

DOI: <http://doi.org/10.32792/utq.jceps.10.01.017>

Polymorphisms of STAT6 Gene and It Association with Asthmatics in Nassiriya, Iraq.

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Received 16/09/2019

Accepted 24/10/2019

Published 20/1/2020



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Abstract:

The present study was carried out in the Labs of college of Science and specialized center for respiratory disease in Nasiriya city, during the period from May to August of 2018. The aim of study was to examine possible association between polymorphism of STAT6 gene in patients with asthma and evaluate immune status to them by measuring the levels of some cytokines (IL4, IL13) and IgE in serum by using a technique enzyme-linked immune sorbent adsorptive (ELISA). The study included a total of 63 patients with asthma and 31 healthy people and their age between 10 - 60 years.

DNA was isolated and RFLP-PCR was performed by using primers specific for genotypes of region of the STAT6 gene, the results showed the presence of mutations in most sample of patients after using gene sequence. The gene polymorphism reveal that a direct relationship between the STAT6 of the asthmatic patients.

The statistical analysis also showed that a high significant increase ($P < 0.05$) in serum IL4 and IL13 and IgE in the sera of asthmatic compared to the control group.

In conclusion, the results indicate that a polymorphism of STAT6 gene with immunological study of IL4, IL13 may be a useful as marker for predicting asthma diseases in Nasiriya/ Iraq population. Accordingly, the study of asthma genetically and immunologically to provide a theoretical basis for asthma protection.

Keyword: STAT6, gene Polymorphisms, IL4, IL13 and IgE.

1. Introduction:

Asthma is a chronic airway inflammation, characterized by variable airway obstruction, reduced lung function, and leading to wheezing and shortness of breath [1]. This inflammation causes the airways to swell and become very sensitive. It can lead to wheezing and chest tightness. Asthma affects people of all ages and genders, with continuously increasing incidence rates, about 300 million people suffer from asthma worldwide [2]. It is thought that 60% of asthma cases are inherited. Other risk factors include tobacco exposure, occupational exposure, air pollution, house dust mite, cesarean section, severe respiratory tract infection, and obesity [3,4].

Asthma is often characterized by enhanced total serum IgE level upon the exposure to allergens, which is known as an atopy. Many studies, through genome-wide linkage studies, confirmed the involvement of genetic predisposition in the development of atopy in asthmatic patients [5]. Among these candidate genes, signal transducer and activator of transcription factor 6 (STAT6) is one of the most widely investigated important genes [6], [7]. The signal transducers and activators of transcription (STATs) including STAT6 are latent cytoplasmic proteins that undergo tyrosine phosphorylation by Janus kinases (JAKs) in response to cytokine exposure in the extracellular milieu. Ligation of cytokines interleukin-4 (IL-4) and interleukin-13 (IL-13) with their receptors that contain the α subunit of the IL-4 receptor (IL-4R α) result in a common STAT6-mediated signaling pathway critical to the development of Th2 inflammation characteristic of asthma and anti-parasitic responses [8,9].

STAT6 gene is located at 12q13.314.1 [10]. One of the most susceptible regions associated with asthma. STAT6 is normally activated by Th2-related cytokines such as interleukin-4 (IL-4) and IL-13 [8]. The elevated IgE production in asthmatic patients results in promotion of acute hypersensitivity responses, chronic eosinophil-predominant allergic inflammation with T helper-2 (Th2) cells cytokine production [11]. Therefore, the aims and objectives of the present study were to estimation relationship of IL4, IL13, IgE and STAT6 gene polymorphism with asthma patient in Al-Nasseryia population.

2. Materials and Method:

2.1. Patient and sampling:

Venous blood samples were collected from 63 patients, in addition to that about 31 healthy volunteers were included as a control group. The age groups for patients included between 10 and 60 years with asthma diagnosis according to Specialized Center for Respiratory Diseases in Al-Nasiriya city. Three milliliters of blood collected directly in a EDTA free plane tube and allowed to clot then serum was separated by centrifugation After centrifugation serum was separated and stored at -20 C to be measure IL-4, IL-13 and IgE concentration using Elabscience kits (Elisa kits).

Tow milliliters of blood collected in sterile tube containing EDTA for DNA extraction, by using Genomic DNA mini kit extraction (Geneaid- Tialand). DNA was detected by Electrophoresis (0.8%). according to the method of [12]. The Conventional Polymerase Chain Reaction (PCR technique) was used to determine the different genotypes of STAT6-2892 by specific Primers were:

Fwd: 5'CTCTTCCCACCCCTGTGTCTATC3' and
Rev: 5'-TCCCATAGATAGCCCTCCTAGGTAC-3'. [13] provide by Bioneer company. the PCR mixture (total, 25 μ l) containing 5 μ L DNA, 3 μ l of each primer, 12.5 μ L 2X Taq master mix (containing Taq DNA Polymerase, dNTPs, MgCl₂ and reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR) and 1.5 Ml nuclease free water. The PCR mixture was incubated at 95°C for 5 minutes, then 35 cycles of 95°C for 30 seconds minute to denature, 72°C for 40 seconds to anneal the primers and 72°C for 8 minute to elongate the strand PCR, amplification kit was obtained from Bioneer company [14]. The products of PCR were analyzed by 1% agarose gel electrophoresis [12]. The PCR product tubes of 20% representative samples with (forward and reverse primer) were sent for DNA sequencing (Bioneer, Korea).

The BLAST program (Basic Local Alignment Search Tool) accessed through the website (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) was used for analysis the sequences data.

Statistical analysis

Data were expressed as mean \pm standard deviation (SD) or median (interquintile range). Differences between groups were tested with the Student's t-test. The values of $P < 0.05$ were considered significant.

3. Results

1.3. Serum IL4, IL13 and IgE Concentration of asthma Patients:

The results of this study showed the presence of a significant increase ($P < 0.05$) in the rate of concentrations of (IL-4) see Table (1) as the mean of concentration of IL-4 in patients (1.45 ± 0.32 pg / ml) compared to the control group (0.60 ± 0.18 pg/ml) with a significant difference (0.00), while IL-13 concentration (4.24 ± 1.64 pg / ml) for patients compared with the healthy control (0.63 ± 1.24 pg / ml) with a significant difference (0.00) in addition, a significant difference in the rate of concentrations of IgE concentration (506 ± 65.15 IU/mL) serum level for patients compared with the healthy control.

Table (1): Levels of Serum IL-4, IL-13, and IgE of the asthma patient and healthy controls group.

Parameter	Subject	No of cases	Mean \pm SD	T-value	Df	P-value
IL – 4	Patients	63	1.45 ± 0.32	13.49	92	0.00
	Control	31	0.60 ± 0.18			
IL – 13	Patients	63	4.24 ± 1.64	12.26	92	0.00
	Control	31	0.63 ± 1.24			
IgE	Patients	63	506 ± 65.155	32.27	92	0.00
	Control	31	121 ± 16.88			

($P < 0.05$).

df: degree freedom

3.2. Genetic study:

Genomic DAN was extracted successfully from all studied groups. Extracted by following the instructions of (Geneaid Kit), The presence of extracted DNA was confirmed by Agarose gel electrophoresis on a 0.8 % as shown in figure (1). while, Figure (2) revealed that amplified STAT6 gene of asthma patients. Bands were fractionated by electrophoresis on a 2 % agarose gel. light after staining with ethidium bromide staining.

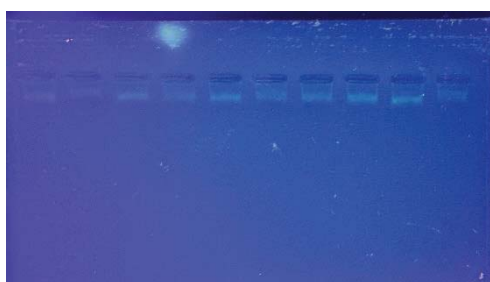


Figure (1): Agarose gel electrophoresis (0.8%) of extracted genomic DNA, after 60 minutes at 100 V, stained with ethidium bromide and visualized by a UV transilluminator. Lanes 1- 10: Genomic DNA.

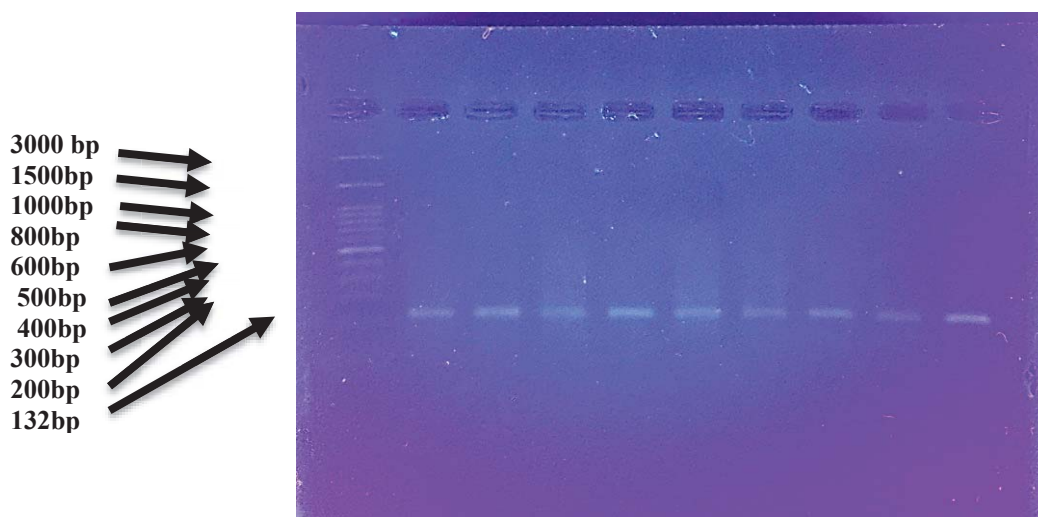


Figure (2): Agarose gel electrophoresis for amplified STAT6 gene of asthma patients. Bands were fractionated by electrophoresis on a 2 % agarose gel (1 h., 80V/cm) and visualized under U.V. light after staining with ethidium bromide staining. (L:100-3000bp M: ladder)
Lane: 1,2,3,4,5,6,7,8 and 9: positive results.

Genetic sequence analysis of asthma-infected samples in Nasirriyah city, presence SNPs in the studied genetic site of activator of transcription 6 (STAT6) gene with Accession Number AH006951.2 , 99% Identity with Transition Polymorphism in location 51 of A--- C nucleotide as shown in Table 2.

Table (2): Homo sapiens chromosome 12 signal transducer and activator of transcription 6 (STAT6) gene.

Gene	Accession Number	Identity	Polymorphism			Range
			Type	Location	Nucleotide	Range
chromosome 12 signal transducer and activator of transcription 6 (STAT6) gene	<u>AH006951.2</u>	99%	Transition	51	A-----C	6566 to 6638

Score	Expect	Identities	Gaps	Strand
130 bits(70)	5e-27	72/73(99%)	0/73(0%)	Plus/Plus

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Query1      GGATGCCCCCTGGTTTAAGGTGGTCTGGCCATGAGTGGTGGTGGGGACGGTACCTAGGAGG 60
|||||
Sbjct6566   GGATGCCCCCTGGTTTAAGGTGGTCTGGCCATGAGTGGTGGTGGGGACGGTACCTAGGAGG 6625
                Query 61      GCTATCTATGGGA      73
                |||||
                Sbjct 6626   GCTATCTATGGGA      6638
    
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4. Discussion:

Cytokines play important role in the development and activation of immune cells, since they act as cell-signaling molecules, especially in autoimmune diseases [15]. In the present study, the results showed a

significant increase in serum levels of IL4, IL13 and IgE. These results are in line with [11] and [16] all they found that significant elevated of serum IL4, IL13 and IgE levels in asthmatics compared to healthy controls.

IL-4 is an important cytokine secreted from T helper type 2 cells, as potent switch factors, that leads to the production and synthesis of IgE, favoring development of type 2 inflammation and the atopic asthma phenotype [17], [18]. Synthesis of IgE from B cells and the differentiation, maturation, migration and survival of eosinophils are induced by the increased proinflammatory cytokines, including interleukins IL-4, IL-5 and IL-13 [19]. One of the more frequent asthma gene is signal transducer and activator of transcription 6 (STAT6) that elevate the development of asthma by increasing serum IgE level and then induced airway hyperresponsiveness [20]. STAT6 is principally activated by two cytokines, interleukin-4 and interleukin-13 [21,22].

The present study demonstrated that a strong linkage disequilibrium between STAT6 gene polymorphism with asthma. Previous studies have shown trends to increase excessive bronchial response and atopic asthma with the STAT6 gene [23], [24]. Expression and activation of STAT6 SNP rs4559 in individuals with asthma has been explained in other studies [24]. These results agree with [14] who found that a significant interaction between of STAT6 rs324011 Gene Polymorphism with susceptibility of atopic bronchial asthma in Egyptian children. In addition, [26] documented a genetic variant in the STAT6 gene significantly contribute to the regulation of serum IgE levels but in contrast with [27], they found that no significant risk of STAT6 with the asthma. There is a significant overlap between STAT6 and the risk of developing asthma in the Chinese population [28]. However, in our study, we observed a significant relationship between polymorphisms of STAT6 (rs324011 SNP) with asthma in the population of Iraq.

5. Conclusion:

In conclusion, our results indicate that a polymorphism of STAT6 gene with immunological study of IL4, IL13 may be a useful as marker for predicting asthma diseases in Iraq population. However, the pathogenesis of asthma is complex, and the exact mechanisms of STAT6 gene polymorphisms and asthma still need to be further studied. Just by studying the gene polymorphism and expression on the whole we can study asthma better from the genetic and immunological perspectives, and provide a better theoretical basis for the protection of the asthma.

6. References:

- 1- Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, Casale TB, Chaney P, Enright PL, Gibson PG, de Jongste JC, Kerstjens HA, Lazarus SC, Levy ML, O'Byrne PM, Partridge MR, Pavord ID, Sears MR, Sterk PJ, Stoloff SW, Sullivan SD, Szeffler SJ, Thomas MD, Wenzel SE; American Thoracic "An official american thoracic society/European respiratory society statement: asthma control and exacerbations". *Am J Respir Crit Care Med*, 180(1):59–99, 2009 July 2009.
- 2- Al-Jahdali H, Wali S, Salem G, Al-Hameed F, Almotair A, Zeitouni M, Aref H, Nadama R, Algethami MM, Al Ghamdy A, Dihan T. "Asthma control and predictive factors among adults in Saudi Arabia: Results from the Epidemiological Study on the Management of Asthma in Asthmatic Middle East Adult Population study". *Ann Thorac Med*. 14(2): 148–154. April, 2019.

- 3- Thomsen SF."Epidemiology and natural history of atopic diseases". Eur Clin Respir J;2: 10.3402/ecrj.v2.June,2015.
- 4- AbdulRazak MR."Chirakalwasan N. Obstructive sleep apnea and asthma". Asian Pac J Allergy Immunol; 34:265-71. December ,2016.
- 5- Ober C and Yao TC. "The genetics of asthma and allergic disease: A 21st century perspective". Immunol Rev 242: 10-30. July ,2011.
- 6- Gao PS, Mao XQ, Roberts MH, Arinobu Y, Akaiwa M, Enomoto T, Dake Y, Kawai M, Sasaki S, Hamasaki N, Izuhara K, Shirakawa T, Hopkin JM."Variants of STAT6 (signal transducer and activator of transcription 6) in atopic asthma". Journal of Medical Genetics 37: 380–382.May ,2000.
- 7- Hu JH, Wu JM, Cui TP, Li YR and Lou DD. "Study on the correlation between STAT6 gene polymorphism and atopic asthma in Chinese Han population of Hubei province". Chinese Journal of Microbiology and Immunology 25: 243–247.May,2005.
- 8- Hershey GK." IL-13 receptors and signaling pathways": an evolving web. J Allergy Clin Immunol; 111:677-90April,2003.
- 9- Zimmermann N, Hershey GK, Foster PS, Rothenberg ME. Chemokines in asthma: cooperative interaction between chemokines and IL-13. J Allergy Clin Immunol; 111:227-42. February,2003.
- 10-Patel BK1, Keck CL, O'Leary RS, Popescu NC, LaRoche WJ." Localization of the human stat6 gene to chromosome 12q13.3-q14.1, a region implicated in multiple solid tumors". Genomics 52: 192–200.September,1998.
- 11-Antczak, A, Domańska-Senderowska D, Górski P, Pastuszek- Lewandoska D, Nielepkowicz-Goździńska A. "Analysis of changes in expression of IL-4/IL-13/STAT6 pathway and correlation with the selected clinical parameters in patients with atopic asthma". Int J Immunopathol Pharmacol 29: 195-204.June ,2016.
- 12-Sambrook, J, Fritsh, EF and Maniatis T. "Molecular cloning, a laboratory manual". 2nd ed . Cold Spring Harbor Laboratory Press. USA.1989.
- 13-Wu X, Li Y, Chen Q, Chen F, Cai P, ." Association and gene-gene interactions of eight common single-nucleotide polymorphisms with pediatric asthma in middle china". J Asthma .47: 238-244.January,2016.
- 14-Rehab M El-Gohary1, Ayman A Wagih1, Hala E Hamouda1, Salwa M El-Melegy1 and Mohamed A. "Association of STAT6 rs324011 Gene Polymorphism with Susceptibility of Atopic Bronchial Asthma in Egyptian Children". Biochemistry and Molecular Biology Journal. Vol.4 No.1:6. February,2018.
- 15-15- Gomes KB. "IL-6 and type 1 diabetes mellitus: T cell responses and increase in IL-6 receptor surface expression". Ann Transl Med. 5(1): 16 November,2016.
- 16-Tavakkol Afshari J, Farid Hosseini R, Hosseini Farahabadi S, Heydarian F, Boskabady MH, Khoshnavaz R, Razavi A, Ghayoor Karimiani E, Ghasemi G. "Association of the expression of IL-4 and IL-13 genes, IL-4 and IgE serum levels with allergic asthma". Iranian Journal of Allergy Asthma Immunology 6: 67–72. June, 2007.
- 17-Simpson JL, Scott R, Boyle MJ, Gibson PG." Inflammatory subtypes in asthma: Assessment and identification using induced sputum". Respirology, 11, 54–61. January,2006.
- 18-Subrahmanyam RM, Srikantaiah C, Krishna P, Delphine Silvia, CR, Thirunavukkarasu S, Devi K, Rao M, Kumar V. "Can bronchial asthma be classified based on the immunological status"? Lung India, 28, 110–113.June,2011.

- 19-Kupryś-Lipińska I, Molińska K, Kuna P. "The effect of omalizumab on eosinophilic inflammation of the respiratory tract in patients with allergic asthma". *Pneumonol Alergol Pol* 84:232-243. February, 2016.
- 20-Kavalari MS, Balantic, M, Silar M, Kosnik M P, Korosec P and M. Rijavec M. "Association of ORMDL3, STAT6 and TBXA2R gene polymorphisms with asthma". Blackwell Publishing Ltd, *International Journal of Immunogenetics* 39, 20–25. February ,2012.
- 21-Hou J, Schindler U, Henzel WJ, Ho TC, Brasseur M, McKnight SL. "An interleukin-4-induced transcription factor": IL-4 Stat. *Science*. 265:1701–6. December,1994.
- 22-Quelle FW, Shimoda K, Thierfelder W, Fischer C, Kim A, Ruben SM, Cleveland JL, Pierce JH, Keegan AD, Nelms K. "Cloning of murine Stat6 and human Stat6, Stat proteins that are tyrosine phosphorylated in responses to IL-4 and IL-3 but are not required for mitogenesis". *Mol Cell Biol*. 15:3336–43. May,1995.
- 23-Martinez FD, Holt PG. "Role of microbial burden in aetiology of allergy and asthma". *The Lancet*; 354: S112-S115. November,1991.
- 24-Miller RL, Eppinger TM, McConnell D, Cunningham-Rundles C, Rothman P. "Analysis of cytokine signaling in patients with extrinsic asthma and hyperimmunoglobulin E". *J Allergy Clin Immunol* 102: 503-511. March,1998.
- 25-Schedel M, Carr D, Klopp N, Woitsch B, Illig T, Stachel D, Schmid I, Fritzsche C, Weiland SK, von Mutius, E. and Kabesch, M. "A signal transducer and activator of transcription 6 haplotype influences the regulation of serum IgE levels". *J Allergy Clin Immunol.*;114(5):1100-5. May ,2004.
- 26-Pykäläinen M1, Kinoshita R, Valkonen S, Rydman P, Kilpeläinen M, Laitinen LA, Karjalainen J, Nieminen M, Hurme M, Kere J, Laitinen T, Laheesmaa R. "Association analysis of common variants of STAT6, GATA3, and STAT4 to asthma and high serum IgE phenotypes". *J Allergy Clin Immunol* 115: 80-87. January,2005.
- 27-Palmer LJ, Cookson WO. "Genomic approaches to understanding asthma". *Gen res*. 10:1280-1287. September,2000.