Cysteine leukotriene receptors type 2 (CyLTR2 M01 V) gene polymorphism and its role in Iraqi asthmatic patients

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***Abstract* :-**

Background: Asthma is currently understood to be a fairly common but complicated chronic respiratory disease with multiple etiologies (Buttaro etal .,2012 and Zhang etal .,2013).Polymorphism in the CysLTR2 gene resulting in a single amino acid substitution, M201V (i.e. amino acid methionine changed for valine at the 201 position of CysLTR2 protein).

Objectives:-The aims of this study included analyzing of polymorphism in CyLTR2 M01 V genes and determined whether there is an interaction and association between these polymorphisms and some parameter (total IgE ,IL-5 ,Eosinophil)for asthma development in Iraqi asthmatic patients

***Methods*:** 100 patients with asthma with ages ranged between (15- 50) years were taken from (Al-Hussein Medical City/Kerbala).Control group consisted of 60 healthy people who were free from signs and symptoms of Asthma who matched in age and gender with patients, and had no history for any asthma problem. Total IgE and IL-5 Euroimmun /Ggermen ,Peprotech UK respectively) was studied using the enzyme-linked immunosorbent assay (ELISA) method and automated blood cell counter (Sysmex XT-200i)for eosinophil counts ,the CyLTR2 M01 V also studied by using RFLP PCR . T-test and ANOVA and Pearson correlation used to analyze results by using SPSS version 20. P-value ≤ 0.05 was considered significant.

***Results*:** Total IgE ,IL-5 levels and Eosinophil counts were increased significantly ($p< 0.05$) in patients compared with control group. Also there were show significant abnormality and complication when compared with control groups, there Regarding to CyLTR2 M01 V gene polymorphism were done by RFLP PCR there is no statistical difference between control and asthmatic patients. also there are a statistically significant difference in serum IgE . serum IL-5 and blood eosinophil between each two groups depending on polymorphism in cysteine leukotriene receptor 2 (CyLTR2 M01 V).

***Conclusion* :** there are a significantly correlation between total IgE ,IL-5 levels and eosinophil counts and polymorphism in cysteine leukotriene receptor 2 (CyLTR2 M01 V)in

asthmatic patients . but CysLTR1 927A/T gene polymorphism no show statistical difference between control and asthmatic patients.

Key words :- Asthma , gene Polymorphism ,CysLTR2

Introduction

Asthma is one of the most common respiratory disorders in clinical practices, affecting up to 13% of people worldwide (Kamble et al.,2009). Most cases of asthma are atopic in nature, with trigger for acute asthma attacks and chronic worsening of inflammation being allergens inducing an immune response through immunoglobulins of IgE class (Ammato et al.,2010).

Asthma is characterized by frequent episodes of whistling or wheezing sound, shortness of breath, chest pain, and persistent coughing. The cough may be dry or mucous containing; sputum may be expelled from the lungs by coughing but is usually hard to bring up (Jindal etal.,2017). These symptoms might worsen at night or after exercise, and may also develop in response to different triggers, such as cold air or exposure to allergens (Janssens etal.,2013).Cysteine leukotriene Leukotrienes (LTs) are considered to assume a significant role in the pathomechanism in a lot of diseases, such as bronchial asthma, ,allergic rhinitis (Poff etal.,2004, Pergola etal.,2010). In the some investigation have demonstration that an increasing generation of the CysLTs in asthma contributes significantly to exacerbations of asthma symptoms (Kanaoka etal., 2014).

Cysteine leukotriene receptor type 2 mRNA is co-communicated alongside CysLRR1 in human blood eosinophils and platelets, and tissue mast cells, macrophages, airway route epithelial cells, and vascular endothelial cells. It is additionally communicated without CysLTR1 all through the heart, including Purkinje cells, adrenal organ, and mind and additionally some vascular endothelial, airway route epithelial, and smooth muscle cells.(Zhang etal., 2006, Singh etal.,2013, Kanaoka etal., 2013, Bankova etal.,2014 ,Liu etal.,2015 and Cattaneo, 2015)

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Patients and Methods

Selection of patients

During the period 1/ march /2017 to 1/November /2017, 100 patients with Astma (with ages ranged between (15-50) years were taken from (Al-Hussain Mediacl City/Kerbala. Control group consisted of 60 healthy people who were free from signs and symptoms of asthma who matched in age and gender with patients, and had no history for any asthma problem.

Sample collection and assay procedure

Blood sample (5ml) was collected from patients and the separated for two tubes ,one of the EDTA tube for PCR analysis and the other in plain tube then left at room temperature and then centrifuge

for 10 min. at (3500 rpm). Serum was then separated and preserved at -70c until time of analysis. Total IgE and IL-5 Euroimmun /Ggermen ,Peprotech UK respectively) was studied using the enzyme-linked immunosorbent assay (ELISA) method and automated blood cell counter (Sysmex XT-200i) for eosinophil counts in serum of patients ,The CyLTR2 M01 V also studied by using RFLP PCR .using commercially available and performed as recommended in leaflet of the kits .

Statistical Analysis :Results are expressed as mean ± standard error mean (SEM), student t-test and ANOVA and Pearson correlation used to analyze results by using SPSS version 20. P-value ≤ 0.05 was considered significant.

Results :-

A total of 100 patients with asthma divided into two groups according to the gender the males patients represented 48 %and females groups represented 52% .

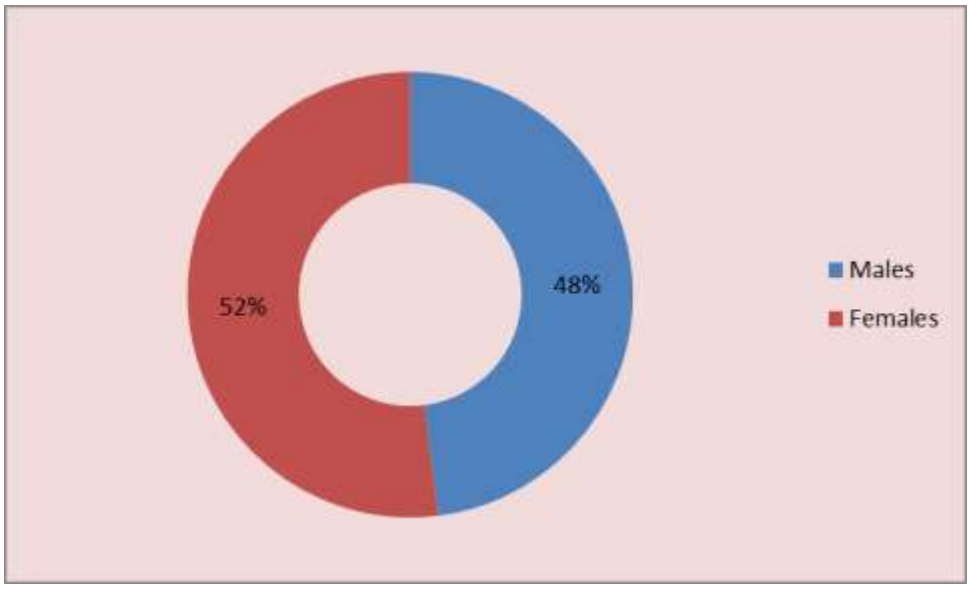


Figure1 : Demographic distribution of subject

The results of table two recorded statically significant difference in three parameters between patients groups and health cases groups .

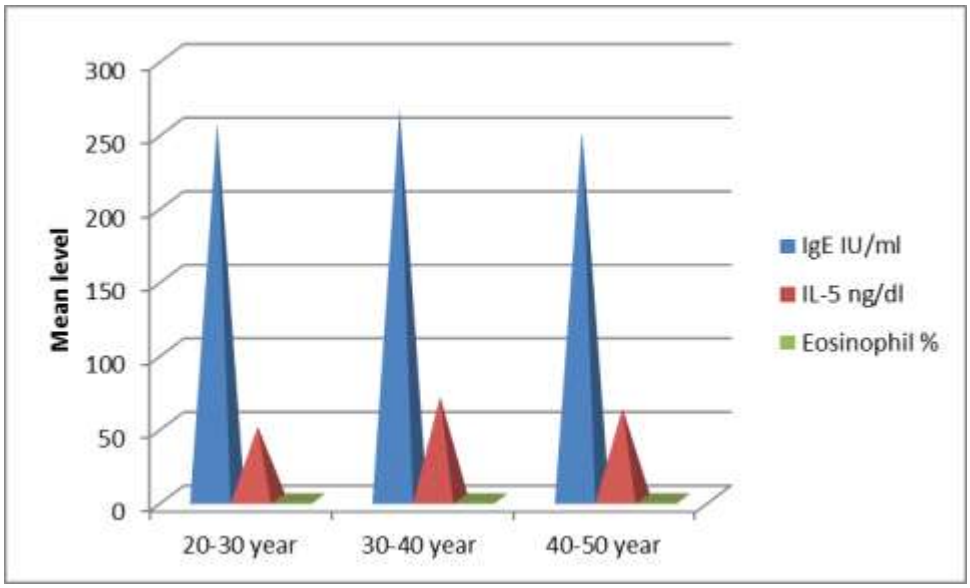


Figure 2:-Differentiation in means of selected outcome measurements between the three age groups of Asthma cases.

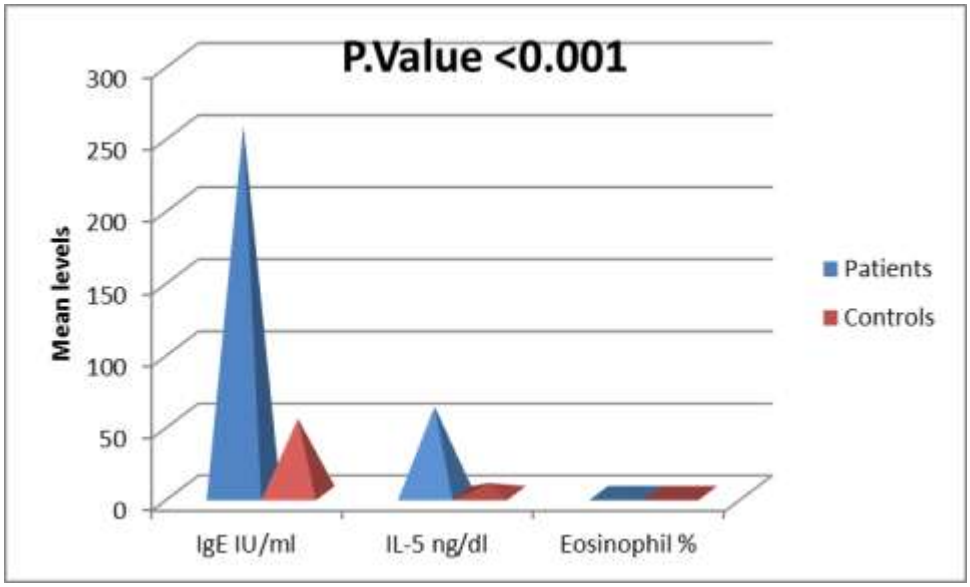


Figure 3 : Distribution of three parameters in control and Patients with asthma .

The results of figure (4) included the polymorphism in cysteine leukotriene receptor gene (CyLTR2 M01 V) in patients and controls groups ,In this results we found that the AG genotypes frequency represented high present and more frequents (60%) ,AA (20%) ,and less frequency in genotypes GG(20%).

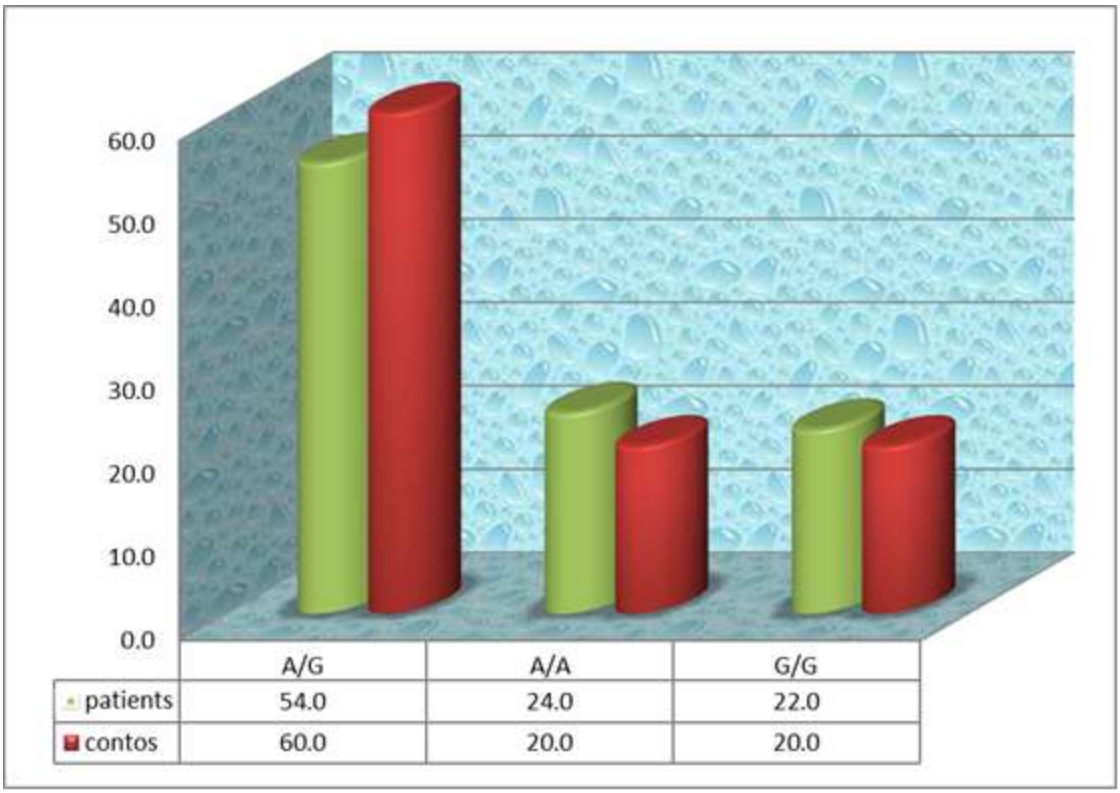


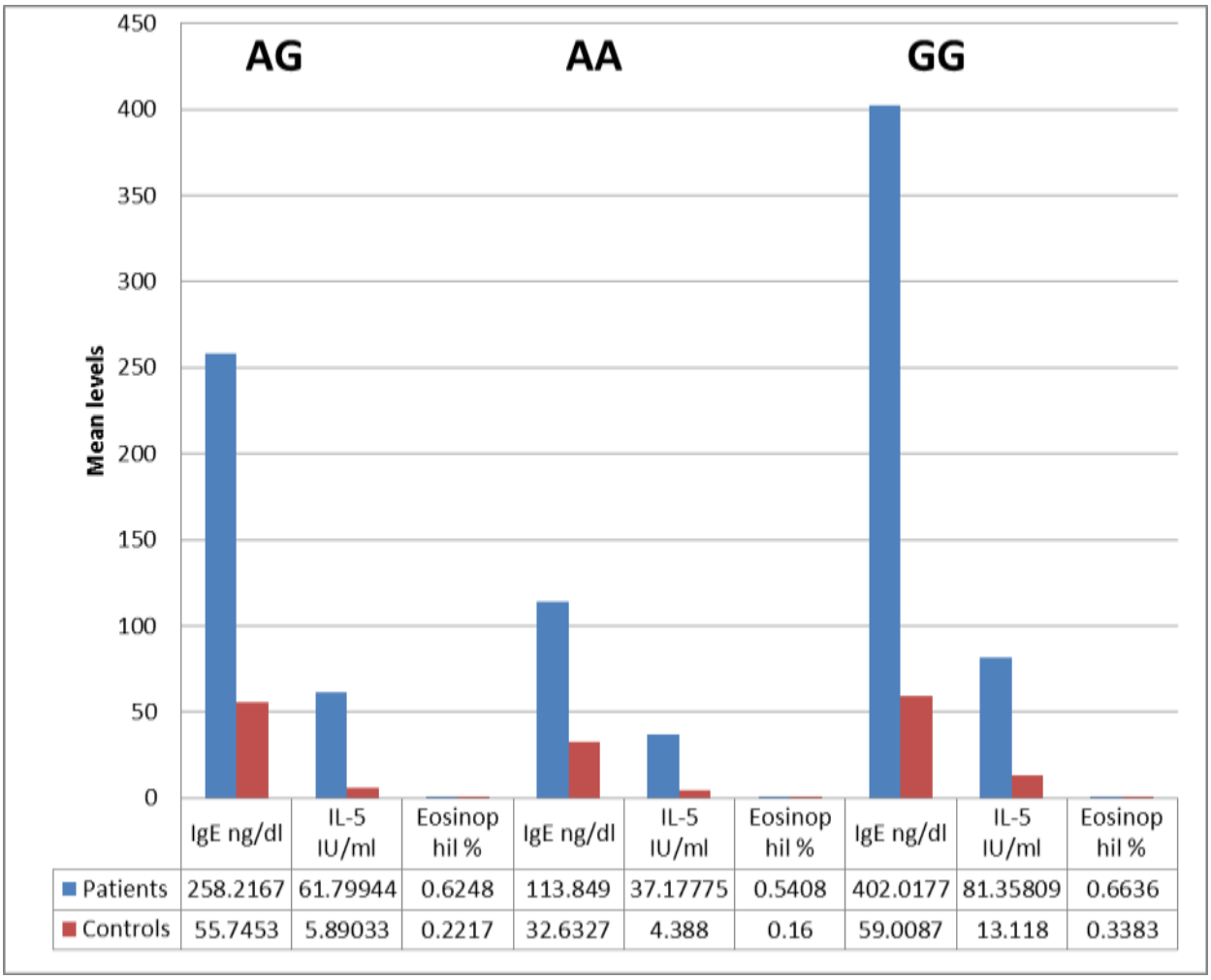
Figure (4) Distribution of percent of Cysteine leukotriene receptors gene type 2 (CyLTR2 M01 V)polymorphism in asthmatic patients and controls groups.

This figure 5 show the results of total serum immunoglobulin IgE levels ,Serum IL-5 levels ,And peripheral blood Eosinophil in asthmatic and control groups according to Cysteine leukotriene receptor 2(CyLTR2 M01 V) gen polymorphism.

These mean levels of serum IgE and serum IL-5 consider statistically highly significant difference among the three Cysteine leukotriene receptor 2gene morphology (AA ,AG, GG) when camper the levels of these parameters between asthmatic cases and health control group.

The mean of blood eosinophil recorded the statistically significant difference in asthmatic patients and healthy control group those having AG cysteine leukotriene receptor 2(CyLTR2 M01 V)

Figure (5)Distribution of three parameters among asthmatic patients & control group according to difference in cysteine leukotriene receptor 2 (CyLTR2 M01 V) genotypes.



	IgE ng/dl	IL-5 IU/ml	Eosinophil %	IgE ng/dl	IL-5 IU/ml	Eosinophil %	IgE ng/dl	IL-5 IU/ml	Eosinophil %
Patients	258.2167	61.79944	0.6248	113.849	37.17775	0.5408	402.0177	81.35809	0.6636
Controls	55.7453	5.89033	0.2217	32.6327	4.388	0.16	59.0087	13.118	0.3383

Discussion:-

The present study conducted at Al- Hussein Medical City / Kerbala , included 100 asthma disease cases and 60 control subjects. Asthma characterized by the IgE-dependent release of mast cell-derived mediators and cellular infiltration particularly of activated eosinophils and T-lymphocytes [11]. IgE play an important role in mediate type-1 hypersensitivity reactions, lead for contribute to the pathogenesis of allergic diseases including asthma[12].The table one recorded majorty of asthmatic cases occur in females than in males and this agreed with [13]who reported the asthma more common in females. There are significant differences in the levels concentration of (T-IgE) among different age groups in asthma, where recorded (30-39 years) give the highest level in camper to other groups which represent increase activity and contact with environmental allergens, these findings are in agreement with the results of [14], in Iraq, who found patients with detectable levels of serum total IgE (>100 IU/ml) gradually increased with age, with a maximum being observed in the 31-40 year old group. In this study the results show an increase in the concentration

of IL-5 and eosinophils in asthmatic patients when this results compared with control group ,the explanation of this results , the immune response in patients with asthma characterized by the increase level of Th2 cell [15] ,Th2 produce a numbers of inflammatory cytokines including IL-5, that play many effects on other cells have an effective role in the pathogenesis of asthma like eosinophils [16] , the genetic variables that associated with asthma ,that difficult to distinguish these variables consider as protective factor for some populations and risk factors for others peoples. That can explain by study done by an endoganous isolation affected by a high frequency of asthma .(Wysocki 2011)) that find the CYSLTR2 M201V SNP was related with some of atopy like sensitization to cockroach, Now we found a possibility of functional CYSLTR2 M201V variants may associated with asthma in causative or protective manner in different population. gene polymorphism variant of CysLTR2 has been associated significantly with development of asthma in a Japanese population; the impact of this polymorphism on the genes expression or product has not been determined.(Fukai etal., 2004) These results suggest that CYSLTR2 contributes to the etiology and development asthma and that drugs targeting CYSLTR2 may work in a manner that differs from those of CYSLTR1 antagonists.(Fukai etal., 2004)

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