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The relationship between the Serum Level and IL-4 Polymorphism (rs2243250) with Susceptibility to Cutaneous Leishmaniasis

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Abstract

Background. Leishmaniasis is a parasitic infection caused by *Leishmania* protozoan parasites. Cytokines is a group of glycoproteins which play a crucial role in the body's defense mechanisms for both innate and adaptive in responding to infection and disease. **Aim.** The present study aimed to elucidate the association between Interleukine-4 (IL-4) serum levels and single nucleotide polymorphisms (SNP) of IL-4 (rs2243250) with the cutaneous leishmaniasis (CL) susceptibility. **Methods.** From Baquba Teaching Hospital which is located in Diyala Governorate/ Iraq, 200 samples of whole blood of patients and controls were collected from October 2022 to February 2023 which were utilized to measure the serum level of IL-4 using enzyme-linked immunosorbent assay and IL-4 SNP (rs2243250) utilized High Resolution Melting Technique. **Results.** The findings showed no significant differences between the serum levels IL-4 of CL in patients and control. Although, the serum levels of IL-4 were slightly elevated in patients when compared with controls. Also, the results showed no significant differences between patients and controls of female and male for all age groups with slight decrease was noticed in the serum levels IL-4 for patients' female compare to the controls. Furthermore, there were significantly differences between patients and control by data of polymorphisms in the genotype models for CT, TT and CT+TT and allele T with ($P < 0.001$) and $OR > 1$. Whilst, the distribution serum levels of IL-4 by SNP (rs2243250) revealed no significant differences of the genotypes between the two groups. **Conclusion.** Tacking together, the SNP IL-4 (rs2243250) had the T allele (P=0.0001, OR=4.696) and the genotype TT (P= 0.0001, OR=9.857) in codominant genetic model which is consider as higher risk factor for the infection of CL than the other genotypes.

Keywords: Cutaneous Leishmaniasis, IL-4 serum level, IL-4 (rs2243250) single nucleotide polymorphisms.

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Introduction

Leishmaniasis is one of the most important infectious diseases that causes serious health concern around the world (1). It can exhibit at least three clinical types: cutaneous, mucocutaneous, and visceral (2). Each condition relies on the host genetic variability and thus their immune responses, the transmitting sandfly vector, environmental factors and on the species of parasite (3). Cutaneous leishmaniasis is caused by various *Leishmania* species, including at least 21 species and subspecies, which can also cause human leishmaniasis (4). *Leishmania* infection progresses into disease due to factors like cytokine suppression, T cell exhaustion, and humoral response defects, with genetic changes determining the progression or control of the infection (5). Cytokines like IFN-γ activate macrophages, enhancing their microbicidal activity to kill intracellular pathogens by producing reactive oxygen and reactive nitrogen species (6). IL-4 is a key immune cytokine involved in adaptive immunity, inhibiting pro-inflammatory cytokines and regulating macrophage function. It also acts as a B cell mitogen and increases the secretion of IgG and IgE (7). IL-4 promotes a Th2-type response during *Leishmania* infection (8). Also, promoting parasite survival and proliferation. IL-4 inhibits the production of IFN-γ by CD4+ T cells, thus suppressing the protective Th1 immune response (9).

Generally, SNPs are the most prevalent genetic variants in the human genome, are widely used molecular markers due to their widespread distribution and low cost (10, 11). SNPs, located within the genome's coding regions, are used as markers in genetic disease research, while those relying on non-coding areas are used in evolutionary studies, playing a crucial role in determining an individual's disease vulnerability and treatment response (12, 13). The IL-4 gene consists of four exons and is located on chromosome 5 and the promoter region contains various polymorphic sites (14), polymorphisms in this gene have been linked to various diseases affecting immune response modulation (7). Numerous human genes have been investigated as potential susceptibility factors for *Leishmania* infection, with numerous genes and regions identified through candidate gene and genome-wide linkage studies (15). The present study aimed to elucidate the association between IL-4 serum levels and single nucleotide polymorphisms (SNP) of IL-4 (rs2243250) with the cutaneous leishmaniasis susceptibility.

Materials and Methods

Study Subject

In the present study, a total 200 samples from whole blood were collected from Baquba Teaching Hospital, which including 100 samples from patients suspected of having the cutaneous form of leishmaniasis and 100 non-infected with it from Diyala Governorate, and from the date October in 2022 to February in 2023. Patients with CL parasite infection were confirmed using ELISA assay and Human Anti-*Leishmania* IgM Ab using a kit commercially available, and the manufacturer's instructions were followed by SUNLONG BIOTECH/China and Catalog No.: SL3382Hu. The data for patients and controls was gathered through a pre-prepared questionnaire. The Patients group include the children and adults aged from (5 to 65 years) whom have been diagnosed with Cutaneous leishmaniasis by a doctor and based on epidemiological data (Figure 1), including the number of lesions from (1 to 3 lesions), duration, location, and type of lesion while the control group include healthy children and adults aged from (5 to 65 years) without Cutaneous leishmaniasis history or scars from similar endemic areas and shared

environments, do not suffer from chronic diseases, auto immune dysfunction, inflammatory diseases, or other systemic disorders. Patients with more than 3 lesions, autoimmune dysfunction, inflammatory disease, chronic disease, and other systemic disorders were excluded.

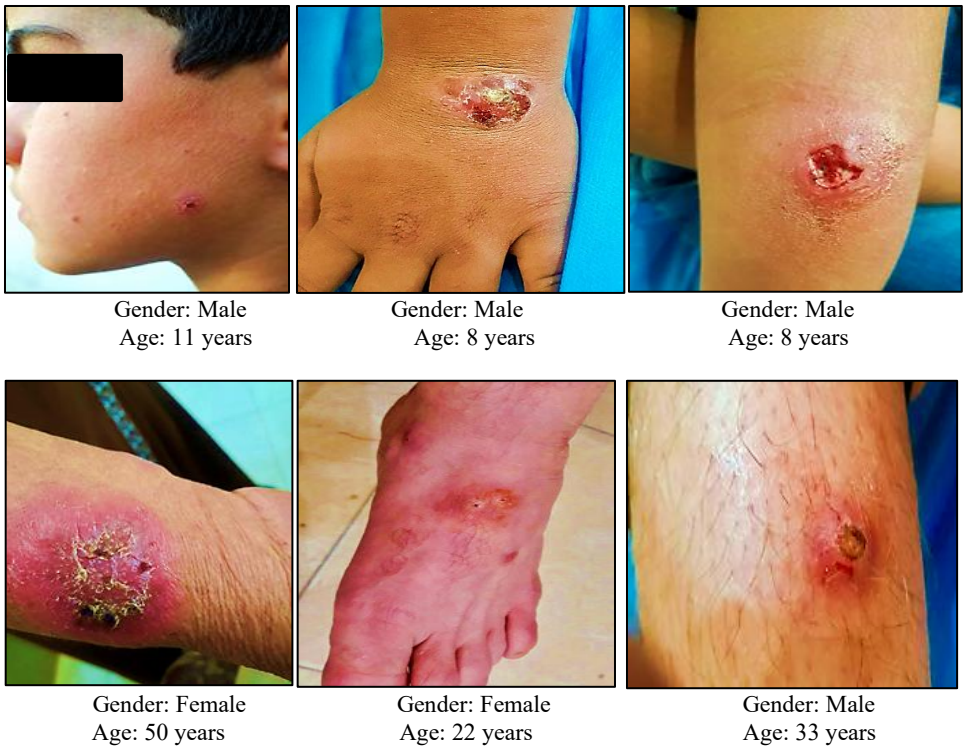

Figure 1: Pictures of patients with Cutaneous leishmaniasis (CL)

Measurement of the IL-4 Concentrations

Five ml of venous blood was collected from patients and controls by venipuncture, 3 ml was clotted, and the remaining 3 ml was centrifuged at 4000 rpm for 10 minutes. The serum was stored at -80°C, and the remaining 2 ml of blood was pipetted into EDTA containing tubes for DNA extraction .The sandwich ELISA kit was utilized to test IL-4 serum levels (from Inova Company/ China) using an ELISA reader (16).

Genetic Analysis

Frozen whole blood stored in an EDTA tube was used for DNA extraction using a kit from the commercial company TransGen Biotech/China. The lyophilized primers were dissolved in nuclease-free water (as per manufacturer's instructions) to create a 10 µM concentration and stored at 20°C until needed, as shown in Table 1. Specific allelic discrimination of IL-4 SNP (rs2243250) was conducted using High Resolution Melting (HRM) technique and real-time qPCR (Qiagen. Germany) to evaluate IL-4 gene genotyping. Additionally, a 2x TransStart® Tip Green qPCR Super Mix containing Eva green was used from (TransGen, biotech. AQ141-01). The HRM reaction was duplicated and allelic differences were established using qPCR-HRM on triple synthetic controls, with differential curves (DC) constructed using the HRM Tool in the integrated program (Rotor gene 4.4) (17,18).

Table 1: The primers Designed of IL-4 gene polymorphisms used of the HRM technique.

SNP	Position	Primer Sequence	Product Size (bp)	Tm (℃)
rs2243250	Promoter	F- 5` CGACCTGTCCTTCTCAAAACA3` R- 5` AACAGGCAGACTCTCCTACCC3`	76	60.28 59.75

Statistical Analysis

The study used Graph Pad software, version 8.0.1 for Windows to analyze IL-4 concentrations and reported as a mean with a standard deviation (SD), with one-way ANOVA for significant differences. Genotype and allele frequencies of SNPs were calculated using direct counting, and Hardy-Weinberg equilibrium was studied using Michael H. Court's online calculator. WINPEPI version 11.65 was used to estimate odds ratios using chi-square and Fischer's exact probability, with P values less than 0.05 considered statistically significant. The population was considered consistent with HWE if P values were greater than 0.05.

Results.

In the current study, the mean serum levels IL-4 for patients and controls were (65.747±9.464pg/ml) and (63.234±15.213pg/ml) respectively, that pointed to non-significantly differences between the two groups (P>0.05). Although, the mean serum levels IL-4 was slightly elevated in patients when compared with controls (Figure 2).

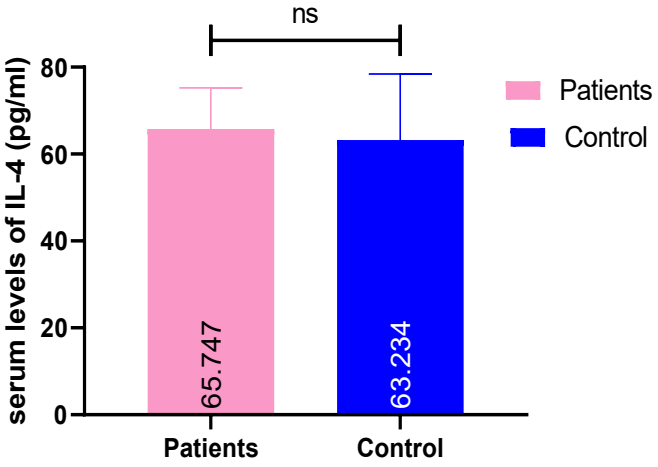

Figure 2: Comparison of IL-4 serum levels among patients and control groups (mean ± standard deviation).

In this study the findings shows the Distribution of Leishmaniasis Cases by Age Groups (Figure 3), that the serum levels IL-4 for all age groups, the results showed non-significantly differences (P>0.05) between patients and controls for female and male. Also noticed a slight decrease in the IL-4 serum levels in female patients' compare to the control with (P>0.05).

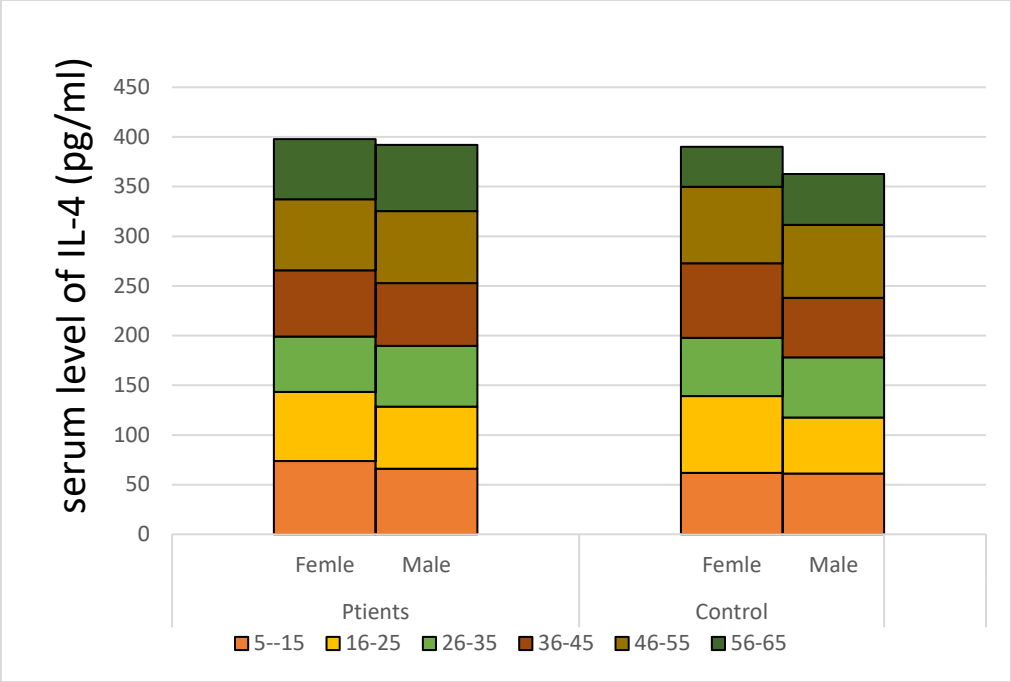

Figure 3: Clustered stacked bar of mean serum level IL-4 in different age according to cutaneous leishmaniasis.

In this part of research, according to the percentage of genotype frequency and Hardy-Weinberg Equilibrium (HWE) of IL-4 (rs2243250) in the studied groups, the findings in Table 2 revealed that IL-4 (rs2243250 C/T) genotype frequencies had a highly significant association in patients' group between the observed and the expected genotypes. Also, the genotypic frequencies of IL-4 (rs2243250 C/T) recorded a highly significant correlation in controls' group between the genotypes for observed and expected.

Table 2: The percentage of genotype frequency and Hardy-Weinberg Equilibrium (HWE) of IL-4 (rs2243250) in the studied groups.

Groups			CC	CT	TT	HWE P>0.05
Patients	Observed	N	28	36	36	0.006**
		%	28%	36%	36%	
	Expected	N	21.16	49.68	29.16	
		%	21.16%	49.68%	29.16%	
Controls	Observed	N	69	22	9	0.002**
		%	69%	22%	9%	
	Expected	N	64	32	4	
		%	64%	32%	4%	

Table 3 demonstrates that the genetic models of codominant, dominant and recessive for the genotypes CT, TT and CT+TT had a highly significant association between patients and controls, with a p-value= 0.0001, and an

OR=4.033, 9.857, 5.724 and 5.688 respectively. Also, the genotypic model of over dominant have showed significantly association between patients and controls in genotype CT with (p-value= 0.3, OR=1.994). In addition, T allele show a highly significant difference with (p-value = 0.0001, and OR=4.696). Also, the results showed in Table 4 that the polymorphic genotypes CT, TT and allele T have appeared high significantly differences between patients and controls with (P-value= 0.0001, and an OR=4.033, 9.857 and 4.696) respectively. The results showed the distribution serum levels of IL-4 by SNP (rs2243250) as revealed in Table 5; there are no differences between genotypes within and between both groups for patients and controls.

Table 3: Alleles association and genotypesof IL-4(rs2243250) in codominant, 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	CC	28	69	-	Reference
	CT	36	22	4.033 (2.025 to 8.029)	0.0001**
	TT	36	9	9.857 (4.203 to 23.12)	0.0001**
Dominant	CC	28	69	-	Reference
	CT+TT	72	31	5.724 (3.115 to 10.52)	0.0001**
Recessive	CC+CT	64	91	-	Reference
	TT	36	9	5.688 (2.562 to 12.62)	0.0001**
Over dominant	CC+TT	64	78	-	Reference
	CT	36	22	1.994 (1.068 to 3.726)	0.03*
Allele	C	0.46 (92)	0.8 (160)	-	Reference
	T	0.54(108)	0.2 (40)	4.696 (3.011 to 7.322)	0.0001**

Table 4: The genotypes and allele frequencyof IL-4 (rs2243250) between cutaneous leishmaniasis and controls.

Genotype	Patients	Controls	OR	P-Value
CC	28	69	-	Reference
CT	36	22	4.033 (2.025 to 8.029)	0.0001**
TT	36	9	9.857 (4.203 to 23.12)	0.0001**
Total	**100**	**100**	-	-
Allele	**Patients**	**Controls**	**OR**	**P-Value**
C	0.46 (92)	0.8 (160)	-	Reference
T	0.54 (108)	0.2 (40)	4.696 (3.011 to 7.322)	0.0001**
Total	**1 (200)**	**1 (200)**	-	-

Table 5: Distributions of serum level for IL-4 pg/ml (Mean± SD) in patients and controls by SNP (rs 2243250) genotypes.

Groups	SNPs genotypes			
	CC	CT	TT	p-value
Patients	32.868±5.171	32.363±3.811	34.058±4.914	0.5
Control	30.782±7.072	34.224±5.066	38.537±5	0.1
	0.4	0.3	0.1	-

Discussion

Leishmania parasites' maintain their presence in immunocompetent hosts through continuous strategies that can manipulate and subvert innate and adaptive immune responses (19). In vitro studies reveal that the *Leishmania* species and host and parasite isolates significantly influence the immune response to CL, characterized by a mixed T-helper 1 and T-helper 2 response, with the Th1 immune response crucial for controlling parasite growth and preventing Leishmania dissemination (20, 21, 22). Th1 typically produces a significant amount of IL-2 and IFN-γ (23). On the other hand, The Th2 immune response characterized by the production of cytokines IL-4 and IL-10, is responsible for the development and chronicity of lesions in *Leishmania* infection (24). The current study demonstrated non-significantly results with slightly elevate for serum levels IL-4 in patients comparing with controls; the reason may be due to the high response of innate immunity in the early stages of acute infection to control the parasite infection. The complement system is a vital part of the innate immune defense, activating classical, alternative, and lectin pathways, triggering proteolytic cascades that produce anaphylatoxins, opsonins, and the membrane attack complex, leading to pathogen lysis and inflammatory response (25). The complement cascade is manipulated by inactivating opsonins to enhance macrophage attraction (26, 27). Studies indicate that lectin pathway is effectively activated by mannose-binding lectin, which binds to lipophosphoglycans in microorganisms like *Leishmania*, initiating a proteolytic cascade causing pathogen lysis (28, 29). The immune response to *Leishmania* infection is complex and varies based on many factors, including the strain of *Leishmania* parasite causing the infection, the host's genetic background and the stage of disease progression (30). Immune response against intracellular parasites is dependent on the balance between Th1 and Th2 cells. In the current study, the first reason of non-significantly findings for IL-4 levels in patients' sera that the disease may be in the early stages of infection and the innate immune response be active in the infection site that indicates to the inflammation in the acute stage. So, IFN-γ inhibit the production of cytokines such as IL-4 and IL-10 which associated with Th2 response in early infection's stages (31). Or the second reason maybe male and female sex hormones, such as estrogen and testosterone, play a role in regulating immune responses through their pro-inflammatory and anti-inflammatory properties (32). Furthermore, studies indicate that 17β-estradiol can enhance nitric oxide

production from macrophages in a pro-inflammatory cytokine-independent manner (33). Perhaps, the third reason concerning with random collection strategy of samples number in this study. Additionally, the current results demonstrate slightly decrease of IL-4 levels in patients' female compared with controls may indicate activation Th1-type response. However, when progression of infection, the research showed that IL-4 levels in patients with cutaneous leishmaniasis vary significantly with age and disease status, with higher levels observed in chronic non-healing lesions compared to healed ones, indicating a correlation between IL-4 and disease severity (34). Also, age significantly impacts the immune response, with elderly patients showing increased IL-4 and decreased IFN-γ production, potentially contributing to disease persistence and severity (35). In this study, according to the percentage of genotype frequency and Hardy-Weinberg Equilibrium (HWE) of IL-4 (rs2243250) in the studied groups, a significant departure from equilibrium was recorded for cutaneous leishmaniasis in Iraq, the reason may be due to sample size limitations and a larger population could provide a more accurate profile of the impact of gene SNPs on serum levels of cytokine (36). This study contradicts the Ad'hiah et al. (36) a study in Iraq, which conducted to that non significantly differences between observed and expected genotype frequencies for certain cytokines including IL-4 (2243250). The findings illustrated that the odds ratio (OD) for allele T and genotypes CT, TT, and (CT+TT) were greater than 1 indicates the potential elevate risk development to infective disease when elevated levels of IL-4 in patients (37). Also, IL-4 is cortical for the forming of Th2 cells, which are linked to an increased risk of cutaneous leishmaniasis (38). The IL-4 levels in the current study were slightly elevated in patients' sera when compared to controls. Frequently, IL-4 is observed as detrimental in the context of cutaneous leishmaniasis, it is important to consider that its role can vary based on the timing and context of immune activation (39). Early IL-4 production in BALB/c mice increases Th2 susceptibility to *Leishmania major* (40), while IL-4 can also instruct dendritic cells to produce IL-12, promoting Th1 responses during initial immune activation (41). The host's ability to produce Th1 or Th2 type cytokines may influence susceptibility to CL and the development of different clinical forms, with genetic polymorphisms in their genes believed to influence the secretion of Th2 (IL-4) and Th1 type cytokines (IFN-γ, TNF-α and TNF-β) (42). Hence, this polymorphisms (rs2243250) in IL-4 gene may potentially influence on susceptibility of CL via make variations in IL-4 production or signaling; so, it may conceder as a risk factor for development of disease infection. The current results were agreed with previous study by Kirik *et al.* (43) in Turkey whom indicated that IL-4 polymorphisms C/T (rs2243250) are significantly associated with susceptibility to CL and individuals carrying T allele at IL-4 gene SNP (rs2243250) are at a higher risk for CL. Another study by Kamali- Sarvestani *et al.* (42) in Iran who suggested that polymorphisms in the IL-4 C/T

(rs2243250) could influence the risk of CL developing. In other parasites, the gene IL-4 (rs2243250) is a susceptibility factor for schistosomiasis in Nigeria, and essential to regulate disease burden, with risk factor for disease among carriers of the genotype TT for IL-4 (rs2243250) variant (44). Mishra *et al.* (45) study in India found no association between IL-4 polymorphisms C/T (rs2243250) and VL, suggesting other genes may offer insights into IL-4 regulation and immune-pathogenesis. Or perhaps, IL-4 significantly influences the immune response by modulating the immune response in body when infected with CL, typically skewing the response towards a Th2 profile; IL-4 plays a crucial role in the regulation of humoral and adaptive immunity, facilitating the differentiation of naive CD4+ T cells into Th2 cells (46). Besides, IL-4 plays a crucial role in regulating the production of Th2 associated cytokines like IL-5, IL-10, and IL-13 (47). IL-4 and IL-13 have been linked to the survival and persistence of parasites (48). IL-4, IL-13, and STAT6 are found to reduce susceptibility by inhibiting the production of protective Th1 immune responses (49). Or perhaps IL-4 may relate to increase parasites burden and the severity of disease in susceptible hosts (50). However, there are other factors concerned with development of CL infection such as the environment, the insect vector are all significant factors to consider (51). The current results of CL patients and controls in Iraqi population according to genotypes and allele frequency of IL-4 (rs2243250) reveals that the genotype TT have (OD=9.857, P value=0.0001) suggest as a risk factor for development CL greater than CT and (CT+TT) genotypes have (OD=4.033, 5.724 and P-value=0.0001) because the genotype TT is homozygote for T allele (OD=4.696, P value=0.0001) which consider as potential risk allele for higher development for CL infection. While, the IL-4 (rs2243250) revealed a significantly difference in genotype frequencies between CL patients and controls in Turkish population (P = 0.001). The genotype TT+CT (OD=2.049, P value= 0.041) indicating more susceptible to CL from the genotypes CT and TT. Additionally, the allele frequency analysis reveals C versus T allele (OR = 2.441, P = 0.002), this suggests that the T allele may increase the risk of developing CL (43). The distribution serum IL-4 levels in CL patients vary significantly based on clinical presentation and genetic factors, with elevated levels observed in patients with chronic non-healing lesions compared to healed lesions and healthy controls, suggesting a correlation between IL-4 and disease severity. Chronic lesions patients have higher IL-4 levels than healed lesions, while healthy controls have significantly lower levels (34). Therefore, the results of the current study (Table 5) do not show any significant differences in the distribution serum levels IL-4 for genotypes between the CL patients and the controls that is according to the limitations of samples' collection for the current study, samples were collected from patients which infected with CL in the early stages of infection and before treatment. While the production of IL-4 correlate with the severity of infection is related to the chronic stages of the disease and Th2 cell activity. In contrast, studies show that

IL-4 do not significantly vary across different clinical presentations of CL, indicating the complexity of immune responses in this disease (52).

Ethical Approval

The study was approved by the local ethics committee of the faculty of College of Science, University of Baghdad (ref: CSEC/0922/0094), after obtaining permission from the Ministry of Health and Environment in Baghdad, Iraq.

Conclusion

The present study revealed the role of IL-4 in the immune response during CL disease in the early stages of infection and before treatment, as the level of interleukin was slightly elevated in patients when compared with the control. The results had shown that the SNP for IL-4 (rs2243250) may be a potential risk development factor for susceptibility infection with CL disease, with the mutant allele T and genotype TT being considered more potential risk factors than other genotypes.

References

1. Bayram, D. M., Hassan, G. M., and Ali, H. Z. (2022). Detection of some CC Chemokine Ligands in Patients with Cutaneous Leishmaniasis. Iraqi Journal of Science, 1431-1437.
2. Yaseen, F. T., and Ali, H. Z. (2019). Identification of Leishmania donovani isolates by polymerase chain reaction. Iraqi journal of biotechnology, 18(2).
3. Ramzi, U. F., and Zghair, K. H. (2020). Histological disturbance in liver and spleen of visceral leishmaniasis infected mice with the progression of infection. Biochemical & Cellular Archives, 20.
4. Bayram, D. M., and Ali, H. Z. (2021). Molecular typing of two suspected cutaneous leishmaniasis isolates in Baghdad. Baghdad Science Journal, 18(1), 0029-0029.
5. Azami, M., Adermanabadi, V. R., Khanahmad, H., Mohaghegh, M. A., Zaherinejad, E., Aghaei, M., and Hejazi, S. H. (2018). Immunology and genetic of Leishmania infantum: The role of endonuclease G in the apoptosis. Journal of Research in Medical Sciences, 23(1), 36.
6. Mohamed, S. T., Sulaiman, H. H. N. M., Kamal, S. B., and Aja, H. A. (2019). Effect of Fusarium graminarum Silver-Nanoparticles on IL-10 and INF-γ Cytokines Levels in the Mice by Leishmania donovani in vivo. Iraqi journal of biotechnology, 18(2).
7. Anovazzi, G., Medeiros, M. C., Pigossi, S. C., Finoti, L. S., Moreira, S., Mayer, M. P. A., and Scarel-Caminaga, R. M. (2017). Functionality and opposite roles of two interleukin 4 haplotypes in immune cells. Genes & Immunity, 18(1), 33-41.
8. Mosmann, T., and Coffman, R. L. (1989). TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annual review of immunology, 7(1), 145-173.
9. Mishra, A., Jha, A. N., van Tong, H., Singh, V. K., Gomes, C. E., Singh, L., and Thangaraj, K. (2014). Analysis of genetic variants in the IL4 promoter and VNTR loci in Indian patients with Visceral Leishmaniasis. Human Immunology, 75(12), 1177-1181.
10. Al-Qiam, Z. H., Al-Saadi, A. H., and Al, A. A. A. K. M. (2019). Association between Ankylosing Spondylitis and the miR-146a Polymorphisms a Samples of Iraqi Patients. Iraqi journal of biotechnology, 18(2).
11. Semagn, K., Babu, R., Hearne, S., and Olsen, M. (2014). Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): Overview of the technology and its application in crop improvement. Molecular Breeding, 33(1): 1-14.
12. Kim, S. and Misra, A. (2007). SNP genotyping: technologies and biomedical applications. Annual Review of Biomedical Engineering, 9(1), 289-320.
13. Stenson, P. D., Mort, M., Ball, E. V., Howells, K., Phillips, A. D., Thomas, N. S., and Cooper, D. N. (2009). The Human Gene Mutation Database: 2008 update. Genome medicine, 1(1), 13.
14. Al-Naseri, M. A., Salman, E. D., and Ad'hiah, A. H. (2022). Genetic analysis of IL4 (rs2070874), IL17A (rs2275913), and

IL33 (rs7044343) polymorphisms in Iraqi multiple sclerosis patients by using T-plex real-time PCR method. Meta Gene, 31, 100986.

15. Blackwell, J. M., Fakiola, M., Ibrahim, M. E., Jamieson, S. E., Jeronimo, S. B., Miller, E. N., and Wilson, M. E. (2009). Genetics and visceral leishmaniasis: of mice and man. Parasite immunology, 31(5), 254-266.
16. Mohammed, M. Q., Alwan, A. H., and Almukhtar, A. A. (2024). Estimation of Serum TLR-9, TNF-α, and IL-6 Levels in the Iraqi Patients Diagnosed as Acute Myelogenous Leukemia. Baghdad Science Journal, 21(7), 2182-2182.
17. Mohammed, M. Q., Alwan, A. H., Almukhtar, A. A., and Al-Saedi, M. K. A. (2024). Revealing of TLR-9 gene polymorphisms by qPCR HRM technique and their influence on TLR-9 serum level in acute myeloid leukemia patients: Case-control study. Cytokine, 182, 156730.
18. Ramzi, U. F., Saheb, E. J., and Hussein, W. M. (2024). The association of serum level and polymorphisms of IFN-γ (rs2069705) with the susceptibility to cutaneous leishmaniasis. Cytokine, 184, 156785.
19. Conceicao, J., Davis, R., Carneiro, P. P., Giudice, A., Muniz, A. C., Wilson, M. E., and Bacellar, O. (2016). Characterization of neutrophil function in human cutaneous leishmaniasis caused by Leishmania braziliensis. PLoS neglected tropical diseases, 10(5), e0004715.
20. Ritter, U., Frischknecht, F., and van Zandbergen, G. (2009). Are neutrophils important host cells for Leishmania parasites. Trends in parasitology, 25(11), 505-510.
21. Castellano, L. R., Correia Filho, D., Argiro, L., Dessein, H., Prata, A., Dessein, A., and Rodrigues, V. (2009). Th1/Th2 immune responses are associated with active cutaneous leishmaniasis and clinical cure is associated with strong interferon-γ production. Human immunology, 70(6), 383-390.
22. Costa, R. S., Carvalho, L. P., Campos, T. M., Magalhaes, A. S., Passos, S. T., Schriefer, A., and Carvalho, E. M. (2018). Early cutaneous leishmaniasis patients infected with Leishmania braziliensis express increased inflammatory responses after antimony therapy. The Journal of infectious diseases, 217(5), 840-850.
23. Meymandi, S., Dabiri, S. H., Meymandi, M., Nikpour, H., and Kharazmi, E. (2009). Immunophenotypic pattern and cytokine profiles of dry type cutaneous leishmaniasis. Archives of Iranian Medicine, 12 (4): 371 – 376
24. Hejazi, S. H., Hoseini, S. G., Javanmard, S. H., Zarkesh, S. H., and Khamesipour, A. (2012). Interleukin-10 and transforming growth factor-β in early and late lesions of patients with Leishmania major induced cutaneous leishmaniasis. Iranian journal of parasitology, 7(2), 53.
25. Gupta, G., Oghumu, S., & Satoskar, A. R. (2013). Mechanisms of immune evasion in leishmaniasis. In Advances in applied microbiology, Vol. 82, pp. 155-184.
26. Lopes, M. F., Costa-da-Silva, A. C., and DosReis, G. A. (2014). Innate immunity to Leishmania infection: within phagocytes. Mediators of inflammation, (1), 754965.
27. Santos-Mateus, D., Passero, F., Rodrigues, A., Valerio-Bolas, A., Silva-Pedrosa, R., Pereira, M., and Santos-Gomes, G. (2016). The battle between Leishmania and the host immune system at a glance. International trends in immunity, 4(1), 28-34.
28. Dominguez, M., Moreno, I., Lopez-Trascasa, M., and Torano, A. (2002). Complement interaction with trypanosomatid promastigotes in normal human serum. The Journal of experimental medicine, 195(4), 451-459.
29. Ambrosio, A. R., & de Messias-Reason, I. J. T. (2005). Leishmania (Viannia) braziliensis: Interaction of mannose-binding lectin with surface glycoconjugates and complement activation. An antibody-independent defence mechanism. Parasite immunology, 27(9), 333-340.
30. Manamperi, N. H., Oghumu, S., Pathirana, N., de Silva, M. V. C., Abeyewickreme, W., Satoskar, A. R., and Karunaweera, N. D. (2017). In situ immunopathological changes in cutaneous leishmaniasis due to Leishmania donovani. Parasite Immunology, 39(3), e12413.
31. Choi, B. S., and Kropf, P. (2008). Evaluation of T cell responses in healing and nonhealing leishmaniasis reveals differences in T helper cell polarisation ex vivo and in vitro. Parasite immunology, 31, 199–209
32. Snider, H., Lezama-Davila, C., Alexander, J., and Satoskar, A. R. (2009). Sex hormones and modulation of immunity against leishmaniasis. Neuroimmuno modulation,

16(2), 106-113.
33. Lezama-Davila, C. M., Isaac-Marquez, A. P., Barbi, J., Oghumu, S., and Satoskar, A. R. (2007). 17β-estradiol increases Leishmania mexicana killing in macrophages from DBA/2 mice by enhancing production of nitric oxide but not pro-inflammatory cytokines. The American journal of tropical medicine and hygiene, 76(6), 1125.
34. Taheri, A. R., Goyonlo, V. M., Nahidi, Y., Moheghi, N., and Afshari, J. T. (2014). Plasma levels of interlukin-4 and interferon-γ in patients with chronic or healed cutaneous leishmaniasis. Iranian Journal of Basic Medical Sciences, 17(3), 216.
35. Carvalho, A. M., Amorim, C. F., Barbosa, J. L., Lago, A. S., and Carvalho, E. M. (2015). Age modifies the immunologic response and clinical presentation of American tegumentary leishmaniasis. The American journal of tropical medicine and hygiene, 92(6), 1173.
36. Ad'hiah, A. H., Al-naseri, M. A., Zahra'a, A. A., Salman, E. D., Al-saffar, O. B., Ahmed, H. S., and Idan, E. M. (2019). Cytokine gene variations and their impact on serum levels of IFN-γ, IL-2, IL-4, IL-10 and IL-12 among Iraqi Arabs. Meta Gene, 19, 98-103.
37. Andrade, C. (2015). Understanding relative risk, odds ratio, and related terms: as simple as it can get. The Journal of clinical psychiatry, 76(7), 21865.
38. Leal, L. M., Moss, D. W., Kuhn, R., Miiller, W., and Liew, F. Y. (1993). Interleukin-4 transgenic mice of resistant background are susceptible to Leishmania major infection. European journal of immunology, 23(2), 566-569.
39. Launois, P., Swihart, K. G., Milon, G., and Louis, J. A. (1997). Early production of IL-4 in susceptible mice infected with Leishmania major rapidly induces IL-12 unresponsiveness. Journal of immunology (Baltimore, Md.: 1950), 158(7), 3317-3324.
40. Launois, P., Ohteki, T., Swihart, K., MacDonald, H. R., and Louis, J. A. (1995). In susceptible mice, Leishmania major induce very rapid interleukin-4 production by CD4+ T cells which are NK1. 1−. European journal of immunology, 25(12), 3298-3307.
41. Biedermann, T., Zimmermann, S., Himmelrich, H., Gumy, A., Egeter, O., Sakrauski, A. K., and Rocken, M. (2001). IL-4 instructs TH1 responses and resistance to Leishmania major in susceptible BALB/c mice. Nature immunology, 2(11), 1054-1060.
42. Kamali-Sarvestani, E., Rasouli, M., Mortazavi, H., and Gharesi-Fard, B. (2006). Cytokine gene polymorphisms and susceptibility to cutaneous leishmaniasis in Iranian patients. Cytokine, 35(3-4), 159-165.
43. Kirik, F. E., Ulger, M., Tezcan Ulger, S., and Aslan, G. (2020). Association of cytokine gene polymorphisms with susceptibility to cutaneous leishmaniasis in a Turkish population. Parasite Immunology, 42(11), e12775.
44. Adedokun, S. A., Seamans, B. N., Cox, N. T., Liou, G., Akindele, A. A., Li, Y., and Thomas, B. N. (2018). Interleukin-4 and STAT6 promoter polymorphisms but not interleukin-10 or 13 are essential for schistosomiasis and associated disease burden among Nigerian children. Infection, Genetics and Evolution, 65, 28-34.
45. Mishra, A., Jha, A. N., van Tong, H., Singh, V. K., Gomes, C. E., Singh, L., and Thangaraj, K. (2014). Analysis of genetic variants in the IL4 promoter and VNTR loci in Indian patients with Visceral Leishmaniasis. Human Immunology, 75(12), 1177-1181.
46. Guo, L., Hu-Li, J., Zhu, J., Watson, C. J., Difilippantonio, M. J., Pannetier, C., and Paul, W. E. (2002). In TH2 cells the Il4 gene has a series of accessibility states associated with distinctive probabilities of IL-4 production. Proceedings of the National Academy of Sciences, 99(16), 10623-10628.
47. Tripathi, P., Singh, V., and Naik, S. (2007). Immune response to leishmania: paradox rather than paradigm. FEMS Immunology & Medical Microbiology, 51(2), 229-242.
48. Rosas, L. E., Keiser, T., Barbi, J., Satoskar, A. A., Septer, A., Kaczmarek, J. and Satoskar, A. R. (2005). Genetic background influences immune responses and disease outcome of cutaneous L. mexicana infection in mice. International immunology, 17(10), 1347-1357.
49. de Araujo Santos, F. J., da Silva, L. S., Junior, J. D. E. S., Ramos de Mesquita, T. G., de Souza, M. L. G., de Andrade Junior, M. C. and Ramasawmy, R. (2019). Single nucleotide polymorphisms of the genes IL-2, IL-2RB, and JAK3 in patients with cutaneous leishmaniasis caused by

Leishmania (V.) guyanensis in Manaus, Amazonas, Brazil. PLoS One, 14(8), e0220572.

50. Satoskar, A., Bluethmann, H., and Alexander, J. (1995). Disruption of the murine interleukin-4 gene inhibits disease progression during Leishmania mexicana infection but does not increase control of Leishmania donovani infection. Infection and immunity, 63(12), 4894-4899.
51. Patz, J. A., Graczyk, T. K., Geller, N., and Vittor, A. Y. (2000). Effects of environmental change on emerging parasitic diseases. International journal for parasitology, 30(12-13), 1395-1405.
52. Yizengaw, E., Takele, Y., Franssen, S. U., Gashaw, B., Yimer, M., Adem, E., and Kropf, P. (2024). Parasite genetic variation and systemic immune responses are not associated with different clinical presentations of cutaneous leishmaniasis caused by Leishmania aethiopica. bioRxiv, 590259.