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SINGLE NUCLEOTIDE POLYMORPHISM OF *IL9* + 176 (RS1799962) IN A SAMPLE OF ASTHMA IN IRAQI PATIENTS

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ABSTRACT : The present study aimed at investigating of frequency of polymorphism of interleukin-9 gene (IL9) at position$_{176+}$SNP) in and in healthy controls subjects beginning of the month October (2020) to the end of March (2021), The study included 74 patients suffering from asthma with an average age of (43.16 ± 2.14), the number of males was (26) and the percentage of (40.6 %) and the number of females was (38) and the rate of (59.4 %), while the control group included (26) apparently healthy people with a rate of My age is (38.35 ± 2.22), if the number of males is (21) with a percentage (80.8%) and the number of females is (5) with a percentage (19.2%) were enrolled in this study, the polymorphism of IL9$_{176+}$ was data waved by polymerase chain reaction-specific sequence primer (PCR-SSP) assay. The results showed a comparison *IL9*$_{176+}$ genotypes & alleles between asthma patients & controls frequencies of CC genotype & C allele 21.67, 20.0%) P = 0.219, respectively). There was a significant increase in patients contrast to controls, (7.5 *vs*. 5.0%; P = 0.093) and associated EF rates were 4.75 & 3.41 respectively. And the In contrast TT genotype & *T allele* (%76,67 *vs*. 78.33%, P = 0.258, respectively). Low frequency was observed in patients compared to healthy subjects (90.00% *vs*. 92.50%; P = 0.093) and the related PF values were 0.37 & 0.29, respectively. Similar observations were made in TC genotype. The study results indicate that the IL9+176 SNP may have a role in events and development of asthma in Iraqi patients.

Key words : Single nucleotide polymorphism, *Interleukine-9* asthma.

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INTRODUCTION

Asthma is one of the allergic diseases and it is a chronic inflammation that affects the airways in the lungs, and it occurs due to the presence of severe sensitivity of the airways in people who suffer from it, in exchange for multiple irritants and allergens when exposed to them. When this inflammation occurs, changes occur in the lungs, where the air ducts narrow to the contraction of the muscles in the bronchial wall and contract and the secretion of mucous material from the bronchi increases, and the membranes lining them swell (Rawi, 2013; Athari, 2019). Most deaths from it occur in poor countries (WHO, 2020). The prevalence of asthma is higher in young children than in adults, as well as higher in females than males, as the number of asthmatic patients in 2015 globally exceeded 358 million people, compared to 183 million people in 1990 and caused the death of about 397,100 in 2015 (Enilari and Sinha, 2019; Global, 2019 and World Health Organization, 2020). Asthma is the most common disease in the West, affecting more than 5 Millions of people are England and Wales alone. The disease affects male children more than females, but affects women slightly more than men among adults (Al-Quraydi, 2020). Asthma is a complex disease with significant genetic predisposition. To date, nearly 100 asthma-related genes have been identified using GWAS, such as IL33 on chromosomal region 9p24, HLA-DR/DQ on 6p21, IL1RL1/IL18R1 on 2q12 and IL13 on 5q31. HLA class II molecules and most of the asthma-prone genes found to date to be involved in immune and inflammatory processes, which are also associated with allergic and hyper responsive airway diseases, reflecting the association between asthma and other diseases at the genetic level. They are involved in triggering asthma and

promote chronic airway inflammation. These genes encoding cytokines are an important type of asthma-susceptible gene and their polymorphisms influence the severity of asthma. Currently, targeted cytokine drugs are being used as new treatments for asthma (Huo and Zhang, 2018). The airway inflammation underlying asthma is regulated by a network of mutually interacting cytokines that activate the inflammatory process, whereby immune cells produce cytokines important for the inflammatory process, and Th2 helper cells play an important role in this process, as they secrete two types of interleukins, IL12, IL-12, IL-5 and IL-4 have an essential role in the initial sensitization process to allergens as well as stimulating airway eosinophilia (Zhou *et al*, 2001) and IL-5 is involved in the activation and transport of eosinophils to the airways leading to bronchitis (Huo and Zhang, 2018; Athari, 2019). Interleukin-9 was first defined the late 1980s as a member of a growing number of cytokines that had pleiotropic effects within the immune system (Goswami and Kaplan, 2011). IL-9 has been considered a Th2 cytokine and has been implicated in asthma and parasitic infections. Within the year 2008, a T helper cell subset was specific to form IL-9 and these cells were called the Th9 cells (Saeki *et al*, 2019). IL-9 is a significant agent in the hypersensitivity response to allergic asthma (Jebur *et al*, 2020).

MATERIALS AND METHODS

Study samples

The current study was conducted in the consultative clinic for respiratory diseases in Diyala governorate for a period from the beginning of the month October (2020) to the end of March (2021). The study included 74 patients suffering from asthma with an average age of (43.16 ± 2.14), the number of males was (26) and the percentage of (40.6%) and the number of females was (38) and the rate of (59.4%), while the control group included (26) apparently healthy people with a rate of My age is (38.35 ± 2.22), if the number of males is (21) with a percentage (80.8%) and the number of females is (5) with a percentage (19.2%). All cases were chronically ill with asthma, and all private information was recorded patients and healthy people according to a form that included (name, age, sex, date of injury, type of disease, diseases chronic) and the samples were numbered according to the above information.

Detection of IL9 polymorphism

Genomic DNA was extracted from EDTA blood using G- Spin TM Total DNA Purification Kit (G- Spin, USA) followed by electrophoresis on 2% agarose-gel by CTS-PCRSSP Tray Kit (Macrogen, USA).

Amplification of IL9 gene

The polymerase chain reaction (PCR) technique was used to amplify the promoter region of the IL9 gene, as a nucleotide primer designed by Dr. Ibtisam Badday Hassan and sent to Macrogen.

Statistical analysis

Genotypes of $IL9_{176+}$ SNP were presented as percentage frequencies; these estimations were calculated by using the WINPEPI computer programs for epidemiologists. The latest version of the WINPEPI package is available free online at http://www.brixtonhealth.com

RESULTS

SNP of IL9gene was determined in the promoter region at position +176 (IL9+176 SNP), it was presented with three genotypes (TT, TC and CC) that correlate with two alleles (T and C). Among asthma patients, no significant difference was observed between the observed and expected frequencies of the three genotypes (a good agreement with Hardy-Weinberg equilibrium; HWE), However, comparing patients to controls results some significant differences (Table 2). The results of the current study showed, as shown in Table 2, that the homozygous genotype CC and C allele recorded a significant increase in the group of patients with asthma, according to the mentioned percentages (20.0% and 21.67%), respectively, compared to the control group (the healthy ones). As follows (5% and 5%) according to the Fasher probability (P=0.219 and P=0.093) among patients compared to the control. As shown in Table 3, the homozygous genotype CC and C allele are considered a causative factor (4.75 and 3. 41) with a risk ratio of (15.8% and 15.7%), respectively, as shown in Table 3. While, the homozygous genotype TT and allele T recorded a significant decrease in patients according to the value (3.33% and 67.12%), respectively. Compared to the control group, which scored (5% and 7.5%) respectively, according to the Fisher probability (P=1,000 and P=0.093),

Table 1 :

Primer name	(5' - 3') Seq	Product size	Annealing temp. (°C)
rs1799962- F	CCTTCGTTAGAACACCCATGA	176+ bp	57
rs1799962-R	AGACAGGGATTCTGGTGTGA		

Table 2 : Numbers and percentage frequencies and Hardy-Weinberg (H-W) equilibrium of (IL9+ 176 rs1799962 genotypes and alleles) in asthma patients and controls.

	Groups		IL9+ 176 genotypes and alleles					H-W X2 P ≤
			TT	TC	CC	T	C	
Asthma patient (s=30)	Observed	No.	23	1	6	47	13	N.S.
		%	76,67	3,33	20,0	78.33	21.67	
	Expected	No.	18.41	10.18	1.41			
		%	61.36	33.94	4.69			
Control (s = 20)	Observed	No.	18	1	1	37	3	0.0042
		%	90	5	5	92.5	7.5	
	Expected	No.	17.11	2.78	0.11			
		%	85.56	13.88	0.56			

Table 3 : Statistical analysis of associations between (IL9+ 176 rs1799962 genotypes and alleles) in asthma patients and controls.

Statistical evaluation				Fisher's Exact Probability	95% confidence intervals
Type of comparison	IL9+ 176 genotypes and alleles	Relative Risk	Etiological or Preventive Fraction		
Diabetes type 1 disease Versus Controls	TT	0.37	7.15%	0.285	0,07-1,90
	TC	0.66	1.7%	1.000	0.04 – 10,47
	CC	4.75	15.8%	0.219	0.55 – 40,71
	T	0.29	65.4%	0.093	0.08 – 1,09
	C	3.41	15.3%	0.093	0.92 - 12,66

so the TT genotype and the T allele are considered a protective factor or protection from disease, as it reached 0.66) and 0.29), respectively. Also, the T C genotype showed a decrease in patients compared to the control (P = 1.000, 3.33 vs 5.0). It is also considered a protective factor or protection from disease.

The current study did not agree with study of children with asthma showed that serum levels of IL-9 were significantly higher in the patient group. Patients with TT and TC genotypes at rs2069882 had significantly higher levels of IL-9. In addition, patients with severe asthma had significantly higher levels of IL-9 in their blood. That genotypes in single nucleotide polymorphisms can influence serum levels of IL-9 in asthmatics, which in turn can influence its severity (Mahneh *et al*, 2016). The genetic map study of asthma patients found a close relationship between the interleukin 9 genes and the severity of asthma, because it showed interleukin 9 as an important growth factor and stimulator of cell types important in the pathogenesis of asthma (Zhou *et al*, 2001).

The current study agreed with the study (Sordillo *et al*, 2015), which indicated that people with the dominant genotype (rs11741137, rs2069885 and rs1859430) of these IL9 polymorphisms are more likely to have severe asthma exacerbations.

The current study did not agree with a study conducted by the researcher (Chen *et al*, 2020). Which indicated that IL9 for locus 2066758rs in homozygous genotype CC and allele C recorded a significant decrease in asthma patients.

The current study did not agree with the study conducted by the researcher (Fatahi *et al*, 2016), which indicated that the IL 9 of rs731476 in the AA homozygous genotype has a protective effect or protection against the risk of developing allergic rhinitis in women in Iran because of its low frequency in infected patients found that IL9 single nucleotide polymorphisms (SNPs) showed different genetic and allelic distributions among patients compared to healthy controls, which confirms the existence of a significant association between mechanisms and genes in disease development (Fatahi *et al*, 2016).

This study is the first in terms of the IL 9 genotype of the site rs1799962 in asthmatic patients in Iraq, where he studied other genetic sites not studied in the current study, which made it difficult for us to discuss the results of our current study because there was no similar study for the same genetic site, the current study concluded

the role of IL-9 in the events and development of asthma in Iraqi patients and this conclusion is the result of international studies between IL 9 and genetic single nucleotide polymorphisms, which indicated its role in the exacerbation and severity of asthma.

One study found revealed the severity of the disease in mice with asthma, which was associated with increased secretion of IL-9 by ILC2s. This led to the activation of dendritic cells (DCs) that can accelerate Th2 cell differentiation and exacerbate asthma (Wan *et al*, 2020). Recently, it is known that different subsets of CD4+ T cells Th17 and Th9 as well as innate immune cells, Such as mast cells and innate type 2 lymphocytes (ILC2s), which can all produce the modern cytokine detected by IL-9. For this disease, IL-9 is a pleiotropic cytokine that affects different and distinct functions of different target cells such as T cells, B cells, mast cells, and airway epithelial cells by activating STAT1, STAT3 and STAT5. Due to its multidirectional functions, IL-9 has been shown to be involved in many diseases, such as cancer, autoimmune diseases and other diseases regulated by the pathogen-mediated immune system and the role of Th9 and IL-9-producing cells in allergic asthma (Zhou *et al*, 2012; Mahneh, 2001; Koch *et al*, 2017; Do-Thi *et al*, 2020; Wan *et al*, 2020).

CONCLUSION

The results of the current study suggest that the IL9 + 176 (rs1799962) SNP may have a role in the events and development of asthma in Iraqi patients. the homozygous genotype CC and C allele recorded a significant increase in the group of patients with asthma are considered a causative factor. While the homozygous genotype TT and allele T recorded a significant decrease in patients are considered a preventive factor or protection from disease. The TC genotype showed a decrease in patients compared to the control. It is also considered a preventive factor or protection from disease.

REFERENCES

Al-Quraydi Munira Ammar Mahmoud (2020) The prevalence of asthma and some physiological changes accompanying him to patients at Sabratha Hospital in western Libya. *Int. Multilingual J. Sci. Tech.***5**(9). ISSN: 2528-9810

Athari S S (2019) Targeting cell signaling in allergic asthma. *Sig Transduct Target Ther* **4**, 45 https://doi.org/10.1038/s41392-019-0079-0.

Chen L-X, Xu C-M, Gao F, Zhu M-F, Xu M-J, Zhang J-R and Qian W-X (2020) Associations of IL-18 and IL-9 expressions and gene polymorphisms with asthma. *Europ. Rev. Med. Pharmacol. Sci*. **24**, 6931-6938.

Do-Thi Van Anh, Lee Jie-Oh, Lee Hayyoung and Kim Young Sang (2020) Crosstalk between the producers and immune targets of IL-9. *Immune Netw*. **20**(6), e45 https://doi.org/10.4110/in.2020.20.e45 pISSN 1598-2629·eISSN 2092-6685.

Fatahi F, Chaleshtori A R S, Ghatreh Samani K, Mousavi S M, Zandi F, Heydari S, Hashemzadeh M Chaleshtori, Amiri M and Khazraee H (2016) Assessment of the effects of *IL9*, *IL9R*, *IL17A* and *IL17F* gene polymorphisms on women with allergic rhinitis in Shahrekord, Iran. *Ann. Med. Health Sci. Res*. **6**(4), 216–223. doi: 10.4103/amhsr.amhsr_485_14.

Global Initiative for Asthma (2019) Global .strategy for asthma managerment and prevention (2019 update), available from. http://www.ginasthma.org.

Goswami R and Kaplan M H (2011) A brief history of IL-9. *The J. Immunol.* **186**(6), 3283-3288.

Huo Yue and Zhang Hong-Yu (2018) Genetic mechanisms of asthma and the implications for drug repositioning. *Genes* **9**(5), 237; https://doi.org/10.3390/genes9050237.

Jebur Mohammed S, Saud Asmaa M, Brakhasc Suad A and Al-Haydari Ahmed S (2020) Level of IL-9 in sample of Iraqi allergic asthma patients. *The 5th International Scientific Conference of Medical and Health Specialties.* 141 Session One (MS) 9 – 10 December 2020.

Koch S, Sopel N and Finotto S (2017) Th9 and other IL-9-producing cells in allergic asthma. *Semin Immunopathol.* **39**, 55–68. https://doi.org/10.1007/s00281-016-0601-1.

Mahneh Sedigheh Bahrami, Movahedi Masoud, Bahar Mohammad Ali and Arezou Rezaei (2015) IL9 single nucleotide polymorphism and serum levels of interleukin 9 in children with asthma. *Pediatric Allergy, Immunology and Pulmonology* **29**(1). DOI: 10.1089/ped.2015.0547.

Rawi Abeer Muhammad (2013) Asthma. *J. Security and Life, Egypt* **375**, 112-117.

Saeki M, Nishimura T, Kitamura N, Hiroi T, Mori A and Kaminuma O (2019) Potential mechanisms of T Cell-mediated and eosinophil-independent bronchial hyperresponsiveness. *Int. J. Mol. Sci.* **20**(12), 2980.

Sordillo Joanne E, Kelly Roxanne, Bunyavanich Supinda, McGeachie Michael, Qiu Weiliang, Croteau-Chonka Damien C, Soto-Quiros Manuel, Avila Lydiana, Celedón Juan C, Brehm John M, Weiss Scott T, Gold Diane R and Litonjua Augusto A (2015) Genome-wide expression profiles identify potential targets for gene by environment interactions in asthma severity. *J. Allergy Clin. Immunol*. **136**(4), 885–92.e2.

Wan Jie, Huang Lan, Ji Xiaoyun, Yao Shun, Abdelaziz Mohamed Hamed, Cai Wei, Wang Huixuan, Cheng Jianjun, Dineshkumar Kesavan, Aparna Vasudevan, Su Zhaoliang, Wang Shengjun and Xu Huaxi (2020) HMGB1-induced ILC2s activate dendritic cells by producing IL-9 in asthmatic mouse model. *Cell. Immunol.* **352**, 104085.

World Health Organization (WHO) (2020) Asthma. https://www.who.int/news-room/fact-sheets/detail/asthma.

Yuhong Zhou, Michael McLane and Roy C Levitt (2001) Th2 cytokines and asthma-interleukin-9 as a therapeutic target for asthma. *Respir. Res.* **2**(2), 80–84.