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Association of IL-4 −590 (C>T) Gene polymorphism with the Levels of Serum IL-4 and IgE on the Risk of Bronchial Asthma in Babylon Province/Iraq

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ABSTRACT

Seventy-five bronchial asthma patients and fifty apparently healthy as a control group. The result of this study showed a highly significant (P≤ 0.01) elevation level of the Total IgE in the serum of asthma between patients and control, (456.45 ± 290.106 vs. 30.08 ± 24.414) respectively. The result shows a highly significant elevation differences in the level of the IL-4 in the patients and control (at P -value ≤ 0.01), (445.1978 ± 356.3931vs 54.4255 ± 19.80227) pg./ml respectively. The DNA was extracted from blood samples. Detection of genotype IL-4 SNP -590 were achieved by ARMS-PCR. The results revealed significant differences in the frequency distributions of the of heterozygous CT among case and control (0.019) at P<0.05, the result show protective role, (O. R: 0.4186; C.I.95%:0.19 – 0.88). Although there were no significant differences in wild homozygous (CC) and mutant homozygous (TT) polymorphisms between case and control, the result show rick role with asthmatic among individuals, (O. R: 1.6; C.I.95%:0.74 – 3.53) and (O. R: 2.441; C.I.95%:0.87 – 6.39) respectively, it's may include asthmatic predisposition factors in Iraqi patients. The present study shows that the presence of IgE, and IL-4 serum levels were significantly associated with genotype of IL4-590 (CC, TC, TT) among patients and control at (P ≤ 0.05).

Keyword: Asthma, Bronchial Asthma, ARAMS, IL-4, SNP.

Introduction

Asthma is a chronic inflammatory condition of the airways in which several different cells and cellular elements are involved. These result in airflow obstruction, which is often reversible either directly or with therapy asthma [1], allergic rhinitis, eczema, and food allergies are all examples of hypersensitivity type1. (which mediated with IgE) [2]. Although allergen-specific IgE can undoubtedly mediate asthmatic responses in allergy sufferers, the correlation between IgE responses and asthmatic symptoms is complicated by a number of other factors that influence airway reactivity and disease persistence [3]. Cytokines are small, unstructured, low-weight proteins with complex regulatory effects on inflammation and immunity [4].Many asthma-related genes have been linked to susceptibility, with interleukin IL-4 gene polymorphisms SNP 2227284 and SNP -590 C>T,the central role of the IL-4 pathway in atopy and asthma, primarily through increased IgE expression and Th2 cell activation, as well as contradictory reports on the role of IL-4 gene polymorphism in the disease, [5]. The objective of this study is to study the combination of IL-4-590 C>T polymorphism with susceptibility of bronchial patients with Iraqi asthma.

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Material and Methods

Study Subjects

The research was carried out between September 2020 – January 2021. Seventy-five bronchial asthma patients (clinically and laboratory confirmed) were adopted from the Specialized Center of Allergy in Babylon city, Iraq, including 26 males and 49 females with age range of (14-65) year. Fifty of healthy people as a control, including 30 females and 20 males with ages ranging from (3-40) years. The peripheral blood for DNA isolation was collected using anticoagulants EDTA tubes.

Measurement of serum IL-4 and total IgE

The levels of IL-4 in the blood were measurement by enzyme-linked immunosorbent assay (ELISA) as directed by the manufacturer (Human Interleukin 2 ELISA/ Biotechnolog-laboratory/ China). While determination of total IgE serum level was by vidas assay (Vidas Total IgE Kit/ Vidas / Korea).

Extraction of Genomic DNA

Genomic DNA of fresh whole blood collected in EDTA was isolated according to gSYNC™ DNA Extraction KIT/ Gene aid /Taiwan. The quantity and purity of the DNA, with pure DNA having a 260/280 optical density ratio of less than 1.8.

ARMS Analysis

The genotyping of IL4 590 (C>T) was done using the ARMS-PCR process.

Primers Selection

Primer sets were selected for ARMS analysis of genotyping of IL4 590 (C>T) according to [6], all the primers were synthesized at (Macrogen /Korea), showed in table (1).

Table 1. Sequences of the primers to detect of IL-4 −590 (C>T) genotyping.

Primer	Primer sequence (5'-3')	Product size(bp)
T allele	ACACTAAACTTGGGAGAACATTGTT	216
C allele	ACACTAAACTTGGGAGAACATTGTC	248
Reverse	GAATTTGTTAGTAATGCAGTCCTCC	-

ARMS Components

Several trials were to optimization of ARMS-PCR reaction according to the following the mixture table (2):

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Table 2. ARMS-PCR components (AccPower Taq PCR PreMix / Korea).

Component	Final Concentration	Volume(µL)
Master mix	1 X	12.5
Each primer (T or C allele)	10 picomols/µL	1
Primer reveres	10 picomols/µL	1
Deionized water	-	Up to 25
DNA Sample	0.05-0.15µg/µL	2
Total volume	-	25

ARMS-PCR Program:
PCR conditions for the all-reaction mixers were described in table (3):

Table 3. ARMS-PCR Program

Step	Temperature (C°)	Time (minutes)	No. of Cycles
Initial denaturation	96	1	1
First initial denaturation	96	15 sec	10
First annealing	60	50 sec	
First extension	72	40 sec	
Second initial denaturation	96	10 sec	20
Second annealing	56	50 sec	

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Second extension	72	50 sec	
Final Extension	72	7	1

Analysis of ARMS-PCR Product

Electrophoresis was used to separate the ARMS- PCR products with a ladder marker (Bioneer/Korea). The product was loaded onto a 2% agarose with a 0.5 g/ml Ethidium bromide and run at 70 volts for an hour and a half. The DNA bands were photographed using a photo documentation system after being visualized on a UV transilluminator.

Biostatistical Analysis

The SAS [7] program of the Statistical Analysis System detected differences in the analysis parameters. The program used this program. Chi-square tests were used to compare the percentage significantly (0.05 and 0.01 probability). Odd ratio and CI estimates in the present study.

Results and Discussion

The present study included blood samples collected from seventy bronchial asthmatic Iraqi patients from the Specialized Center of Allergy in Babylon city, Iraq. The study subjects were patients of ages mean of 36.85 ±13.50 SD. Additionally, 50 samples were collected from healthy individuals' subjects, as control group, with mean age of 32.06±11.77 SD, table (4).

Table 4. Distribution of bronchial asthmatic patients and control according to age categories

Age (years)	**Patient No = 70**	**Control No = 50**	**Total No = 175**
10-20	8 (10.6%)	8 (16%)	16 (12.8 %)
21-30	20 (26.66%)	19 (38%)	39 (31.2%)
31-40	15 (20%)	10 (20%)	25 (20%)
41-50	22 (29.3%)	10 (20%)	32(25.6%)
51-60	10 (13.3%)	3 (6%)	13 (10.4%)
Age (mean± SD)	36.85 ±13.50	32.06±11.77	P.value /0.3457
Significance differences (P≤ 0.05)			

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Results listed in table (4) indicated that the age of bronchial asthmatic patients and control ranged from 15-65 year, the highest percentage of the asthma cases was found in age group patients (41-50) which reached to 29.3% of the total patients, followed by 26.6% for the second age group (21-30) included 20 patients and 20% for the third age group (31-40 year) included 15 patients, with no significant difference was observed between the five age groups between patents and control. World Health Organization estimated that about 338 000 asthma deaths have been reported in 2015, and that most deaths occur in older persons more than fifty [8]. Furthermore, there are different environmental and life habits factors that could be related to this subject, work together and each one with different affect to determine the severity of asthma [9,10].

Estimation of serum total IgE and IL4

The result of this study showed in bronchial asthma patients, the mean serum level of IL-4 was significantly higher ($P \leq 0.01$) than in controls (445.1978 ± 356.39316pg/ml vs. 54.4255 ± 19.80227pg/ml) respectively. Additionally, a highly significant ($P \leq 0.01$) elevation level of the Total IgE in the serum of asthma between patients and control, (456.45 ± 290.106 vs. 30.08 ± 24.414) IU /ml respectively, table (5).

Table 5. Serum level ofIgE and IL-4 in patients and controls with bronchial asthma.

	Control (N=50) (Mean ± SD)	**Patients (N=75) (Mean ± SD)**	**P value**
IgE/ IU /ml	30.08 ± 24.414	456.45 ± 290.106	0.0000 **
IL-4(pg/ml)	54.4255 ± 19.80227	445.1978 ± 356.39316	0.0000 **
**** means high significance differences (P ≤ 0.01)**			

Total IgE have a key role in asthma and may consider a worth monitoring, the total T. IgE is well known component of allergic reactions since it is produced through the process of sensitization which is initiated in the first exposure to the allergen [11]. Re-exposure to allergens cross linked with IgE antibodies that coated mast cell, IgE mediated mast cell degranulation and released vasoactive amines such as histamine, leukotrienes and chemokines, mast cell-produced mediators have been shown to be important during the development of allergic airway diseases [12]. [13], (2011) pointed to the relationship between levels of IgE, IgM, IgG and demonstrated that the concentration of IgE was significantly increased in asthmatic patients, IgG decreased and the IgM was unchanged compared to the control group.

IL-4 has acritical role in the pathogenesis of allergic rhinitis (AR), particularly in the late phase of the disease [14,15]. Mast cells and T helper cells are the main source of IL-4 promoting the differentiation of Th2 cells and IgE production. The observed correlation between high titers of IgE and IL-4 in asthmatics is consistent with the hypothesis that IL-4 is at least partially responsible for maintaining elevated IgE synthesis in these patients [16].

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Isolation of Genomic DNA

The blood samples have been used to extract genomic DNA. The high purity sample concentrations of DNA ranged from 1,7 – 1,9 were approximately 80-200 ng/µL, figure (1).

Genotype of IL-4 −59 0 (C>T) Polymorphism

Analysis of IL-4 −59 0 (C>T) Polymorphism by ARMS-PCR Analysis

In the current study, genotyping was performed by allele-specific ARMS– PCR the polymorphism of IL-4 −59 0 (C>T) showed the presence of C allele with molecular(248bp) and T allele with molecular size (216bp) and three genotypes (CC, CT, and TT) by using the specific C, specific T, and reverse primers. This technique used specific primer for IL-4 gene fragment according to [6]. The size of PCR product was revealed by using100bp DNA ladder, Figure (2).

Distribution of Genotype and Allele Frequency of the IL-4 SNP-590 C>T

The distribution of the observed IL-4 -590 C>T genotype and alleles frequencies in the case group was heterozygous CT (42.7%), while (36.0%) for homozygous CC genotype, and TT (21.3%) table (6).

From table (6) showssignificant differencesin the frequency distributions of the of heterozygous among case and control (0.019) at P<0.05, the result show protective role, (O. R: 0.4186; C.I.95%:0.19 – 0.88) for CT genotype.While the results raveled CC, TT genotypes there were no significant differences in the frequency distributions of gene IL-4. Although there were no significant differences in wild homozygous (CC) and mutant homozygous (TT) polymorphisms between case and control, the result show rick role with asthmatic among individuals, (O. R: 1.6; C.I.95%:0.74 – 3.53) and (O. R: 2.441; C.I.95%:0.87 – 6.39) respectively.The promoter region of the IL-4 gene contains a 590C/T SNP that is understood to interact with nuclear transcription factors and regulate IL-4 expression. Specifically, the T allele has been shown to enhance the binding of nuclear transcription factors to the promoter region, ultimately upregulating the IL-4 expression [17,18,15].The substitution of C with T in the -590 position of the IL-4 promoter has been observed to increase IgE levels in patients with asthma [19,20].[21]., (2013) reported that in allergic rhinitis (AR) patients from an Iranian population in Tehran, the CC genotype in the -590C/T SNP within IL-4 gene was associated with increased risk of AR.In China, asthmatic patients with the TT polymorphism have been found to have significantly higher levels of IgE and an increased risk of AR than those with the CT/CC genotypes [18].A separate study observed a protective effect for the CC genotype when compared to the TC and TT genotypes of patients with allergic-related disorders, including AR, asthma, and atopic dermatitis [22].

Association between Genotypes of IL4-590 and with serum IL-4 and IgE Levels

The association between IL4-590 genotype and IgE, IL-4, in asthmatic patients and control were investigated. The present study shows that the presence of IgE, and IL-4 were significantly associated with genotype of IL4-590 (CC, TC, TT) among patients and control at ($P \leq 0.05$). table (7).

The severity and pathogenicity of allergic asthma are primarily determined by the innate and adaptive immune responses. IgE is well known component of allergic reactions since it is produced through the process of sensitization which is initiated in the first exposure to the allergen [18]. The IL-4 snp-590C/T polymorphism has been related to increased luciferase activity and total IgE levels [23]. According to a study conducted on atopic allergy patients in the Philippines, the IL-4 snp590C/T allele predisposes to allergy risk. In addition, increased IgE levels were associated with the IL-4 590 TT genotype [24].

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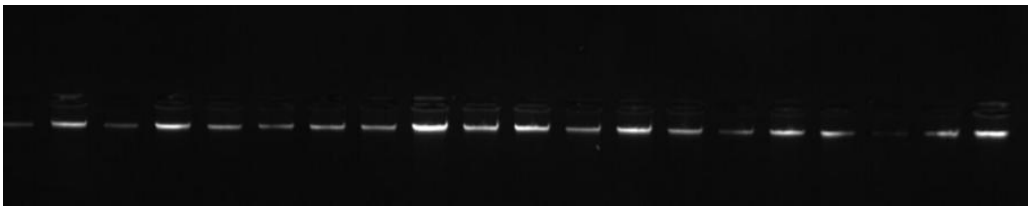

Figure (1): Gel electrophoresis for DNA human blood samples on 1% agarose gel at 70 volts for1 hour

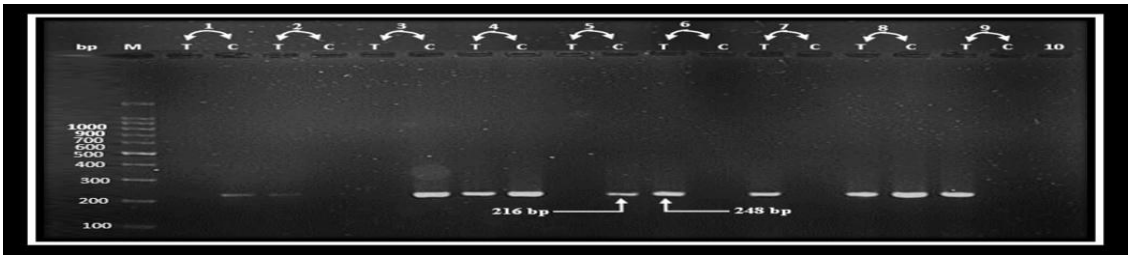

Figure (2): Electrophoresis of ARMS PCR products of IL-4 gene −590 (C>T)on 1.5 % agarose at 70 volt for one and half hour.Presence of one band in C lane and absence of this band in T lane refer to the genotype CC. In contrast, presence of one band in T lane and absence of this band in C lane refer to the genotype TT. Presence of two bands in both lanes refers to the genotype CT.)

Table 6. Distribution of genotype and allele frequency of IL-4 gene in case and control.

Genotype IL-4 SNP-590	**Case No. (%)**	**Control No. (%)**	**Sig.**	**O. R**	**C.I (95%)**
CC	27(36.0%)	13(26.0%)	0.24	1.6	(0.74 – 3.53)
CT	32 (42.7.0%)	32 (64.0%)	0.019*	0.4186	(0.19 – 0.88)
TT	16(21.3%)	5(10.0%)	0.0968	2.441	(0.87 – 6.39)
Total No.	75	50	---	---	---

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Allele	Frequency	Frequency			
T	0.426	0.42	---	----	----
C	0.573	0.58	**-----**	**----**	**-----**
***means significance differences (P<0.05)**					

Table 7. Genotype association of IL4-590-590 with IgE, IL-4,.in patients and control group

		IgE	**IL4**
Group	**IL4-590**	**Mean ± SD**	**Mean ± SD**
Patient	**CC**	493.85 ±317.991	545.3424 ±351.46989
	CT	447.44 ±289.755	350.3627 ±321.81630
	TT	411.37 ±248.099	465.8740 ±400.95095
Control	**CC**	26.46 ±24.949	51.4502 ±18.14276
	CT	32.72 ±25.222	57.5367 ±20.99130
	TT	22.60 ±18.325	42.2494 ±10.47215
P. value		**0.004***	**0.026***
***means significance differences (P ≤0.05).**			

Conclusion

The present results showed the TT and CC genotype of IL-4 -590C/T gene SNP might include asthmatic predisposition factors in Iraqi patients.

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