseases. The usefulness of total serum IgE or allergen-specific IgE measurements for diagnosis and therapy, however, varies. It is crucial to understand that total IgE levels rarely reveal information regarding IgE to particular allergens, and the presence of IgE to a particular allergen does not always indicate a clinically significant allergic reaction to that (4). Numerous genes have been linked to Allergic rhinitis (AR) through genetic research and genome-wide association studies (GWASs). Identified

SNP (single nucleotide polymorphisms) connected to its development (5, 6). Immunoglobulin E that is allergen-specific is essential to the pathophysiology of allergic diseases. The usefulness of total serum IgE or allergen-specific IgE measurement for diagnosis and therapy, however, varies. It is crucial to understand that overall IgE levels infrequently reveal information regarding IgE to particular allergens, and that the presence of IgE to a particular allergen does not always indicate a clinically significant allergic reaction to that substance. Single nucleotide polymorphisms in the IL-4 gene have been reported to be associated with allergic responses (7,8). The C-590T (rs2243250) SNP has previously been shown to be related with a greater risk of AR in the promoter region of IL-4. The IL-4 gene's increased expression, which boosts plasma IgE levels and worsens AR symptoms, is known to be induced by this SNP (9). This study aimed to investigate the relationship between total IgE and allergic patients. Also, investigation the role the polymorphism rs2243250 (IL-4) in allergic-rhinitis patients.

Materials and methods

Study population

Patients in this study who made up the population totaled 58 (allergic rhinitis patients =35, allergic food patients = 23), with ages ranged from 16 to 60 year. Include in this study those patients who have been diagnosed with allergies based on a clinical examination by a doctor and have been attending an allergic center in Baghdad Alrusafa city, particularly those with clinical symptoms of allergies. A healthy control group of 31 people were enlisted; their ages varied from 16 to 41 year, the following exclusion criteria; a febrile illness or chest infection, smoking, a family history of allergies, childhood allergies, and a serum total IgE level less than100 IU/ml.

Measurement of serum total IgE

Blood sample (3 ml) was taken from each subject to estimate IgE levels. Sera was extracted from the blood, divided into Eppendorf tube, refrigerated at -20°C, and then thawed right before analysis. The enzyme-linked immunosorbent assay (ELISA) Assay was used to quantify the total IgE levels in the serum (Euroimmun Company/ Germany**).**

Extraction of DNA

Tow ml of venous blood was taken from the patient and control groups in falcon tubes consisting of EDTA. Using an extraction kit (QIAGEN /Germany), DNA was extracted. The Qubit 4 Fluorometer was then used to measure the purity and concentration of the DNA.

Primers

Two primers of IL-4 gene were designed for the present study as, listed in (Table 1). The two primers designed based on the Bioinformatics tools by using the international databases (NCBI) and a number of tools that are available on website (online tools).

Table (1): The primer of SNP (rs2243250), their sequences, PCR product size.

Primer	Primer sequence to rs2243250	Product size
Forward primer	5'-CTCAGAATAGACCTACCTTGC -3'	575 bp
Ravers primer	5' -TTACACCAGATTGTCAGTCAC -3'	

bp: base pair.

Genotyping

Polymerase chain reaction (PCR) was used to genotype the SNP rs2243250 of the IL-4 gene, which was followed by sequence analysis. Table 1 lists the traits of the SNPs and the

primer sequences. The following PCR Thermo cycler conditions were carried out using the PCR Thermo cycler system: Initial-denaturation, denaturation, Annealing, extension and final-extension for 20 cycles. After electrophoresis on a 2% agarose gel stained with Red Safe dye, amplifications were examined at Gel Doc. (Biorad). Utilizing the ABI Big Dye v.3.2 terminator sequencing kit, PCR products were sequenced (Applied Bio systems). Using Geneious software, sequence data were compared to the reference sequence (NCBI-RefSeq; http://www.ncbi cbi.nlm.nih.gov) as show in (Table 2).

Table (2): Program for the IL-4 Gene PCR Profile.

Step	Temperature	Time	Cycle
Initial denaturation	94ºC	5 minutes	1
Denaturation	94ºC	30 second	20x
Annealing	56 ºC	1minutes	
Extension	72ºC	30 second	
Final extension	72ºC	7 minutes	1

Statistical analysis

Spatial data analysis with SPSS-software (version 13). The data on the levels of circulating IgE are displayed as mean ± S.E. Asthma patients and controls were compared using a paired Mann-Whitney test. P values of less than 0.05 were judged to be statistically significant. SPSS software was used for data-analysis. Calculate of OR: Odds ratios and CI: Confidence intervals by winPepi software.

Results and discussion

Distribution of patients according to gender and age

The distribution of allergic patients by age and gender as shown in (Table 3). The mean of age of Allergic (AR; 28.2±12.7 AF: 27.2±11.1) compare with controls (25.9± 9.3). The gender female patients more than male while in this study control male more than female (64.5% vs. 35.5%) as showed in (Table 3).

Table (3): Distribution of patients according to gender and age.

Group	N, (%)	Mean ± S.E. of Age	Gender N, (%)	
			Females	Males
Allergic rhinitis	35 (39)	28.2±12.7	18 (51)	17 (49)
Allergic food	23 (26)	27.2±11.1	12 (52)	11 (42)
Controls	31 (35)	25.9± 9.3	11 (35.5)	20 (64.5)
Total	89 (100)	p-value= 0.72	41 (46)	48 (54)

S.E. = Standard error, N= Frequency, %= Percent, p= Probability

Serum concentrations of IgE

Total IgE Serum level in Allergic Food patients compared with controls at high significant, total IgE Serum level in Allergic Food patients comper with Allergic Rhinitis at high non-significant and total IgE Serum level in Allergic Rhinitis patients comper with controls at high-significant. As shown in (Table 4) and (Figure 1).

Table (4): Total IgE IU/ml serum levels.

Total IgE IU/ml Serum levels			
Group	**N**	**Mean ± S.E.**	**P-Value**
Allergic Rhinitis	35	253.4 ±29.1	**a, c= <0.001**
Allergic Food	23	234.4 ± 35.8	**b,=0.5**
Controls	31	48.0 ± 5.7	

(a). Serum level of Total IgE IU/ml in patients AR compared with controls. (b). Serum level of Total IgE IU/ml in AR compared with AF. (c). Serum level of Total IgE IU/ml in patient AF compare with controls.

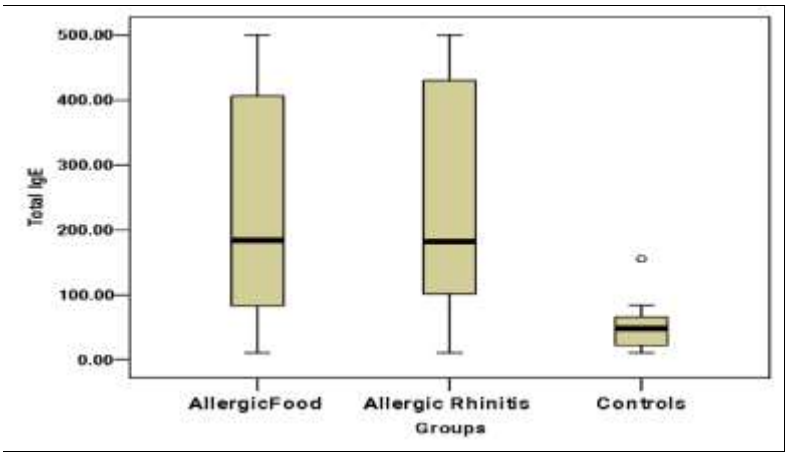

Figure (1): Serum concentrations of T-IgE in patients and controls.

The term allergy involves a great ranged of situations, it is not a disease in itself but it is a number of disorders, due to exposure to an ordinarily harmless substance called an allergen such as dust mold, mites, grass pollen and tree weed, as well as food allergens such as egg, milk, soy, fish proteins, wheat, nut, etc (10). The level of T-IgE increases significantly in patients with allergy compared with healthy control, as (Table 4) shows, and this is consistent with Iraqi studies (11-13). The results of their studies agreed with the current study. Also had a high IgE level in patients with (allergic rhinitis) when compared with allergic food patients and control. The result of this study also is consistent with the results of several non-Iraqi studies study (14-16), who found in their studies high levels of IgE in allergic patients with highly significant differences in comparison with healthy. All of the above studies support the primary role of IgE in maintaining symptom severity and immune response in allergic diseases. Also, IgE mediates type 1 hypersensitivity reactions and the association of this antibody by a special receptor (Fc Epsilon RI) with the surface of the basophils and mast cells leads to the release of inflammatory mediators and effectors of allergic reactions from cells. As a result, allergies appeared within minutes to several hours (17). IgE was involved in the pathogenicity of allergy such as allergic rhinitis, urticaria as well as asthma (18).

Agarose gel electrophoresis of DNA and detection of gene

Electrophoresis has been used to determine DNA pieces after the extraction process or to detect the result of the interaction of PCR when standard DNA is present. The detection of IL-4 in this study as shown in (Figure 2).

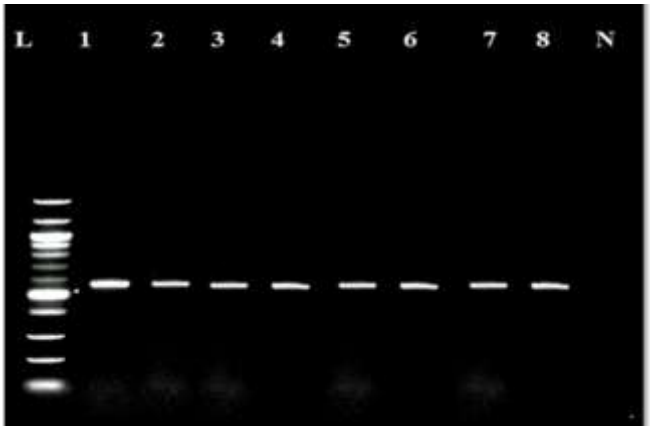

Figure (2): Agarose gel (2%) stained with RedSafe® and 80V electrophoresis for detection of IL-4 gene detected as in lane 1-8 (patients) with PCR product, L: DNA ladder (100 bp). N: Negative controls DNA

IL-4 gene polymorphisms in AR patients and controls

Analysis of the genotype and allele frequencies of the IL-4 (rs2243250) SNP using the C allele as the reference allele showed, that AR patients has no different between of homozygous CC genotypes than healthy controls (100% vs. 100%). Also, this difference was not statistically-significant of AR-patients carried the C allele, compared to the control group (OR = 1.00, p = 1.0), as show in (Table 5) and (Figure 3). Additionally, the polymorphism (rs2243250 = C-590T) in the IL-4 gene had no correlation with AR in the sample.

Table (5): Gene polymorphisms (rs2243250) in AR and controls

Genotype and Allele	Patients AR (N=20)		Controls (N=20)		OR	CI	P value
	No.	%	No.	%			
CC	20	100	20	100	1.00	0.11-52.8	1.0
C	40	100	40	100	0.32	0.19-51.6	1.0

CI: 95% confidence-interval, OR: odds-ratio, P-value= probability value

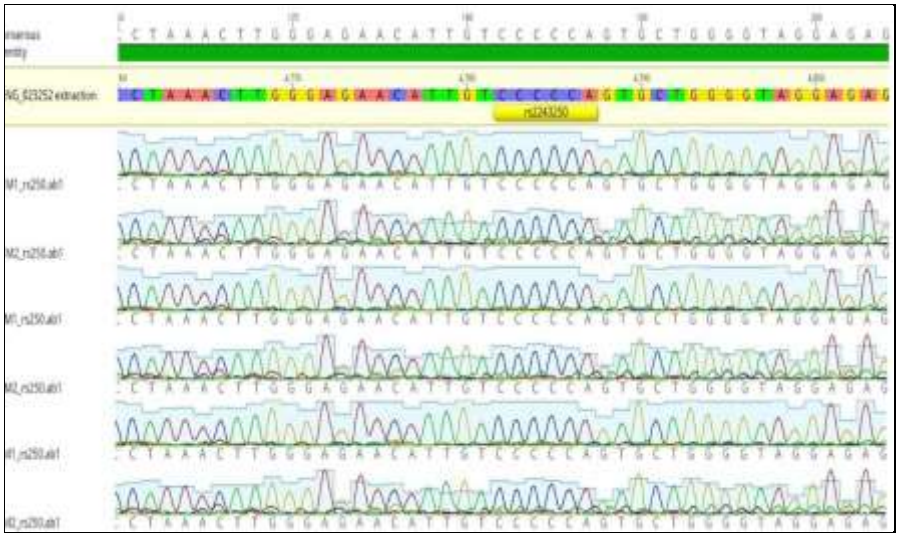

Figure (3): Patients Sequence Data.

The complex combination of genetic and environmental variables that might cause immune system dysregulation underlies the complicated disorder known as Allergic Rhinitis and Allergic-Asthma (6, 19, and 20). The objective of the current investigation was to assess the relationship between SNPs in the IL-4 promoter (rs2243250; C-590T) and the prevalence of AR in patients from certain samples from Iraq-Baghdad. It is widely known that IL-4, particularly in the late stages of the illness, plays a crucial function in the pathophysiology of AR. The primary sources of IL-4, which promotes the development of Th2 cells and the generation of IgE, are mast cells and T helper cells (4). Due to the reduced size of the study's sample, our findings indicated no relationship between the C-590T SNP in the IL-4 promoter gene and the risk of AR in individuals with AR as show in (Table 5). These results are not consistent with research from other studies assessing the association between IL-4 SNPs and the risk of different allergic-related illnesses. The T allele and TT genotype of the C-590T polymorphism in the IL-4 gene promoter have a favorable correlation with AR risk, according to a significant meta-analysis that included multiple published human studies (21). Additionally IgE levels were shown to be substantially greater in AR patients with the TT polymorphism in China than in those with the CT/CC genotypes, and their chance of developing AR was also higher (7). In contrast to earlier research, Movahedi et al. found that the CC genotype in the -590C/T SNP inside the IL-4 gene was related with higher risk of AR in AR patients from an Iranian population in Tehran. It was discovered that patients with the TT genotype had a poor correlation with AR (8). The discrepancies in allele frequencies between various ethnic groups could be the cause of the inconsistencies. As a result, it's possible that the TT genotype is more prevalent among the population of north-eastern Iran than it is in Tehran. Our study's and the Tehran study's contradicting findings are corroborated by earlier research that also produced contradictory outcomes. Patients with allergic illnesses such AR, asthma, and atopic dermatitis showed a protective effect for the CC genotype when compared to the TC and TT genotypes in a different study (22). Additionally, research on patients with hay fever and rhino conjunctivitis revealed no connection between the -590C/T SNP with allergy (23).

Conclusion

The polymorphism (rs2243250=C-590T) in the IL-4 gene had no correlation with AR in the sample being examined due to the sample size of the subject was small. IgE is a unique immunoglobulin that plays a central role in the pathophysiology of acute and chronic allergic reactions. The SNP (rs2243250) has not been investigated in relation to AR in the Iraqi community, and this study is the first of its kind at the level of the Iraqi people.

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