Original Article

Polymorphism of Interleukin -17 Gene in Scabies

A Serum Level and Polymorphism of Interleukin -17 Gene (RS 8193036, RS 2275913) in Scabies Patients

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ABSTRACT

Objective: To assess how genotyping affects an individual's likelihood of developing scabies.
Study Design: Cross-sectional study.
Place and Duration of Study: This study was conducted at the Baquba Teaching Hospital- Consultation Clinic-Dermatology Unit/ Diyala Governorate from 4th of December, 2022 to 10th June, 2023.
Methods: Seventy-five male and female volunteer patients who clinically diagnosed with scabies and 75 non-infected individuals as a control were enrolled.
Results: There was an increase in IL-17 concentration of the patients' serum compared with control and there was genotype variation in both studied single nucleotide polymorphisms.
Conclusion: The serum level of IL-17 was increased in scabies group, the GA genotype and G allele of IL-17 SNPs rs 8193036 and the TT,CT genotypes and T allele of rs 2275913 were increased too with high OR value, the genotypes and alleles might be a risk factors from scabies infestation.
Key Words: Scabies, Genetic polymorphism, IL-17, Allele-specific, Serum levels

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INTRODUCTION

Scabies is one of cutaneous disease mediated by inflammatory and allergic reaction caused by a skin parasitic infection with parasite named Sarcoptes scabieivarhominis.[1] The disease is cosmopolitan in distribution as it affects millions (more than 300 million people) It is endemic in many countries and it although infects all age groups in both sexes , it is more frequent in children and elderly in low economic countries.[2] It is easily transmits by direct skin contact, in addition to indirectly methods using clothing, blankets, and towels.[3] Severe nocturnal itching occurs as a result of the females burrows digging skin tunnels to lay their eggs inside them, as well as result of the parasite's secretions and remains which is the most important symptom of the disease.[4]

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Burrows and papules are frequently signs in patients.[5] Skin scratching may leads to worsen the disease as a result of Secondary infection.[6] There are three clinical forms of scabies including classic scabies, Norwegian scabies and Nodular scabies.[7] Symptoms of the disease appear 2-4 weeks after infection.[8]

Mite infestation was stated to induce allergic and inflammatory immune response as a parasite-host relationship.[8] The Interleukin-17(IL-17) is a cytokine that was noticed after expose to allergens and it is involved in skin allergic reaction. It is a cytokine secreted by (Th-17) T cells and is a glycoprotein with a molecular weight of 20 kilo Daltons.[9] IL-17 stimulates immune cells fibroblasts, macrophages, neutrophils, and endothelial cells to produce pro-inflammatory mediators such as IL-1, TNF-a, and IL-6.[10] IL-17 has a role in protecting against bacterial and parasitic infections and has a role in recruiting neutrophils and producing antimicrobial peptides.[11,12]

Association between the polymorphism of IL-17 and inflammatory and infectious dermal disease such as psoriasis, basal cell carcinoma, lichen planus and cutaneous leishmaniasis was previously recorded.[13-14] The current study was designed to evaluate the role of serum and IL-17 genotyping in susceptibility to scabies in humans.

METHODS

The study conducted at Baquba Teaching Hospital, Consultation Clinic, Dermatology Unit/Diyala Governorate from December 2022 to June 2023, and

the policies and ethics followed by the Ministry of Health were taken into consideration. Seventy-five samples (41 males and 34 females) were collected from patients infected with scabies after they were diagnosed by Dermatologist and confirmed the diagnosis by a dermoscope as well as to 75 samples were collected from healthy people (45 males and 30 females), and after ensuring that they were free of all infectious and chronic diseases they underwent a CBC test (control group). Five ml of blood was drawn and divided into two parts. Three ml was used to serological test, and 2 ml was placed in an EDTA tube to detect polymorphisms of the IL-17 gene, SNP rs 8193036 and rs 2275913. The purity and concentration of the DNA were estimated using Nanodrop. The primers used in the study were designed according the NCBI-Blast online website by the authors and were proceeded according to the instructions of Alpha-DNA (Canada), as shown in (Table No. 1).

Table No. 1: IL-17 SNP of rs 8193036 and rs 2275913 Primers Information

rs 8193036	**Sequence(5´>3´)**	**Product length**
Forward primer	ACTGGCCCTTCCTTGTCCTA	
Reverse 1 primer	GATAGAGACTGGACAAAGGTGATGG	217bp
Reverse 2 primer	GATAGAGACTGGACAAAGGTGATGA	
rs 2275913	**Sequence(5´>3´)**	
Forward primer	AACACCTGGCCAAGGAATC	
Reverse1 primer	CAATGAGGTCATAGAAGAATCTCTC	298bp
Reverse2 primer	CAATGAGGTCATAGAAGAATCTCTT	

The polymerase chain reaction/allele-specific primer (ASP-PCR) technique was used to detect single nucleotide polymorphisms (SNPs) of the IL-17 rs 8193036, rs 2275913 gene. Amplicons product was visualized by ultraviolet transilluminator. IBM SPSS version 26.0 was used for statistical analysis, with the Duncan test, student's t-test, and ANOVA table utilized to compute probability at the $p<0.05$ level.

RESULTS

A significant increase in the mean concentration of IL-17 was seen among patients in comparison with control 759.63±172.27 and 20.95±0.93 pg/ml. There was also a insignificant raise in the IL-17 mean serum concentration of males infected with scabies compared to females, 948.95±247.62 and 531.33±233.01 pg/ml respectively, while insignificant reduction in the level in the level of IL-17 concentration in the sera of males compared to females in the control group 20.08±1.18, 22.34±1.50 pg/ml respectively (Table No. 2).

The IL-17 gene (SNPs) rs8193036 and rs2275913 were the two genetic variants that were examined in both studied groups. The study examined the genetic variation of (SNP)rs 8193036 in two alleles, G and A, which corresponded to three genotypes: AA, GA, and GG. When the well on the agarose gel was loaded with Reverse 2 primer in this technique, the homozygote AA showed up as a single band. The homozygote GG genotype emerged as a single band loaded with Reverse 1 primer on an agarose gel, but the heterozygote GA genotype presented as two bands, the first loaded with Reverse 1 primer and the second band with Reverse 2 primer (Fig.1).

The frequencies of genotypes and alleles of IL-17 rs8193036 SNPs for the patient group are incompatible with Hardy Weinberg equilibrium while it is compatible with a control group $1.0x10^{-12}$ and 0.1258 respectively (Table 3).

A non-significantly decreased frequency percentage of the GG genotyping in the scabies patient group compared to the control (9.3 vs. 13.3, OR:0.67, Pc value: 0.452) respectively (Table.4). There was a significant decrease in the frequency of the AA genotyping and the A allele in the patient group compared to the control (0 vs. 29.3, OR: 0.02, Pc value $3.3x10^{-8}$) (45.0 vs. 58.0, OR:0.06, Pc value: 0.029) respectively. It also showed a significant increase in genotyping frequencies percentage GA and G allele in the scabies patients group compared to the control (90.7 vs. 57.3, OR:7.23, Pc value: $2.2x10^{-6}$) and (55.0 vs. 42.0, OR:1.67, Pc value: 0.029) respectively. According to the results the high OR value of GA genotype and G allele referred to this genotype and allele may be risk factors for scabies, while GG, AA, and A allele may be protective agents from the disease development. The significant increase in the proportions of people carrying the GG and AG genotyping in the patient group compared to the control when measuring the serum levels of the IL-17 gene SNPs rs 8193036 among the study groups (456.57±278.67 vs. $20.56±9.91^{A}$ pg/ml and (790.83±187.83vs. $21.25±826^{A}$ pg/ml respectively (Table 5).

When the genotype is homozygote, CC, and TT, the genotype appears loaded in the first primer, Reverse 1 or 2 respectively when loaded in the agarose gel. If the genotype has heterozygote CT appears in two bands, one of them loaded in the first primer reverse1 and the other with the well loaded with Reverse 2 (Fig. 2).

In addition, the genotyping and allele frequencies for IL-17 rs 2275913 showed incompatible genotypes for both groups with Hardy-Weinberg equilibrium (Table 6). The TT, CT genotypes and T allele were non

significantly increased frequency percentage in scabies patients compared with control groups (1.3 vs. 0, OR:3.04, Pc value:0.75) and (12.0 vs. 8.0, OR:1.57, Pc value:0.429) respectively, and the T allele (7.0 vs.4.0, OR:1.90, Pc value:0.223) (Table 7).

The CC genotyping and C allele appeared non significantly decrease frequency percentage in the scabies patients group compared to a control group (86.7 vs. 92.0 OR: 0.57, Pc value:0.303) (93.0 vs. 96.0, OR:0.53, Pc value:0.223) respectively. The high OR value of TT, CT, genotypes, and T allele referred to this genotyping and allele might be risk factors for scabies, also the low OR value of CC genotype and C allele in patients group compared to control group might be a protective role from scabies disease. The significant increase in the level of IL-17 for the CC genotype in the patient group compared to the control group 866.73 ± 159.49^{A} and 20.95±8.15pg/ml respectively while there was a non-significant increase in the level of the CT genotype in the patient group compared with the control group,23.14 ± 12.49^{B} and 20.96±7.48 pg/ml (Table 8).

Table No. 2: The serum level of IL-17 between the study groups

Gender	IL-17 concentration (pg/ml)		Probability
	Scabies Patients (n=75)	Control (n=75)	
Males	948.95±247.62	20.08±1.18	0.000065
Females	531.33±233.01	22.34±1.50	0.057
Total	759.63±172.27	20.95±0.93	0.000032
Probability	0.089	0.993	

Table No. 3:The IL-17 SNPs rs8193036 genotyping frequencies and Hardy Weinberg compatibility in the patients' group compared to controls

Genotyping frequencies of IL-17	Patients group (n=75)		Control group (n=75)	
	Observed	Expected	Observed	Expected
GG	7 (9.3%)	22.4 (29.9%)	10 (13.3%)	13.2 (17.6%)
GA	68 (90.7%)	37.2 (49.6%)	43 (57.3%)	36.5 (48.7%)
AA	-	15.4 (20.6%)	22 (29.3%)	25.2 (33.6%)
P-HWE	1.0×10^{-12}		0.1258	

P-HWE: probability of Hardy-Weinberg equilibrium

Table No. 4: The IL-17 SNPs rs8193036 genotypes and alleles frequencies in the patients' group compared to controls

Genotyping and alleles frequencies of IL-17 rs8193036	Patients group (n=75)	Control group (n=75)	OR (95% CI)	P-value	Pc-value
G	82 (55.0)	63 (42.0)	1.67 (1.06-2.62)	0.037	0.029
A	68 (45.0)	87 (58.0)	0.06 (0.38-0.95)	0.037	0.029
GG	7 (9.3)	10 (13.3)	0.67 (0.24-1.85)	0.608	0.452
GA	68 (90.7)	43 (57.3)	7.23 (2.95-17.72)	4.5×10^{-6}	2.2×10^{-6}
AA	0 (0.0)	22 (29.3)	0.02 (0.0-0.26)	7.8×10^{-8}	3.3×10^{-8}

OR: odd ratio, 95% CI: 95% confidence intervals, P-value: Fisher's exact probability value, Pc-value: Bonferroni corrected probability value

Table No. 5: IL-17 serum levels according to the IL-17 SNPs rs8193036 of the studied groups

Genotyping frequencies of IL-17 rs8193036	IL-17 serum level (pg/ml)		Probability
	Patient Group	Control Group	
GG	456.57±278.67	20.56 ± 9.91^{A}	0.004
GA	790.83±187.83	21.25 ± 8.26^{A}	0.000290
AA		20.55 ± 7.04^{A}	
Probability	0.576		

Duncan test: The similar letters referred to non-significant differences (P > 0.05) between the genotyping of the control group

Table No. 6: The IL-17 SNPs rs 2275913 genotyping frequencies and Hardy Weinberg compatibility in the patients' group compared to controls

Genotyping frequencies of IL-17 rs2275913	Patients group (n=75)		Control group (n=75)	
	Observed	Expected	Observed	Expected
CC	65 (86.7%)	64.4 (85.9%)	69 (92%)	69.1 (92.2%)
CT	9 (12%)	10.2 (13.6%)	6 (8%)	5.8 (7.7%)
TT	1 (1.3%)	0.4 (0.5%)	-	0.1 (0.2%)
P-HWE	0.3107		0.7182	

P-HWE: Probability of Hardy-Weinberg equilibrium

Table No. 7: The IL-17 SNPs rs2275913 genotypes and alleles frequencies in the patients' group compared to controls

Genotyping and alleles frequencies of IL-17 rs2275913	Patients group (n=75)	Control group (n=75)	OR (95% CI)	P-value	Pc-value
C	139 (93%)	144 (96%)	0.53 (0.19-1.46)	0.318	0.223
T	11 (7%)	6 (4%)	1.90 (0.69-5.26)	0.318	0.223
CC	65 (86.7%)	69 (92%)	0.57 (0.20-1.63)	0.428	0.303
CT	9 (12%)	6 (8%)	1.57 (0.53-4.62)	0.558	0.429
TT	1 (1.3%)	-	3.04 (0.12-74.24)	1.0	0.750

OR: odd ratio, 95% CI: 95% confidence intervals, P-value: Fisher's exact probability value, Pc-value: Bonferroni corrected probability value

Table No. 8: IL-17 serum levels according to the IL-17 SNPs rs2275913 of the studied groups

Genotyping frequencies of IL-17 rs8193036	IL-17 serum level (pg/ml)		Probability
	Patient Group	Control Group	
CC	866.73±195.49 ^{A}	20.95±8.15	0.000017
CT	23.14±12.49 ^{B}	20.96±7.48	0.708
TT	426.40^{A}		
Probability		0.999	

Duncan test: The similar letters referred to non-significant differences (P > 0.05) between the genotyping of the control group

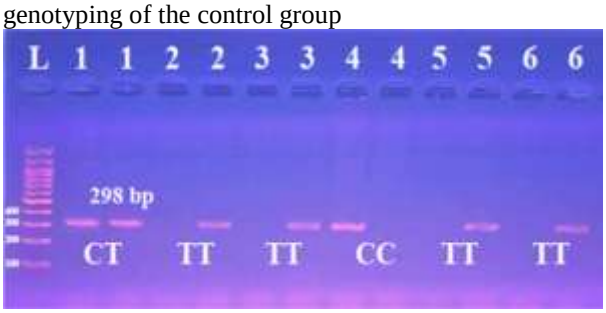

Figure No. 1: Electrophoresis of the IL-17 SNP rs8193036 gene resulting from PCR-ASP

DISCUSSION

Scabies is a parasitic skin disease brought by the mite Sarcoptes scabies var hominis.[15,15] The study indicated higher levels of IL-17 in the sera of scabies patients compared to the control group, and this agreed with previous studies.[5,17] IL-17 is a proinflammatory cytokine that is associated with allergic and inflammatory diseases, and it works to increase the secretion of IL-2, TNF-a, and IL-8, which have a major role in exacerbating the disease and amplifying the immune response.[18]

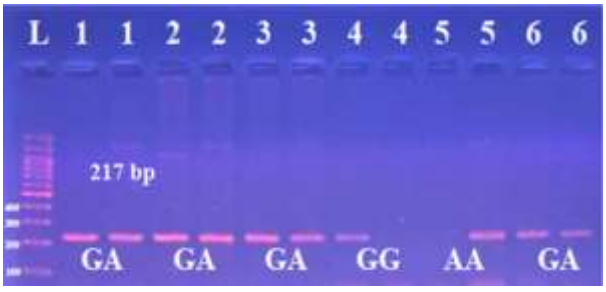

Figure No. 2: Electrophoresis of the IL-17SNP rs2275913 gene resulting from PCR-ASP

IL-17 also works to stimulate keratinocytes and many inflammatory cells near the sites of the parasite in the skin infected with the disease and thus works to induce an inflammatory response as the disease progresses.[19] The current study may be the first to study the polymorphism of the IL-17 gene, SNPs rs 8193036 and

rs 2275913, among scabies patients. Both SNPs were related with some disease,

IL-17A increased in sera of Iraqi patients with asthma. As well as to the rs2275913 variant was associated with a higher risk of pathogenesis of asthma, while the rs8193036 variant was possibly associated with protection from asthma in Iraqi patients.[20] A meta-analysis showed that there was significantly increase in circulating IL-17in patient with rheumatoid arthritis and there was evidence of associations between IL-17A rs2275913 polymorphism and the pathogenesis.[21] A study was reported on Erysipelas disease that the CT genotypes and the T allele have an important role as a risk factor for the disease.[22] Another study was also recorded on COVID-19 disease, indicating the active role of the AA genotype and the A allele as risk factors in the development of the disease.[21] Xie et al[23] reported that genetic variation of the IL-17 gene rs 2275913 for endometrial cancer in women. The AA genotype and the A allele are risk factors in the development of the disease, and a high level of this genotype is associated with a higher risk of developing uterine cancer.

CONCLUSION

IL-17 levels were high among scabies patients, as polymorphisms genotyping rs 8193036, according to the results based on the OR value the high OR value of GA genotype and G allele referred to this genotype and allele may be risk factors for scabies, while GG, AA genotypes, and A allele may be protective agents from the disease development. while the genotyping of rs 2275913 the high OR value of TT, CT, genotypes, and T allele referred to this genotyping and allele might be risk factors for scabies, also the low OR value of CC genotype and C allele in the patients group compared to control group might be a protective role from scabies disease.

Author's Contribution:

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Data Analysis:	Ali H. Abbas
Revisiting Critically:	Noora D. Abd, Nagham Y. Albayati
Final Approval of version:	By all above authors

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