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Investigate The Relation Between Polymorphism of *(CTLA-4*) Gene and Asthma In Some Patients at Thi-Qar Province / IRAQ

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Abstract:

The present study was carried out at the Biology Department laboratories, for the period of (December 2017 to November 2018). The aim of the current study was to investigate polymorphism of CTLA-4 gene in patients with Asthma. The study subject consisted of patients with asthma (74 females) and (26 males) were their aged between (15-55) years, with (50 persons as control group. After DNA extraction, PCR was made by using specific primers for the *CTLA-4* gene, the study results explained the incidence of polymorphisms in a samples of 25 out of asthma patients when using the restriction enzyme (*BSTEII*). After analyzing the results by statistical analysis results showed that no association between the incidence of Asthma and appearance of mutation in *CTLA*-4 gene when compared to the control group.

Key words: *CTLA*-4, polymorphism, Asthma**.**

1-Introduction:

Asthma is a general complex disorder that is characterized by airflow obstruction, airway hyper-reactivity, persistent inflammation of the respiratory system and certain stimulants induce these signs, such as infection, exercises, occupational exposures, and allergens (Lambrecht& Hammad,2012). Asthma affects more than 23 million adults in the United State only, and females than in males (Kynyk *et al*. ,2011). Symptoms of asthma are versatile and consist of out of breath, coughing, hyperpnea, and feeling of repression in the chest (Moorman *et al.*,2012). In the previous study available evidence recommended that both genetic variations and environmental components may contribute to asthma susceptibility (Moorman *et al.*,2012). In recent times, the relations between gene polymorphisms asthma have become an important part of studies that has make essential insights into the pathogenesis of asthma (Temesi,2014). Many studies have recommended that the pathogenesis of asthma caused by interaction between environmental factors and different vulnerability genes, include air pollution, genetic variation, and allergens (Bouzigon *et al*. ,2015). T cells play essential function in asthma pathogenesis when TH1-type response to allergens and cytokines generated (Cao *et al* .,2011).Several researches have indicated that *CTLA4* acts an essential function in asthma pathogenesis (Munthe-Kaas *et al* .,2004),(Botturi *et al* .,2011) .*CTLA-4* located on chromosome 2q33 and expressed on activated T cells, polymorphism in this gene has been established to influence the development of asthma (Kawayama *et al* .,2013).*CTLA-4* plays an important role in the negative regulation of the immune ,and it is associated with TH2 cell differentiation and activation (Wang *et al* .,2015).Many researchers have identified numerous of SNP, and have documented a relationship between asthma and these polymorphisms (Yang *et al* .,2006) . SNP in *CTLA4*+49A/G linked with asthma severity and high levels of IgE which is a main factor in asthma,

and increasing the transcript *of CTLA4*, which possibly will play a role in advanced bronchoconstriction (Hizawa et *al.*, 2001) & (Lee *et al.*, 2002).

2-Materials & Methods

A total of 100 (26 males &74 females) Asthma patients their age between (15-55) years and (50) individuals as a healthy group. These samples were obtained from venous blood and put it in special tubes (EDTA), after that used to extract DNA.

DNA was extracted by using a special kit (Geneaid Biotech) according to the manufacturers protocol. Polymorphisms *in CTLA-4*+49A/G analyzed by using PCR-RFLP technique followed by RFLP method. The primers were used to amplifying in Table 1

Table (1): Oligonucleotide primer sequences used for *CTLA-4* gene amplification.

Primers	Primer sequences'
Reverse	5'-C T G C T G A A A C A A A T G A A A C C C-3
Forward	5'-A A G G C T C A G C T G A A C C T G GT-3

DNA template prepared as the follow:
- Primers (Bioneer company).
- Master Mix (Bioneer company).
- Sterilized D.W, PCR carried out with many cycles as inTable 2, after that 5U of the restriction enzyme *BstEII* incubated with 10 Ml of the pcr product (60 C^{o}) for 4 hours then run on a 3% agarose gel.

Table (2): PCR condition.

No. of Steps	Steps	Temperature	Time
1	Denaturation 1	94	5 min
2	Denaturation 2		
3	Annealing	58	30sec
4	Extension	72	30sec
5	Final Extension	72	10 min

Detecting of Deoxyribonucleic acid (DNA):

Technique is used(Electrophoresis) according to (Sambrook *et al.*,1989). The PCR products were separated on 1.5% agarose gel electrophoresis and visualized by ultraviolet light (302nm) after staining with Ethidium bromide stain. The 152 bp product was digested for 4 h at 60°C with 5 U of the restriction enzyme *BstEII*. The digested products were separated on 3.0% agarose gel.

3-Results:

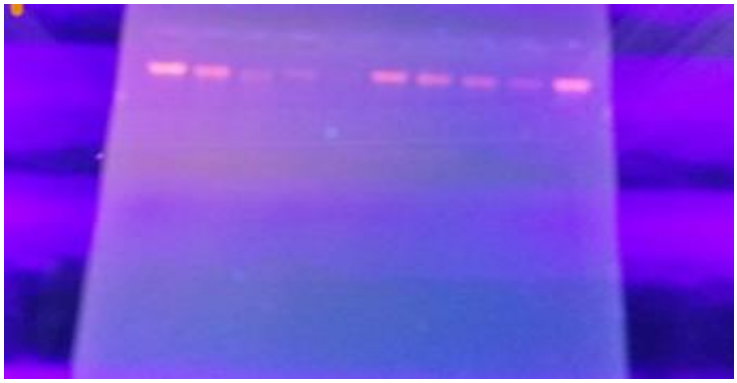

Figure (1): Eelectrophoresis of Extract DNA on a 0.8 % agarose gel.

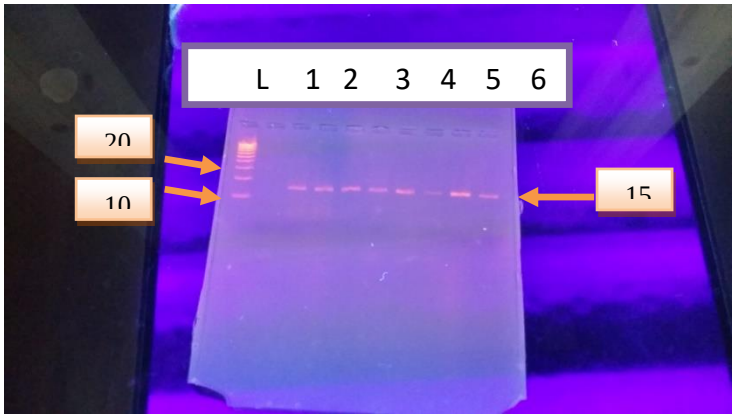

Figure (2): Electrophoresis for amplified *CTLA4* gene for asthmatic patients. Bands were fractionated on a 2 % agarose gel (80V/cm ,1 h., 1X Tris-acetic buffer), visualized under U.V after staining by ethidium bromide stain. (L:100 - 2000bp ladder); Lane 1-9 product band(152bp).

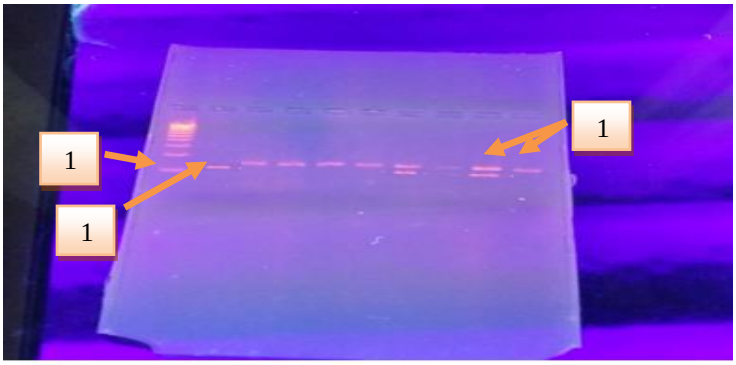

Figure (3) Electrophoresis of amplified *CTLA-4* + 49A\G gene of Asthma patients. Bands were separated on a 3% agarose gel (80V/cm ,1 h., 1X Tris-acetic) and visualized under U.V. after staining with ethidium bromide stain. (L1 :100 - 2000bp ladder) Lane: 1, 2,6,8,9 are PCR samples treated with restriction enzyme (BstEII); Lane: 3,4,5,7, Lane untreated samples Lane (6,8) represent a samples for A\G heterozygous genotype Lane (2,9) represent a samples for A\A homozygous genotype, Lane (1) represent a sample for G\G homozygous genotype.

1. **Frequency *of CTLA-4* gene genotypes.**

The results of the present study show no association between the genotypes of the *CTLA*-4gene and the incidence of Asthma, as the results showed no significant difference between healthy and patients group when genotype A\G (OR=0.9067). The genotype G\G also showed no significant difference between the two groups (OR=0.6951) as in table (3).

Table (3) : Distribution of *CTLA-4* gene genotypes for controls and patients .

Geno type	*Control*	Percentage %	Patients	percentage %	OR	95% CI
AA	34	68%	75	75%	1.0	
AG	1	2%	2	2%	0.9067	0.0795 to 10.3448
GG	15	30%	23	23%	0.6951	0.3230 to 1.4959
Total	50	100%	100	100%		

2. The distribution of *CTLA-4* gene genotypes in patient according to sex.

The results of the present study, showed there are no significant differences for sex and damage of Asthma, there were no significant difference in the A\G genotype (OR= 0.3273), while G\G genotype showed a significant difference (OR=1.7182), as seems in Table (4)

Table (**4**): Distribution of *CTLA-4* gene genotypes for the of patients group according to the sex.

Genotype	*Male*	%	*Female*	%	*OR*	*95% CI*
AA	18	18%	55	55%	1.0	
A\G	1	1%	1	1%	0.3273	0.0195 to 5.5043
G\G	4	4%	21	21%	1.7182	0.5204 to 5.6727

4. The distribution of *CTLA-4* gene genotypes of the patient according to smoking.

The results of the present study showed no correlation between genotypes in people smoking and the risk of Asthma in genotype A\G where there were not significant differences (OR=0.3273), While genotype G\G showed a significant difference (OR=1.7182), as in Table (5).

Table (5): Distribution of *CTLA-4* gene genotypes for the patients according to smoking.

Genotype	Smoking	%	Nonsmoking	%	OR	95% CI
A\A	2	2%	71	71%	1.0	
A\G	1	1%	1	1%	0.0282	0.0013 to 0.6302
G\G	2	2%	23	23%	0.3239	0.0432 to 2.4313

5. The distribution of *CTLA-4* gene genotypes in patient according to family history.
The results of the present study, showed a correlation between patients with Asthma who have a family history and genotype A\G and significant difference more than two times (OR=2.3333), While genotype G\G does not appear any significant difference(OR=1.0000) Table (6)

Table (6): Distribution of *CTLA-4* gene genotypes of patients according to the family history.

Genotype	Family history	%	Non – history	%	OR	95% CI
A\A	21	21%	39	39%	1.0	
A\G	7	7%	27	27%	2.3333	0.8311 to 6.5507
G\G	2	2%	4	4%	1.0000	0.1686 to 5.9314

4-Discussion

As Asthma is a multifactorial, polygenic disease, there are many factors involved in its incidence. These factors are both environmental and genetic. *CTLA-4* represents significant role in the T-cell regulation. In the current study demonstrated that the association between normal control group and asthmatic group as regard of the *CTLA*-4 (A/G 49 in exon 1) genotyping, there was no significant difference between control and patient's groups. This is in agreement with (Munthe-Kaas *et al.*,2004) who also did not find significant difference between control and patients groups. In addition, (Heinzmann *et al.*,2000) had denoted that SNPs in the *CTLA*-4 gene are not linked with atopy or asthma. Also (Nakao *et al.*,2000) established no relationship between atopic asthma and *CTLA*-4 gene polymorphisms in Japanese population. on the other hand, this result differs of the result of (Lee *et al.*,2002) who established that the *CTLA*-4 A/G 49 exon 1 polymorphism may has a disease-modifying in asthmatic airways.

As well as (Sohn *et al.*,2007) had found significant relations between atopic asthma and +49 A/G polymorphism in *CTLA*-4 in Korean children. *(*Yao *et al.*, 2015) suggested that *CTLA*-4 +49A/G in exon 1 polymorphism as risk factor for asthma susceptibility. Also this study showed no significant difference in genotypes of *CTLA4* gene between males and females in the genotype A\G, while genotype G\G showed significant difference between males and females. This result may be due to the subject of study that mostly females.

When we examination of the distribution of genotypes of patients according to smoking results shows statistically non-significant differences between smokers and non-smokers for individuals who have genotype G\G, also genotype A\G showed no significant difference, perhaps this result occurs because of the largest number of our subjects are female and our customs and traditions doesn't allow to the female to smoke or they hide a true information.

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