ARTICLE

JOURNAL OF UNIVERSITY OF BABYLON
For Pure and Applied Sciences (JUBPAS)

Vol.31; No.1.| 2023

Polymorphism in IL-6(-174 G/C) Gene in Scabietic Patients

Amerah Abdel Hadi[1]* **Alaa Jawad Hassan[2]**

[1]Al-Safwa University College. Ameraganmy24@gmail.com,Karbala , Iraq
[2] College of Science, University of Babylon , alnajaralaa71@gmail.com ,Hila, Iraq
1*Corresponding Author email: Ameraganmy24@gmail.com mobile:0780757145

التغايرات الوراثية للحركي الخلوي الانترلوكين – 6 (-174G/C) في القطعة الجينية لمرضى الجرب في العراق

عامرة عبد الهادي رحمن [1] علاء جواد حسن [2]

1كلية الصفوة الجامعة . كربلاء ، العراق Ameraganmy24@gmail.com

2 كلية العلوم ، جامعة بابل، الحلة ، العراق ، alnajaralaa71@gmail.com

Received:	21/ 1 /2023	Accepted:	10/2/2023	Published:	31/3/2023

ABSTRACT

Background : Scabies (*Sarcoptes scabiei* var. *hominis*) is one of the parasitic diseases that is often ignored. It results in problems that trigger an allergic and inflammatory immunological response. Cytokines are crucial to the development of disease and its pathophysiology. This investigation looked at how scabies risk and a variation in the gene IL-6 affected IL-6 production.

Materials and Methods : The goal of the current investigation was to determine the level of IL-6 by ELISA and its linked with IL-6(-174 G/C) polymorphisms in 60 scabietic patients and 30 healthy individuals.

Results : In contrast, the IL-6(-174 G/C) CC genotype and C allele carrier frequencies were highly significant (P0.05) in the patients who had their first infestation. The results showed a significant increase (P0.05) in the IL-6(-174 G/C) GG genotype and G allele in the re-infestation group of patients compared to the healthy group. According to IL-6 genetic variations, low serum levels were connected with the CC genotype.

Conclusion : IL-6(-174 G/C) Scabies susceptibility and polymorphism were related, providing evidence for the hereditary basis of the illness.

Keywords: interleukin-6, Scabies and Gene polymorphism.

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

Introduction

S. scabiei, the scabies mite, continues to spread around the world, affecting an estimated 300 million people. This infection typically causes significant pruritus that must be treated with prescription scabicidal therapy since it worsens at night[1].The S. scabiei that causes scabies in humans and sarcoptic mange in many other animals, on the other hand, are physiological variations of the same species. They don't completely have a host specificity, but they typically only last a short time on other hosts[2]. Pleiotropic cytokine IL-6 has important functions in both acute and chronic inflammation as well as having a variety of impacts on both immune and non-immune cell types. This cytokine is released by T-cells and macrophages to promote an immunological response[3] . IL-6 plays harmful roles in a number of disorders, including inflammatory, autoimmune, and cancerous diseases[4] . On chromosome 7p21, the human IL-6 gene spans 5 kb, has five exons, four introns, and a proximal promoter region. The IL-6 gene promoter region has more than 50 single nucleotide polymorphisms (SNPs), specifically IL-6(174 G/C)[5 and 6] . The purpose of this study was to use the ARMS-PCR technology to examine the relationship between the promoter polymorphism of interleukin-6 (174 G/C) among some Iraqi scabietic patients.

Materials and Methods

1. Patients and control:

The current study comprised 30 healthy persons (15 males and 15 females) as well as 60 people who have *Sarcoptes scabiei* variant. *hominis* infection (35 females and 25 males). The patients were divided into two groups: first infestation and re-infestation.

2. Blood samples :

Five milliliters of blood were drawn from each patient hospitalized to the AL-Diwaniyah Teaching Hospital between February and April of 2019; healthy subject samples were obtained from private labs. While the other portion of the sample was allowed to coagulate at room temperature before being centrifuged at 3000 rpm for 10 minutes, one component of the sample was divided into two and put in an EDTA tube to extract DNA for genetic analysis. Sera samples were then put into eppendrof tubes and maintained in a deep freezer until required..

3. Laboratory investigations :

A.**Immunological assays :**

IL-6 levels were determined manually using ELISA kits from the UK's Bioassay Technology Laboratory to estimate their levels.

B. PCR amplification

Genomic DNA was recovered from the blood of scabietic patients and controls using a DNA micro kit (favergen,korea). Using amplification refractory mutation

systems (ARMS)-PCR, the interleukin-6 gene was amplified for IL-6(-174 G/C)[7] . Table summarizes the primers used to amplify different types of polymorphism(1).
The master mix for the PCR amplification of the IL-6 polymorphism (-174 G/C) was composed of 5 l of genomic DNA, 1 l of each primer (10 pmol/1 l), and 11 l of nuclease-free water. The reaction was conducted at 95 °C for two minutes, followed by 35 cycles of 95 °C for one minute of denaturation, 63 °C for one minute of annealing, and 72 °C for one minute of extension, with the final two minutes of each cycle being conducted at 72 °C. The initial annealing temperature for touchdown reactions was 72°C. Once the annealing temperature was reached, the temperature was decreased by 1°C per cycle until the end of the cycles, when it was kept there. After the PCR result had been stained with ethidium bromide and electrophoresed on a 2% agarose gel, a picture had been taken.

Table 1: Primers of IL-6(-174 G/C)

IL-6(174 G/C)	Sequences	Product size
forward inner primer(G allele)	5'-GCA CTT TTC CCC CTA GTT GTG TCT TCCG-3'	205 bp (G allele
reverse inner primer (C allele)	5'-ATT GTG CAA TGT GAC GTC CTT TAG CTTG-3	176 bp (C allele
forward external primer	5'-GAC TTC AGC TTT ACT CTT TGT CAA GACA-3'	326 bp (from two outer primers)
reverse external primer	5'-GAA TGA GCC TCA GAC ATC TCC AGT CCTA-3'	

4.Statistical Analysis:

Utilizing the statistical system SPSS version -18, the data were examined[7]. Standard deviation (SD) and means were used to express the data. Least significant differences were used to evaluate differences between patient and control groups (L.S.D) [8].

Results and Discussion

The levels of IL-6 in the patient and control groups were significantly different, as shown in table 2, with the levels of the first infestation and re-infestation groups reaching 83.8810.58 ng/L and 230.3072.10 ng/L, respectively, and the healthy group reaching 85.449.35 ng/L.

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

Table 2: The levels of IL-6 in the control, re-infestation, and first infestation groups

Groups	Mean	S.D.	LSD	P.value
First Infestation(n.30)	83.88	10.58	22.395	0.001
Re-infestation(n.30)	230.30	72.10		
Control(n.30)	85.44	9.35		

The data showed that there were significant variations in the levels of IL-6 between the patient and control groups (P>O.O5); nonetheless, it was hypothesized that because IL-6 inhibits TNF-, it operates as an anti-inflammatory cytokine rather than a pro-inflammatory cytokine in scabietic cases[9] .Meanwhile, the interleukin IL-6 has both pro- and anti-inflammatory properties, and it exerts its anti-inflammatory effects by inhibiting TNF-α in individuals with atopic dermatitis [10] . While the major disparities between scabietic patients and healthy groups were explained by the fact that IL-6 does not inhibit TNF- α, acting instead as a pro-inflammatory cytokine, Since TNF- α is inhibited by IL-6, this means that IL-6's anti-inflammatory cytokine activity is mediated via TNF- α[11] .

The inhibitory actions of IL-6 on TNF-α mediate its anti-inflammatory cytokine activity.as evidenced by the significant differences between scabietic patients and healthy groups, in which they explained that the due to its inability to stop TNF- α, IL-6 works as a pro-inflammatory cytokine rather than an anti-inflammatory cytokine. In the meantime, the first infestation, re-infestation, and control group patients' GC genotype frequencies were 33.3%, 33.3%, and 35.5%, respectively, with a non-significant difference in OR of 1.909; table 3 also showed non-significant differences with OR of 0.909 between the first infestation, re-infestation, and control group patients. In the patient (original infestation, re-infestation) and control groups, the GG genotype frequencies were 6.7%, 63.3%, and 61.3%, respectively. This is not statistically significant, although there are small significant differences between the two groups (OR=0.105).

76.7%, 30.0%, and 19.76% of the first infestation, re-infestation, and control groups, respectively, had the IL-6 (-174 G/C) genotype, respectively. This allele was significantly distributed with a high OR between the first infestation and control groups, but not between the re-infestation and control groups, where the OR was 1.000 and the distribution was non-significant.

While the frequencies of the G allele were 23.3%, 80.0%, and 80.3% in the initial infestation, re-infestation, and control groups, respectively, the distribution of the IL-6 (-174 G/C) allele was significant without an OR., in the other groups.

info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com ISSN: 2312-8135 | Print ISSN: 1992-0652

Table 3: Genotype and allele frequencies of IL-6 (-174 G/C)variants in scabies patients and controls .

IL-6 SNPS	Study groups			Total	P value	OR	95% C.I. for OR	
	First infest	Re-infestation	Control				Lower	Upper
CC	18	1	1	20	0.005	18.000	2.403	134.834
	60.0%	3.3%	3.2%	22.0%	0.005	18.000	2.403	134.834
					0.001	1.000	0.063	15.988
					0.005	18.000	2.403	134.834
GC	10	10	11	31	0.082	1.909	0.920	3.959
	33.3%	33.3%	35.5%	34.1%	0.827	0.909	0.386	2.141
					0.827	0.909	0.386	2.141
					1.000	1.000	0.416	2.403
GG	2	19	19	40	0.752	1.105	0.594	2.056
	6.7%	63.3%	61.3%	44.0%	0.002	0.105	0.025	0.452
					1.000	1.000	0.529	1.889
					0.002	0.105	0.025	0.452
Total	46	11	11	91				
	100.0%	100.0%	100.0%	100.0%				
C	46	12	12	70	0.001	4.833	2.596	8.999
	76.7%	20.0%	19.7%	38.7%	0.001	3.833	2.031	7.236
					1.000	1.000	0.449	2.226
					0.001	3.833	2.031	7.236
G	14	48	49	111	0.218	1.265	0.870	1.840
	23.3%	80.0%	80.3%	61.3%	0.001	0.286	0.158	0.517
					0.919	0.980	0.658	1.459
					0.001	0.292	0.161	0.529
Total	60	60	62	182				
	100.0%	100.0%	100.0%	100.0%				

*scabies patients /control
*first infestation/control
*re-infestation/control
*first infestation/re-infestation

ISSN: 2312-8135 | Print ISSN: 1992-0652
jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com
info@journalofbabylon.com

ISSN: 2312-8135 | Print ISSN: 1992-0652
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com

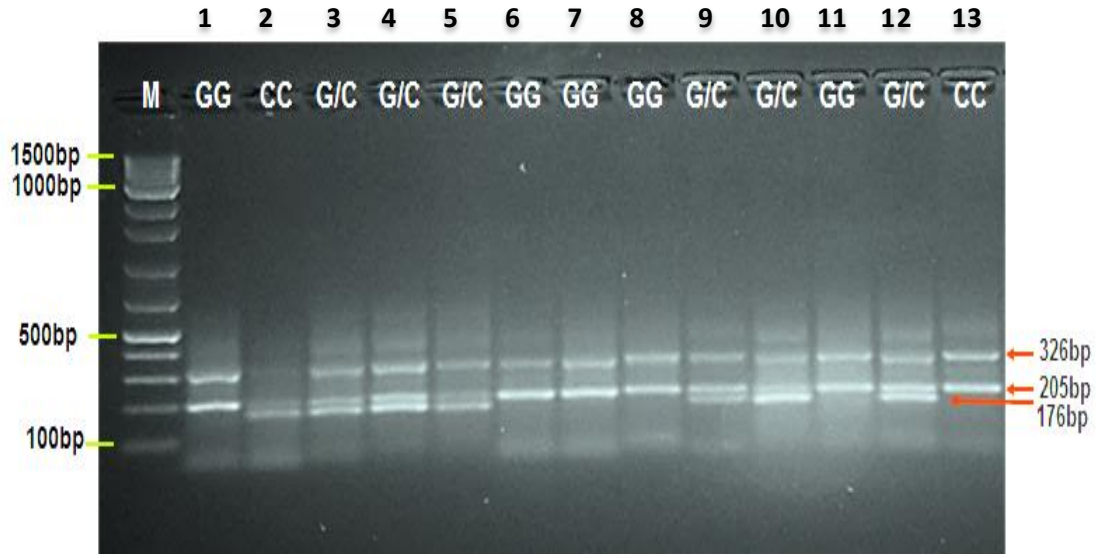

Figure1: Image from a single experiment investigating the IL-6 (-174) (G/C) gene polymorphism using agarose gel electrophoresis. 2% agarose at 100V for 1 hour, with marker M. (100-1500bp). The internal control product was discernible at 326 bp in all samples. The GG wild type homozygote genotype lanes were visible at 205bp ARMS PCR product size. The homozygote genotype lanes of the CC mutant type were visible at a 176bp ARMS PCR product size. The G and C alleles, however, were amplified at 205 and 176 bp product sizes, respectively, in the G/C heterozygote genotype lanes.

The IL-6 levels for the first infestation, re-infestation, and control groups were, respectively, 69.175, 258.27, and 90.60 ng/L; 64.137, 182.00, and 75.49 ng/L for GG and GC genotypes. However, the IL-6 levels for the first infestation, re-infestation, and control groups were all statistically higher in patients who carry the G allele. In the first infestation, re-infestation, and control groups, respectively, the serum levels of IL-6 in patients with the C allele (CC homozygote mutant type) were tested and found to be slightly higher at 64.959, 34.5 ng/L, and 60.2 ng/L.

Table 4: The relationship between IL-6 genotype (-174 G/C) and IL-6 ng/L levels in patient and control groups products' various sizes.

IL-6 SNPS	**First infestation**	**Re-infestation**	**Control**	**Sig. multivariant**
	Mean±SD	**Mean±SD**	**Mean±SD**	
CC	64.959±19.545	34.5± NA	60.2± NA	<0.001
GC	64.137±17.659	182.00±64.68	75.49±4.14	
GG	69.175±1.33	258.27±61.65	90.60±6.40	
SIG.	0.081	0.003	<0.001	

It was used in the scabies investigations since IL-6 (-174 G/C) polymorphism for people with psoriasis and atopic dermatitis as dermatologic disorders had not previously been explored. The most studied SNP at position -174 (IL-6 -174G/C), which modifies

مجلة جامعة بابل للعلوم الصرفة والتطبيقية مجلة جامعة بابل للعلوم الصرفة والتطبيقية مجلة جامعة بابل للعلوم الصرفة والتطبيقية

transcriptional regulation and cytokine levels, has an impact on inflammatory phenotypes[12].Psoriasis susceptibility can be determined by the IL6-174 G/C polymorphism, with the GG genotype carrying a nearly two-fold greater chance of developing the condition[13],Additionally, a single nucleotide change from G to C influences gene transcription, and people with the -174GG genotype have plasma levels of IL-6 that are twice as high as people with the homozygous CC allele[12] . The IL6 (-174G/C) polymorphism's G allele causes an increase in transcriptional activity that raises the serum level of IL-6.; [14 and 12].

IL-6 blood levels have been linked to an SNP in the IL-6 gene's -174 promoter region, albeit few studies have linked allele C to high IL-6 production[15];.[16] On the other hand, research has also shown that allele C leads to a reduced production of IL-6 , additionally, research on the IL-6 SNP revealed that the G allele at position -174 may contribute to an inflammatory response that is worsened in homozygous G allele genotypes, such as in AD patients[17].The G allele and GG genotype of the IL-6 -174C/G polymorphism were associated with an increased risk of AD[18],Although there is no association between the polymorphisms nt565A/G and IL-6 -174C/G and the risk of AD[19],Nevertheless, IL-6 174C/G and nt565A/G polymorphisms and the risk of AD[20].

Acknowledgments :

My great thanks and appreciation for all staff and members of AL-Diwaniyah teaching hospital / department of dermatology for their help .

Conflict of interests :

There are no-conflicts of interest **.**

Conflict of interests; -

There are no conflicts of interest.

References

[1] .J.L.Bolognia, J.L.Jorizzo, J.V. Schaffer, J.P. Callen, L. Cerroni, W.R. Heymann, G.J. Hruza, A.J. Mancini, J.W. Patterson, M. Rocken, and T.Schwarz, Dermatology, 3rd Edition, Elsevier Limited. 2012.

[2] .C.E.M. Griffiths, J.Barker, T. Bleiker, R. Chalmers, and D. Creamerm, Rook's Textbook of Dermatology,Ninth Edition, Registered office: John Wiley and Sons, Ltd. 2016

[3] .M.A. Ullah, J.A. Revez, Z. Loh, J. Simpson, V. Zhang, and L. Bain, Allergen-induced IL-6 trans-signaling activates T cells to promote type 2 and type 17 airway inflammation. *Journal of Allergy and Clinical Immunology*, vol.136,pp.1065-73, 2015

[4] .B.K. Pedersen, A. Steensberg, C. Fischer, and et al., Searching for the exercise factor - is IL-6 acandidate, *Journal of Muscle Research Cell Motility* ,vol. 24,pp. 113-119, 2003

[5] .C.F. Terry, V. Loukaci, and F.R. Green, Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. Journal of Biological Chemistry, vol.275,pp.18138–44, 2000

[6] .F. Totaro, F. Cimmino, P. Pignataro, and G. Acierno, Impact of interleukin-6 -174 G>C gene promoter polymorphism on neuroblastoma. *PLoS One* , vol.8, pp. e76810, 2013.

info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com ISSN: 2312-8135 | Print ISSN: 1992-0652

[7]. S.Ye, S. Dhillon, X. Ke, A.R. Collins, and I.N.M. Day, An efficient procedure for genotyping single nucleotide polymorphisms, *Nucleic Acids Research*,vol.29,no.17,pp. e88, 2001.
[8]. M. Jawad, Statistical analysis by using SPSS . Dar AL-Awael/Jordan ,Amman. 2008.
[9] .A.A. Abd EL-Aal, M.A. Hassan, H.I. Gawdat , M.A. Ali, and M. Barakat , Immunomodulatory impression of anti and pro-inflammatory cytokines in relation to humoral immunity in human scabies International Journal of Immunopathology and Pharmacology, vol. 29,no.2,pp. 188-194, 2016
[10] .H.D. Shin, B.L. Park, L.H. Kim, J.W. Kim, and J.S. Kim, Interleukin-10 haplotype associated with total serum IgE in atopic dermatitis patients, *Allergy,* vol. 60,pp. 1146–1151, 2005
[11] .A.A.A Hamdan, and A.J. Ali, The indirect induction of human scabies to initiation autoimmune disease (SLE): Comprehensive study in marshes population of Thie- Qar. *Kerbala journal of pharmaceutical Sciences*,vol.13,pp.155-166, 2017
[12] .D.Fishman, G. Faulds, R. Jeffery, V. Mohamed-Ali, J.S. Yudkin, and S.Humphries, The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis, *Journal of Clinical Investigations*, vol.102,no.7,pp.1369–76, 1998
[13] . M. Białecka, R. Ostasz, M. Kurzawski, A. Klimowicz, H. Fabia_nczyk, P Bojko, V. Dziedziejko , K. Safranow, and M. Dro_zdzik. , IL6 _174G>C polymorphism is associated with an increased risk of psoriasis but not response to treatment, John Wiley and Sons Ltd, *Experimental Dermatology*, vol. 24,pp. 146–159, 2015
[14] .N.C. Wood, J.A. Symons, E. Dickens, and G.W Duff, In situ hybridization of IL-6 in rheumatoid arthritis. *Clinical and Experimental Immunology* ,vol.87,no.2,pp. 183–9,1992
[15] .E.Mariani, S. Neri, L. Cattini, and M. Sadagiani, Effect of zinc supplementation on plasma IL-6 and MCP-1 production and NK cell function in healthy elderly: Interactive influence of +647 MT1a and -174 IL-6 polymorphic alleles, *Experimental Geronologyt*, vol. 43,pp.462–471, 2008
[16] .R.K. Galimudi, M.K. Spurthi, C. Padala, K.G. Kumar, S. Mudigonda, S.G Reddy, T.M. Aiyengar, S.K. Sahu, and S.H. Rani, Interleukin 6 (-174G/C) variant and its circulating levels in coronary artery disease patients and their first degree relatives, *Inflammation*. vol. 37,no.2,pp.314–21,2014
[17] .C.F. Terry, V. Loukaci, and F.R. Green, Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation, *Journal of Biological Chemistry* ,vol.275,pp.18138–44, 2000
[18] .A.A Settin, H.A. Hassan, R.A. El-Baz,. and T.A. Hassan , Association of cytokine gene polymorphisms with psoriasis in cases from the nile delta of egypt. *Indian Journal of Dermatology*, vol.56,pp.272–7, 2011
[19] .M. Gharagozlou, E. Farhadi, M. Khaledi, N. Behniafard, S. Sotoudeh, and R. Salari, Association between the interleukin 6 genotype at position-174 and atopic dermatitis, *Journal of Investigational Allergology and Clinical Immunology*, vol. 23,pp. 89-93, 2013
[20] .Stavric, K.; Peova, S.; Trajkov ,D. and Spiroski, MGene polymorphisms of 22 cytokines in Macedonian children with atopic dermatitis. *Iranian Journal of Allergy, Asthma and Immunology*, vol. 11,pp. 37-50, 2012

مجلة جامعة بابل للعلوم الصرفة والتطبيقية مجلة جامعة بابل للعلوم الصرفة والتطبيقية مجلة جامعة بابل للعلوم الصرفة والتطبيقية
info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com ISSN: 2312-8135 | Print ISSN: 1992-0652

الخلاصة

الجرب هو احد الامراض الجلدية المتوطنة ، اذ يسبب مضاعفات تؤدي الى حدوث استجابة مناعية التهابية وارجية . تلعب الحركيات الخلوية دورا مهما في نشوء وتقدم المرض . تهدف هذه الدراسة الى معرفة العلاقة بين التغاير الوراثي لجين الانترلوكين – 6 وخطورة الجرب وتأثيره على انتاج الانترلوكين–6 .

الاستنتاجات : التغاير الوراثي للانترلوكين–6 يكون مرتبطا مع حساسية الاصابة بمرض الجرب ، وهذا يعطي دعم اضافي للأساس الوراثي لهذا المرض .

الكلمات المفتاحية : الجرب ، الانترلوكين – 6 ، التغاير الوراثي .

info@journalofbabylon.com | jub@itnet.uobabylon.edu.iq | www.journalofbabylon.com ISSN: 2312-8135 | Print ISSN: 1992-0652