Polymorphism in genes coded for cells surface receptors(cysteine leukotriene receptor type I) among Iraqi asthmatic patients

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***Abstract* :-**

Background: Asthma is a heterogeneous disease ,in which family of potent inflammatory lipid mediators called cysteine leukotrienes , these mediators bind specifically with cells surface receptors (G- proteins-coupled receptors) that include CysLT1.the genes polymorphism in cysteine leukotriene receptors 1 927 T/C have been implicated in susceptibility to asthma .In this study was to analyze the different single nucleotide polymorphism and to determine whether there is an association between these polymorphisms and asthma development.

Objectives:-The aims of this study included analyzing of polymorphism in CysLT1 927 T/C 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 CysLT1 927 T/C 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 CysLTR1 927A/T 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 1 (CysLR1 972 T/C).

Conclusion **:** there are a significantly correlation between total IgE ,IL-5 levels and eosinophil counts and polymorphism in cysteine leukotriene receptor 1 (CysLR1 972 T/C)in asthmatic patients . but CysLTR1 927A/T gene polymorphism no show statistical difference between control and asthmatic patients.

Key words :- Asthma , Polymorphism ,CysLTRI

Introduction

Asthma affects about 300 million people in world and included chronic inflammation of airways .which consider groups of various phenotypes also can be classified according to clinical severity ,age of onset ,and differentiation in immune response (T2-associated and non –T2 asthma[Wenzel SE ;2012].T2-associated asthma is associated with release of IL-5 and IL-13,assistance of eosinophilic infiltration , pro-inflammatory loops, proliferation of epithelial cells ,metaplasia of goblet-cells and ciliary beating alterations .Non-T2 asthma usually more related with neutrophilic and mixed of T17 and T1 cytokine milieu.[Lambrecht BN;*etal* 2015]

Leukotrienes (LTs) mediators play an important activity in developments of the pathomechanism in different types of diseases, such as(bronchial asthma, allergic rhinitis, psoriasis, rheumatoid arthritis, osteoporosis, as well as cardiovascular diseases, neurodegenerative diseases and certain types of cancer) [Harris R.R ;*etal* 1995, Poff C.D ;*etal* 2004 , Pergola C *etal* ;2010] that usually occur and consider as one of the substantial pathways of LTs production is binding to the oxidation of arachidonic acid (AA) by 5-lipoxygenase (LOX) using 5-lipoxygenase-activating protein (FLAP) that lead to increases the affinity of 5-LOX to AA [Peters-Golden M,2007]. 5- LOX converts AA to leukotriene A4 (LTA4), and finally enzymatically transformed into leukotrienes C4 (LTC4), D4 (LTD4) and E4 (LTE4). This category of LTs is called cysteinyl leukotrienes , in away from leukotriene B4 (LTB4) that formed from LTA4 by LTA4 hydrolase [Meirer K;*etal* 2007]these leukotriene's bind specifically to two receptors included CysLT1 and CysLT2 receptors are wildly expressed by immunological and hematopoietic cells. Some cell types (vascular smooth muscle) express specifically the Cys-LT1 receptors [Heise CE;*etal* 2000], otherwise others (endothelial cells) dominantly express CysLT2 receptors [Hui Y;*etal* 2001]. Both receptors are expressed on the cells of the innate (macrophages, monocytes, eosinophils, basophils, mast cells, dendritic cells) and on cells of adaptive (T cells, B cells) immune system, play role in functions of immunity and inflammation [Kanaoka Y;etal 2004].

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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 CysLT1 927 T/C 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% .

Table1: Information of Patients with asthma and control groups .

	patients		Controls	
Gender	**N**	**%**	**N**	**%**
Male	**48**	48.0	28	46..7
Female	**52**	52.0	32	53.3
Total	100	100.0	60	100.0

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

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

	Age			
	<30 y	**31-40 y**	**>40 y**	**P**
IgE				0.980 [NS]
Range	(10.65 to 752.88)	(20.52to 833.30)	(15.40to 689.60)	
Median	241.54	232.19	278.56	
Inter-quartile range	(33.53 to 400.40)	(31.65 to 434.20)	(83.68 to 367.05)	
N	26	24	50	
Mean=	255.56	265.32	250.18	
IL-5				0.088 [NS]
Range	(30.12 to 121.76)	(23.58 to 110.34)	(24.53 to 93.99)	
Median	41.83	65.88	60.52	
Inter-quartile range	(38.54to 52.44)	(49.77 to 93.86)	(41.77 to 79.32)	
N	26	24	50	
Mean=	49.12	69.52	61.47	
Eosinophil				0.002 [HS]
Range	(0.13 to .96)	(0.41 to 1.41)	(0.12 to 1.23)	
Median	0.56	0.87	0.52	
Inter-quartile range	(0.47 to 0.62)	(0.58 to 1.09)	(0.40 to 0.63)	
N	26	24	50	
Mean=	0.53	0.86	0.54	

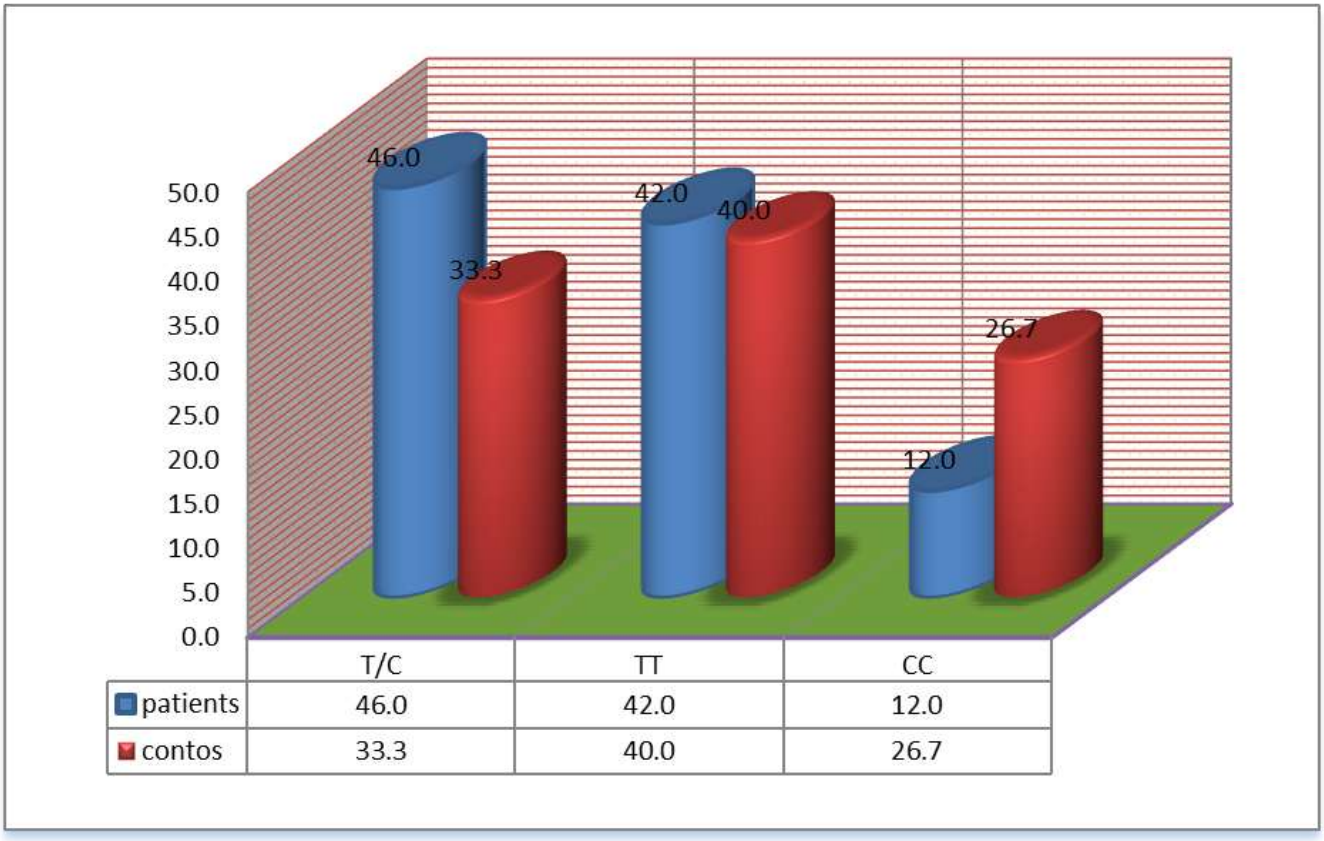
Table 3: Distribution of three parameters in control and Patients with asthma

	groups	**N**	**Mean**	**Std. Deviation**	**Std. Error Mean**	**P-Value**

IgE	Patients	100	255.2047	208.90848	29.54412	.000
	controls	60	51.7754	27.33165	4.99005	
IL-5	patients	100	60.19314	23.588299	3.335889	.000
	controls	60	7.03540	3.670064	.670059	
Eosinophil	patients	100	.6132	.28590	.04043	.000
	controls	60	.2327	.07674	.01401	

The results of figure (1) included the polymorphism in cysteine leukotriene receptor gene (CyLTR1 972 T/C) in patients and controls groups ,In this results we found that the T/C genotypes frequency represented high present and more frequents (46.0%) ,TT (42. 0%) ,and less frequency in genotypes CC(12.0%).

Figure (1) Distribution of percent of Cysteine leukotriene receptors gene type 1 (CyLTR1 972 T/C)polymorphism in asthmatic patients and controls groups.



This table 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 1(CysLR1 972 T/C) gen polymorphism.

These mean levels of serum IgE and serum IL-5 consider statistically highly significant difference among the three Cysteine leukotriene receptor 1gene morphology (T/C ,TT, C/C) 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 T/C cysteine leukotriene receptor 1(CysLR1 972 T/C)

Table (4)Distribution of three parameters among asthmatic patients & control group according to difference in cysteine leukotriene receptor 1 (CysLR1 972 T/C) genotypes.

Group Statistics								
CysLR1 positive		**groups**	**N**	**Mean**	**Std. Deviation**	**Std. Error Mean**	**F-test**	**P-value**
TC	**IgE**	patients	46	243.09	205.92493	42.93832	16.34	**0.000 .HS**
		controls	20	56.237	30.47604	9.63737		
	IL-5	patients	46	56.206	25.464430	5.309701	17.50	**0.000 .HS**

		controls	10	8.0872	3.836939	1.213347		
	Eosinophil	patients	46	.5422	.31445	.06557	5.558	**0.025.S**
		controls	20	.2450	.06852	.02167		
TT	**IgE**	patients	42	309.58	215.84235	47.10066	10.79	**0.003.HS**
		controls	24	52.3945	32.32188	9.33052		
	IL-5	patients	42	66.815	23.115736	5.044267	14.18	**0.001.HS**
		controls	24	6.18142	3.818082	1.102185		
	Eosinophil	patients	42	.6638	.26183	.05714	11.84	**0.002.HS**
		controls	24	.2133	.07572	.02186		
CC	**IgE**	patients	12	111.29	129.67612	52.94005	42.59	**0.000.HS**
		controls	16	45.2691	13.47139	4.76286		
	IL-5	patients	12	52.299	11.413595	4.659580	9.627	**0.009.HS**
		controls	16	7.0016	3.336684	1.179696		
	Eosinophil	patients	12	.7083	.21665	.08845	10.84	**0.006.HS**
		controls	16	.2463	.09102	.03218		

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 [Foley, S.;etal 2009]. IgE play an important role in mediate type-1 hypersensitivity reactions, lead for contribute to the pathogenesis of allergic diseases including asthma[Manohar, S.;etal 2012].The table one recorded majorty of asthmatic cases occur in females than in males and this agreed with (Kalpaklioğlu et al (2008) 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 [Brakhas, S. A etal 2015], 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 (Akdis *et al*.,2004) ,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 (Jacobsen *et al*.,2012) , Regarding to CysLTR1 927A/T gene polymorphism were done by RFLP PCR there is no statistical difference between control and asthmatic patients and these result was agreed with Kadry *et al*;2014. The CysLTR1 927T/C variant have been associated with asthmatic patients in population (Hong *et al*;2009) .The polymorphism occur represented by this SNP was also found in Tristan da Cunha population (Thompson *et al*;2013),Usually not replicated in all studies about atopic asthma(Kadry *et al*;2014).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 1 (CysLR1 972 T/C) .May study demonstrated that the expression of CysLTRs in eosinophils was not unexpected (Bandeira-Melo and Weller, 2003) that due to the contribution of cysteinyl LTs to their accumulation in airways of asthmatic patients.

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