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Effect of cutaneous Leishmaniasis infection on polymorphisms of IL-17A (rs2275913)

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Abstract

Leishmaniasis, a disease triggered by the parasitic protozoan Leishmania, is spread through the bite of infected female sandflies, specifically those of the Phlebotomus and Lutzomyia species. The body's defense mechanisms, both innate and adaptive, rely heavily on cytokines, a category of glycoproteins or modulatory proteins that orchestrate a crucial response to infection and disease. This study aimed to elucidate the relationship between serum IL-17A levels and single nucleotide polymorphisms (SNP) of IL-17A (rs2275913) with the susceptibility to cutaneous leishmaniasis. A total 200 samples of whole blood were collected form patients and controls at Baquba Teaching Hospital /Diyala Governorate/ Iraq from October 2022 to February 2023 which were used to measure serum IL-17A level using enzyme-linked immunosorbent assay and IL-17A SNP using High Resolution Melting Technique. The results revealed no significant differences in serum IL-17A levels between patients with cutaneous leishmaniasis and controls. However, high serum levels of IL-17A for patients were detected when compared with controls. Notably, younger patients (ages 16-25 and 26-35) showed decrease mean levels IL-17A when compared with controls. While, an increase statistically of levels IL-17A for adults and elderly (46-55) and (56-65) years old for patients when compared with controls. Additionally, there was an observed increase in IL-17A of patients' male versus with female compared with controls group. However, the distribution of serum IL-17A levels by SNP (rs2275913) demonstrated no differences between the genotypes of the groups. This study showed that the level of IL-17A was slightly elevated in patients when compared with controls. Interestingly, the results had revealed that the SNP for IL-17A (rs2275913) maybe a risk factor for susceptibility infection with cutaneous leishmaniasis, and considered the mutant allele A as a risk allele and genotype AA as more risk factor than the rest genotypes.

Keywords: Cutaneous Leishmaniasis, IL-17A serum level, IL-17A single nucleotide polymorphisms.

تأثير الإصابة بداء الليشمانيات الجلدي على تعدد الأشكال للأنترلوكين 17A (rs2275913)

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الخلاصة

داء الليشمانيات، وهو مرض يسببه طفيلي الليشمانيا الأبتدائي، والذي ينتقل عن طريق لدغة أنثى ذبابة الرمل المصابة، وخاصة تلك التي تنتمي إلى فصيلتي الفواصد و لتزوميا. وتعتمد آليات الدفاع في الجسم، سواء الفطرية أو التكيفية، بشكل كبير على السيتوكينات، وهي فئة من البروتينات السكرية أو البروتينات المعدلة التي تنظم الاستجابة الحاسمة للعدوى والمرض. تهدف هذه الدراسة إلى توضيح العلاقة بين مستوى المصل وتعدد الأشكال للأنترلوكين (rs2275913) 17A مع القابلية للإصابة بداء الليشمانيات الجلدي. تم جمع 200 عينة دم من المرضى و مجموعة السيطرة من مستشفى بعقوبة التعليمي / محافظة ديالى / العراق في الفترة من أكتوبر 2022 إلى فبراير 2023 حيث تم استخدام عينات الدم لقياس مستوى الأنترلوكين 17A في المصل باستخدام مقايسة الممتز المناعي المرتبط بالإنزيم، وكذلك حساب تعدد الاشكال للأنترلوكين 17A باستخدام تقنية HRM. كشفت النتائج عدم وجود فروق ذات دلالة احصائية بين مستويات الانترلوكين في مصل المرضى الذين يعانون من داء الليشمانيات الجلدي و مجموعة السيطرة. والجدير بالذكر أن المرضى الأصغر سنا (الذين تتراوح أعمارهم بين 16−25 سنة و26 −35 سنة) أظهروا انخفاضا في متوسط مستويات IL−17A عند مقارنتهم بمجموعة السيطرة. في حين أن هناك زيادة إحصائية في مستويات IL−17A للبالغين وكبار السن (46−55 سنة) و(56−65 سنة) للمرضى عند مقارنتهم بمجموعة السيطرة. بالاضافة الى ذلك، لوحظ ان هنالك زيادة في بيانات الأنترلوكين للمرضى الذكور مقابل الإناث عند مقارنتهم مع مجموعة السيطرة. ومع ذلك، فإن توزيع مستويات مصل الانترلوكين17Aبواسطة (rs2275913) SNP أظهرت عدم وجود فروق بين الانماط الوراثية للمجموعات. أظهرت هذه الدراسة أن مستوى الأنترلوكين 17A كان مرتفعًا قليلاً لدى المرضى بالمقارنة مع مجموعة السيطرة. ومن المثير للاهتمام أن النتائج كشفت أن تعدد الاشكال للأنترلوكين 17A (rs2275913) ربما يكون عامل خطر للإصابة بداء الليشمانيات الجلدي، واعتبرت الأليل الطافر A أليل بمثابة أليل خطر والنمط الوراثي AA كعامل خطر أكثر من بقية الأنماط الوراثية في هذه الدراسة.

1 - Introduction

Leishmaniasis is a serious parasitic disease characterized by a broad range of clinical manifestations. These can vary from localized skin lesions to severe and systemic visceral infections that affect internal organs [1]. Sandflies transmit infection to humans by inoculating metacyclic *Leishmania* promastigotes into exposed skin [2]. *Leishmania* parasite has numerous kinds of infections fluctuating between cutaneous and visceral, in which the epidemiological state, clinical features, prognosis and treatment response varies depending on the species [3]. Cutaneous leishmaniasis (CL) disease is endemic in tropical and subtropical regions and presents with two distinct clinical manifestations based on the species of *Leishmania spp.*: anthroponotic cutaneous leishmaniasis and zoonotic cutaneous leishmaniasis [4, 5]. Diverse cells (macrophages, CD4+T cells, CD8+ T cells, dendritic cells, and natural killer cells) in addition some cytokines (interferon-γ, interleukin 10) and effector molecules (nitric oxide and superoxide anion) have been reported to mediate the protective effects in mice and humans [6, 7]. Recently, a study by Naemah *et al.* indicated that nitric oxide levels (NO) were elevated in macrophages in patients with visceral leishmaniasis (VL) compared to controls [8]. Immunity against leishmaniasis in humans and animals is mediated by T lymphocytes. T lymphocytes play an important role in the development of a specific immune response and the formation of memory T lymphocytes against intracellular parasitic infections [9]. There is a strong correlation between the activation of different T cell subsets and disease progression [10]. The helper T17 cells are independently regulated CD4+ T lymphocytes, characterized as producing cytokines of the interleukin 17 family [11]. The interleukin-17 (IL-17) family of cytokines comprises six members, from IL-17A to F, all of which are crucial in combating against microbial organisms and in the progression of

inflammatory diseases. The pro-inflammatory cytokine IL-17 or IL-17A helps recruit neutrophils to the infection site [12].
SNPs are widely used molecular markers due to their wide distribution in the genome [13]. Genome sequencing databases reveal numerous polymorphisms in genes involved in immunity or disease resistance [14]. The IL-17A gene located on chromosome 6p12 in humans and consists of three exons and two introns [15]. Several diseases have been linked to single nucleotide polymorphisms (SNPs) that are critical in determining an individual's vulnerability to various diseases and responses to treatment [16]. The genes of human have been studied for potential susceptibility to Leishmania infection, with most related to the immune and some non-immune systems. Studies have identified several genes and genetic regions contributing to disease susceptibility [17]. This study aimed to elucidate the relationship between serum IL-17A levels and single nucleotide polymorphisms (SNP) of IL-17A (rs2275913) with the susceptibility to cutaneous leishmaniasis.

2. Materials and Methods

2.1. Study Subject

In the present study, 200 whole blood samples were collected from Baquba Teaching Hospital, consisting of 100 samples from patients suspected of having the cutaneous form of leishmaniasis and 100 non-infected with it from Diyala Governorate, and from October 12 in 2022 to February 12 in 2023. Infection was confirmed with cutaneous leishmaniasis parasite in patients by ELISA assay and using anti-Leishmania IgM antibodies using a commercially available kit, and the manufacturer's instructions were followed by SUNLONG BIOTECH/China. Information was collected from controls and patients using a pre-prepared questionnaire. Inclusion criteria included patients' age from 5 to 65 years, location of lesion, number of lesions, duration of lesion, and type of lesion. The exclusion of criteria included auto-immune dysfunction, inflammatory disease, genetic anomalies, and other systemic disorders.

2.2. Ethical Approval

Permission was granted by the Ministry of Health and Environment, Baghdad, Iraq. The study was approved by the local ethics committee of the Faculty of College of Science, University of Baghdad (ref: CSEC/0922/0094).

2.3. Measurement of the IL-17A Concentrations

Five ml of venous blood was collected from patients and controls by venipuncture, of which 3 ml was then placed in plain tubes and allowed to clot before being centrifuged at 4000 rpm through 10 minutes. The serum collected was stored at -80°C until it was needed for analysis. Additionally, 2 ml of the remaining blood was pipetted into EDTA-containing tubes for DNA extraction. The sandwich ELISA kit from (Inova Company, China) was used to test serum from the research groups for IL-17A levels by ELISA reader (Rayto RT-2100c Microplate Reader, 450 nm, China) [18].

2.4. Genetic Analysis

DNA was extracted from whole blood samples that had been frozen in EDTA tubes. The extraction process was carried out using a commercial kit manufactured by TransGen Biotech, a company based in China. The lyophilized primers were dissolved in nuclease-free water (according to instructions of manufacturer's), resulting in a 10 μM concentration, and stored at 20°C until needed for used. The primer used in this study is presented in Table 1.
Specific allelic discrimination of IL-17A SNP (rs2275913) was conducted using High Resolution Melting (HRM) technique via real-time qPCR (Qiagen. Germany) to evaluate IL-

17A genotyping. A reaction mix used for HRM component were, a 2x TransStart® Tip Green qPCR Super Mix containing Eva green was used from (TransGen, biotech. AQ141-01) (10 µl), Nuclease-free water (5 µl), Forward primer (1 µl), Reverse primer (1 µl), and DNA (3 µl) with a final volume 20 µl.

The study employed a Rotor gene Q Real-time CYTO PCR System (QIAGEN) and the thermal profile of the HRM technique includes one cycle of Enzyme Activation at 94℃ for 10 sec, followed by 40 cycles of Denaturation at 94℃ for 5sec, Annealing at 54℃ for 15sec, Extension at 72℃ for 20sec. Finally, the HRM investigation was conducted for (0.2 degrees, 1 sec) between 55 and 95 ◦C.

The HRM reaction was evaluated in duplicate, and allelic differences were established using qPCR-HRM on triple synthetic controls. Differential curves (DC) were constructed using the HRM Tool provided in the integrated program (Rotor gene 4.4).

Table 1: The primers Designed of IL-17A gene polymorphisms of the HRM technique.

SNP	Position	Primer Sequence	Product Size (bp)	Tm (°C)
rs2275913	2KB upstream variant	F- 5`ATTTCTGCCCTTCCCATTTT 3` R- 5`CCCCCAATGAGGTCATAGAA 3`	63	59.78 59.74

2.5. Statistical Analysis

The results were analyzed using Graph Pad software, version 8.0.1 for Windows. The IL-17A concentration was reported as a mean with a standard deviation (SD). One-way ANOVA analysis scrutinized significant differences. The study used a direct counting method to calculate genotype and allele frequencies of SNPs, and Hardy-Weinberg equilibrium (HWE) was studied using Michael H. Court's online calculator. The current study used WINPEPI version 11.65 to estimate odds ratios (OR) using chi-square and Fischer's exact probability, with P values less than 0.05 considered statistically significant. The population was consistent with HWE if P values were greater than 0.05.

3. Results

3.1. Serum level of IL-17A in the studied groups

The results revealed to non-significantly differences in the mean concentration serum levels of IL-17A ($p>0.05$) of cutaneous leishmaniasis patients and controls groups which were (83.091±16.922 pg/ml) and (80.350±14.859 pg/ml) respectively. However, the mean serum levels of IL-17A were higher in patients compared with controls (Figure 1).

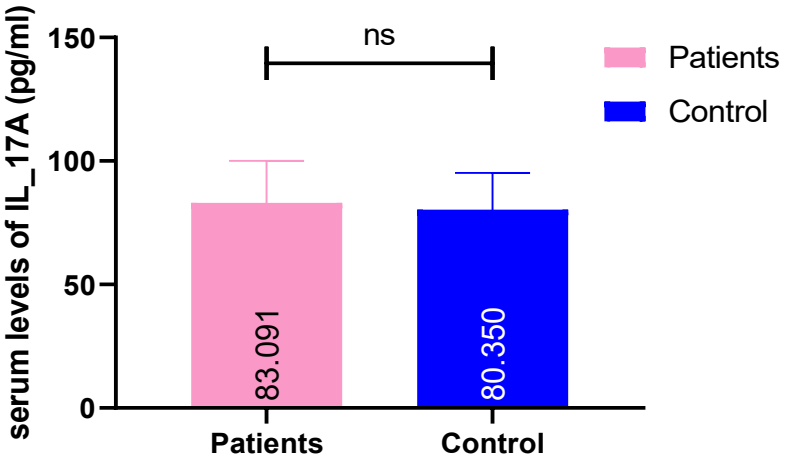

Figure 1: Comparison of IL-17A serum levels among patients and control groups (mean ± standard deviation).

3.1.1. Distribution of Leishmaniasis Cases by Age Groups

According to the age groups, figure 2 shows that the mean levels IL-17A concentration for (16-25) and (26-35) years old of patients' female and male were (79.00pg/ml, 82.396pg/ml) and (68.132pg/ml, 61.107pg/ml) comparing with controls (105.780pg/ml, 106.527pg/ml) and (79.380pg/ml, 77.173pg/ml) respectively. These findings indicated significant differences ($p< 0.01$). While the mean levels IL-17A concentration of adults and elderly (46-55) and (56-65) years old for patients' female and male were (91.793pg/ml, 102.012pg/ml) and (80.660pg/ml, 82.00pg/ml) comparing with controls (82.687pg/ml, 75.773pg/ml) and (57.340pg/ml, 68.330pg/ml) respectively. This pointed to increase data for IL-17A of patients' male versus with female compared with controls group ($p< 0.01$).

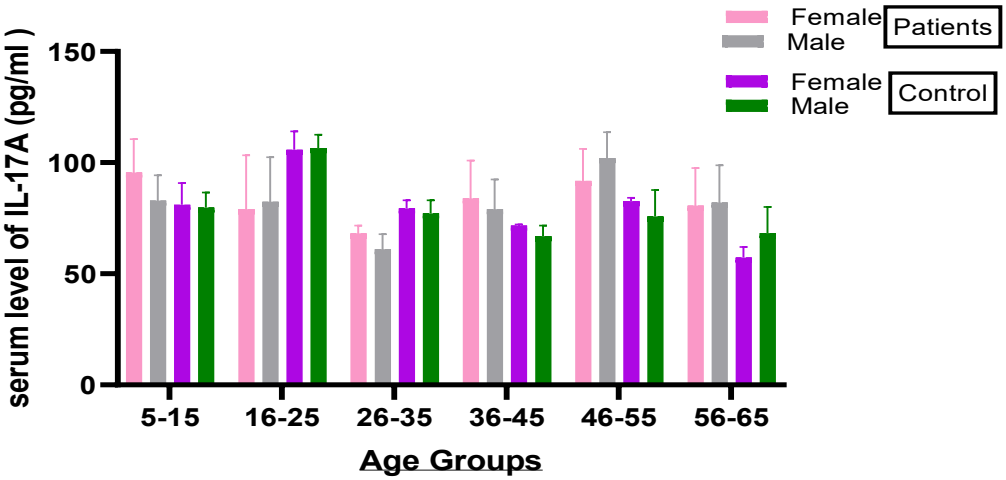

Figure 2: The mean serum level of IL-17A in different age according to cutaneous leishmaniasis.

3.2. SNPs of IL-17A in the studied groups

Figures 3A and 4A illustrate the amplification curves generated by High Resolution Melting (HRM) real-time PCR detection, comparing the DNA samples from patient and control groups, respectively, for the IL-17A single nucleotide polymorphism (SNP) at rs2275913. While, the HRM RT-PCR output for both patients and controls were depicted in Figure 3 (B) and Figure 4 (B) respectively, the separation double strands DNA are depending nitrogen base difference in temperatures where Guanine and cytosine require a higher temperature compared to adenine and thymine.

The results presented in table 2 indicated that IL-17A (rs2275913 G/A) genotype frequencies had a highly significant association in patients' group between the observed and the expected genotypes. Table 3 shows that the codominant genotypic model for GA and AA genotypes had a highly significant association between patients and controls, with a p-value= 0.01 and 0.0001, and an OR= 2.304 and 8.133 respectively. The genotypic dominant model appeared in the (GA+AA) genotypes show a high significant association between patients and controls with a p-value= 0.0001, OR= 3.650. Also, Recessive model revealed a high significantly association for genotype AA with a p-value= 0.0001, OR= 5.688 between patients and controls. The results showed that the over dominant genotypic model did not show significant association between patients and controls in genotype GA with (p-value= 0.5, OR=1.202). In addition, A allele show significant differences with (p-value = 0.0001, and OR=3.571). Also, the results revealed in table 4 that the polymorphic genotypes GA, AA and allele A have appeared a high significantly difference between cutaneous leishmaniasis patients and controls with (P-value= 0.01and 0.0001, and an OR=2.304 and 8.133 and 3.571)

respectively. Furthermore, the results showed the distribution of IL-17A serum levels by SNP (rs2275913) as shown in table 5; there are no differences between genotypes within and between both groups for patients and controls.

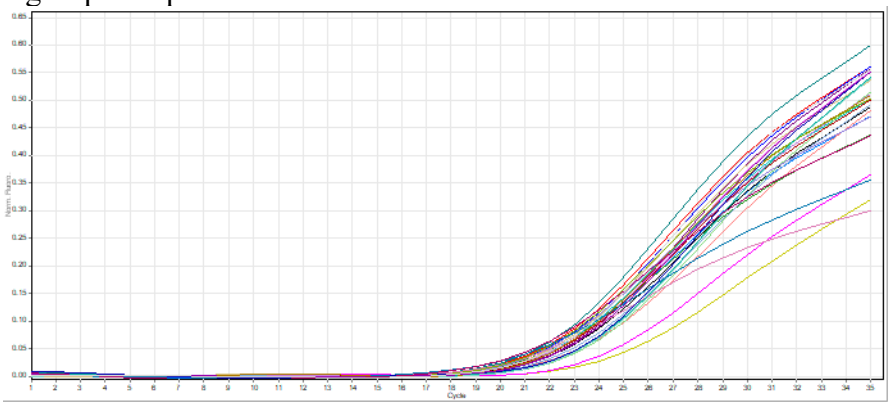

Figure 3: (A): Detection SNP IL -17A (rs2275913) amplification was plotted using qPCR samples that covered patients' group**.**

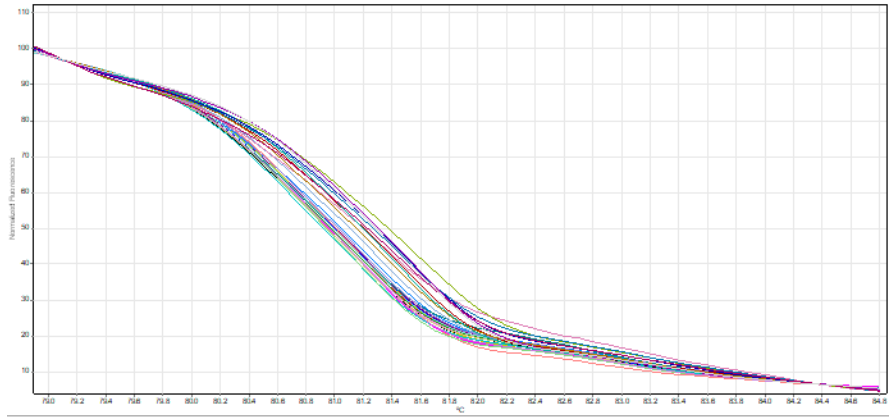

Figure 3: (B): The result output of HRM for the genotypes in SNP (rs2275913).

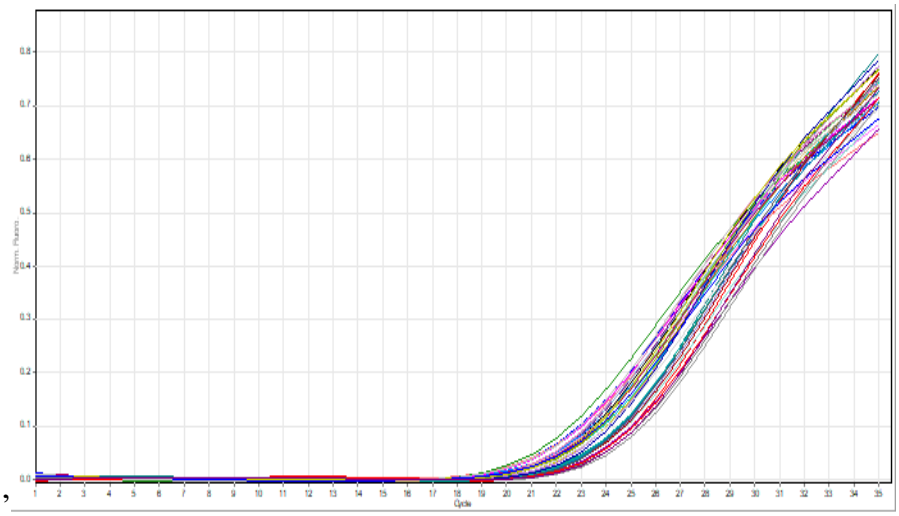

Figure 4: A: Detection SNP IL-17A (rs2275913) amplification was plotted using qPCR samples that covered controls' group.

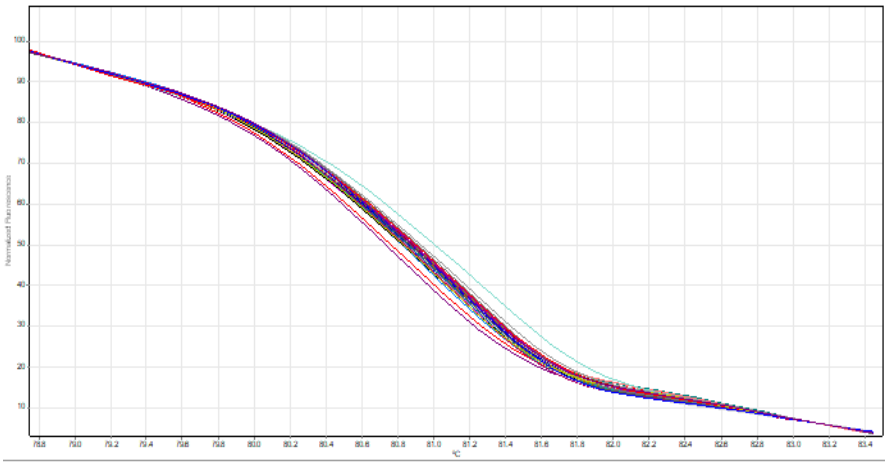

Figure 4: (B): The result output of HRM for the genotypes in SNP IL-17A (rs2275913).

Table 2: The percentage frequencies of genotype and Hardy-Weinberg Equilibrium (HWE) of IL-17A (rs 2275913) in the studied groups.

Groups			GG	GA	AA	HWE P>0.05
Patients	Observed	N	30	34	36	0.01**
		%	30%	34%	36%	
	Expected	N	22.09	49.82	28.09	
		%	22.09%	49.82%	28.09%	
Controls	Observed	N	61	30	9	0.07
		%	61%	30%	9%	
	Expected	N	57.76	36.48	5.76	
		%	57.76%	36.48%	5.76%	

Table 3: The genetic model of the alleles association and genotypes of IL-17A (rs2275913) in co-dominant, dominant, recessive, and over dominant models between cutaneous leishmaniasis and controls.

Genetic model	Genotype and allele	Patients N=100(%)	Controls N=100(%)	OR	P-Value
Codominant	GG	30	61	-	Reference
	GA	34	30	2.304 (1.194 to 4.447)	0.01**
	AA	36	9	8.133 (3.472 to 19.053)	0.0001**
Dominant	GG	30	61	-	Reference
	GA+AA	70	39	3.650 (2.029 to 6.563)	0.0001**
Recessive	GG+GA	64	91	-	Reference
	AA	36	9	5.688 (2.562 to 12.625)	0.0001**
Over dominant	GG+AA	66	70	-	Reference
	GA	34	30	1.202 (0.663 to 2.180)	0.5
Allele	G	0.47 (94)	0.76 (152)	-	Reference
	A	0.53 (106)	0.24 (48)	3.571 (2.330 to 5.474)	0.0001**

Explanations: OR: Odds ratio; CI: Confidence interval; P-value <0.05

Table 4: Comparison of the genotypes and allele frequency of IL-17A (rs2275913) between cutaneous leishmaniasis and controls.

Genotype	Patients	Controls	OR	P-Value
GG	30	61	-	Reference
GA	34	30	2.304 (1.194 to 4.447)	0.01**
AA	36	9	8.133 (3.472 to 19.053)	0.0001**
Total	100	100	-	-
Allele	**Patients**	**Controls**	**OR**	**P-Value**
G	0.47 (94)	0.76 (152)	-	Reference
A	0.53 (106)	0.24 (48)	3.571 (2.330 to 5.474)	0.0001**
Total	1.0 (200)	1.0 (200)	-	-

Explanations: OR: Odds ratio; CI: Confidence interval; P-value <0.05

Table 5: The distributions of IL-17Apg/ml (Mean± SD) serum level in patients and controls by SNP (rs2275913) genotypes.

Groups	SNPs genotypes			
	GG	GA	AA	p-value
Patients	40.738±0.0	42.790±9.159	40.992±9.190	0.7
Controls	39.485±7.710	41.127±6.752	45.955±6.668	0.4
p-value	0.6	0.5	0.4	-

OR: Odds ratio; CI: Confidence interval; P value <0.05

4. Discussion

Research has shown that cells producing Interleukin-17 play a crucial role in inflammation in skin lesions of CL patients, indicating that they may be involved in regulating the parasite's presence in skin tissue [19]. IL-17 is expressed at a higher level in the peripheral blood and tissues of patients with mucosal and cutaneous leishmaniasis [20]. IL-17 in humans is associated with inflammation and recruitment of neutrophils to the site of infection during infection with *L. major* and *L. braziliensis* [21]. This current result (Figure 1) is in agreement with the previous study by Oliveira *et al.* [22]. Researchers discovered that IL-17 cells facilitate neutrophil infiltration in human mucosal lesions caused on by *L. braziliensis*. Bacellar *et al.* found that IL-17 expression increases in peripheral blood mononuclear cell cultures after *Leishmania* antigen stimulation [23], suggesting despite its defense role, also this cytokine contributes to disease severity [24]. Moreover, in mice the Th17 cells contribute to the progression of CL [22], and this cytokine (IL-17) contributes to the severity of the disease. While, IL-17 mediates neutrophil attraction and release proteinases, causing tissue damage [25].

The observed decrease in mean serum levels IL-17A concentration levels among young patients (Figure 2) in this study may be attributed to several factors: deference number of samples between patients and controls, or perhaps several factors other than parasite dose may be important in promoting pathology, such as patient genetic background, vector influence, site of infection, co-infection, and skin micro flora (presence or absence of secondary bacterial infection) and/or the species/strain of the parasite, each of which may contribute to a much more pronounced early immune response following natural infection [26, 27]. Previous study agrees with these results about low level of IL-17A for young's

patients when compared with controls; Hussein *et al.* show that the IL-17 level in positive group of cutaneous leishmaniasis and co-infection was (53.42 ± 29.11) compared to the without ulceration group (83.80 ± 40.82), the difference was statistically significant (p = 0.043) between the positive culture (co-infection) and the absence of ulceration. Ulceration with secondary bacterial infection is one of the complications of the disease that can increase tissue destruction and lead to scarring, so the possible presence of co-infection has an effect on the lower level of IL-17 [28], thus leading to CL ulceration and survival long-term period of illness.

The findings of the current study, specifically in the 46-55 and 56-65 age groups for both female and male patients (Figure 2), are in line with previous research on mucosal leishmaniasis and American cutaneous leishmaniasis, which have consistently shown that higher levels of IL-17 are produced [23]. Also, Katara *et al.* showed that IL-17 levels were found to be significantly higher in CL samples compared to controls [29]. AL-Saady, in Diyala province in Iraq found increase IL-17 concentration of patients with CL compared with control group (67.02. ± 38.3pg/ ml), (38.21±1.87pg/ml) respectively [30]. Besides, the study by Flaih *et al.* in Thi-Qar province in Iraq recorded a significant high level of IL-17A in patients of early dermal lesion infected with *L. major* in comparison with healthy individuals which recorded (240.5± 12.14pg/ml) and the serum level of IL-17A in patients was (374.3 ± 43.7pg/ml) [31]. Also, this finding was consistent with Flaih *et al.* [32]. As well as, the current results pointed to increase data for IL-17A of patients' male versus with female when compared with controls group ($p< 0.01$) and this agree with previous study by Ali and Al-Hadraawy, whom mentioned that the mean concentration of IL-17 was highest significant ($P< 0.05$) between patients and control groups (360.92 ±82. 766pg /ml) versus (38.974± 4.922pg/ml) respectively; in addition, the concentration of IL-17 was increased significantly ($p<0.05$) between male and female in CL patients' group. The increased expression maybe attributed to a higher level of cellular activation or a rise in the number of cytokine-producing cells [33].

A significant deviation from equilibrium (Table 2) was observed for cutaneous leishmaniasis in Iraq, which may be attributed to limitations in sample size limitations and a larger population could provide a better profile of gene SNP impact on cytokine serum levels [34]. While, the genotypic frequencies of IL-17A (rs2275913 G/A) for controls had been no significant association between the observed and the expected genotypic frequencies. This finding agrees with Ad'hiah *et al.* study in Iraq, which found that no significant differences between observed and expected genotype frequencies for ten cytokines [34], indicating good agreement with the equilibrium.

In this study, it was demonstrated that an odds ratio (OD) for the allele A and genotypes GA, AA and (GA+AA) was greater than 1, indicating that the risk of infectious disease increases when IL-17A levels are elevated in patients [35]. The present study (Table 3), (Table 4) and (Table 5) reported for the first time in Iraq, showed a significantly higher frequency of allele A and GA, AA and (GA+AA) genotypes for IL-17A (rs2275913) with an elevation in the IL-17A level in patients' sera compared to controls. Therefore, this level of IL-17A elevation suggests a risk factor for cutaneous leishmaniasis susceptibility infection. Interestingly, the genotype AA suggest as risk factor for infection more than GA and (GA+AA) genotypes because the genotype AA is homozygote for A allele which suggested as risk allele by which should be higher risk of Cl infection. This is in disagreement with a previous study conducted in Brazil by de Albuquerque *et al.* who showed that the allele A increases the production of IL-17 in the healthy individuals. Whilst, IL-17 production was

decreased in *L. braziliensis* -infected individuals carrying the mutant allele and these patients retained higher parasite loads. Also, de Albuquerque *et al.* demonstrates that the allele A is not strongly linked to CL susceptibility [36]. The reason for the elevation of IL-17A in patient sera may be that the SNP rs2275913 exchange of guanine for adenine at the 197th nucleotide position upstream of the start codon of the human IL17A gene. Despite of IL-17 plays a critical role in autoimmunity, allergic and infectious diseases, this mutation has been linked with a great number of pathologies [37, 38]. Single nucleotide polymorphism rs2275913, also known as G-197A, is an SNP located within a binding motif for nuclear factor of activated T cells (NFAT), which is an essential regulator of the IL-17 promoter and therefore has an effect on the regulation of the IL-17 gene. Genetic studies have shown that the IL-17 197A allele is associated with higher IL-17 production and higher affinity for NFAT [39]. IL-17 is linked to inflammation and neutrophil recruitment during *L. major* infection in humans, potentially causing tissue damage at the infection site [21]. Variations in genes encoding cytokines can significantly influence an individual's susceptibility or resistance to disease. This influence is particularly pronounced when these genetic variants occur within pathways involved in protecting against infections [40]. In the case of leishmaniasis, salivary components of the vector species, parasite virulence, and nutritional status are other determinants of susceptibility [41]. Studies show that *Leishmania spp.* infections significantly increase IL-17 production [23, 42]. The presence of various T cells in PBMCs may contribute to immunological interactions that enhance or block Th17 differentiation in *L. braziliensis* infection [43]. Khatonier *et al.* showed that the allele A was significantly associated with an increased risk of kala-azar in India [13]. Given the complexity of the host immune system and parasite biology, SNP analyses suggest that it is unlikely that genetic variation at a single locus is the cause, is sufficient to explain variations host immunological responses in individuals, which give rise to a range of clinical reactions symptoms. As a result, the discovery of gene-gene interactions can improve the accuracy and potency of complicated disease prognosis [44]. Functional evaluation of illness associated polymorphisms is critical for providing a clearer understanding of the genetic architecture of disease susceptibility and for unambiguously identifying the variables involved for both causation and predisposition to a disease. Noncoding polymorphisms significantly influence the variance and etiology of a characteristic by regulating the expression of neighboring genes, and that variations in gene expression may be a significant source of phenotypic variability in complex disorders [45]. The protozoan parasite modulates the host innate immune response into its own benefit that reflects the need for a highly efficient proinflammatory response for protection. Cytokines are essential in the host immune response and host parasite interaction [46, 47]. IL-17A (rs2275913) polymorphisms have been linked to various diseases, including rheumatoid arthritis [48]. As well the polymorphism of IL17A could be associated with Chagas disease, caused by the infection of *Typanosoma cruzi*, can lead to chronic cardiomyopathy due to the differential expression of the cytokine IL-17A [49]. Whilst, another study exposed a protective effect of IL-17 (rs2275913) polymorphisms in Brazilian population tuberculosis [50].

Conclusion

This study highlighted the role of IL-17A in the immune response during cutaneous leishmaniasis infection before treatment, as interleukin levels were slightly elevated in patients compared with controls. The findings indicated that the SNP for IL-17A (rs2275913) may serve as a risk factor for susceptibility infection with cutaneous leishmaniasis, and considered the mutant allele A as a risk allele and genotype AA as more risk factor than the rest genotypes that appeared in the study.

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