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Evaluation of Interleukin-4 in Patients with Visceral Leishmaniasis

Zahraa Fahmi Zamakh[1*], Esraa Fadhel Wathah[2]

[1]Department of Biology, Collage of Science, University of Al Qadisiyah, Iraq

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***Corresponding author:** Zahraa Fahmi Zamakh
Department of Biology, Collage of Science, University of Al Qadisiyah, Iraq

Abstract | **Original Research Article**

Background: *Visceral leishmaniasis* is one of endemic illness in Iraq, and the immunosuppression is one and important clinical symptom of disease. ***Aim:*** The current study aims to measuring the level of IL-4 in patient with Kala-azar. ***Methods:*** 60 sample from patients with visceral leishmaniasis who were admitted to hospital that were let in the study from the period between September 2024 to march 2025, and other groups consist of 30 apparently healthy individuals. A five ml of blood samples were collected, for IL-4 ELISA test. ***Results****:* The Mean levels of Interleukin-4 were 1443.92 ± 112.67 and 946.13 ± 190.52, respectively, patients and healthy controls; the level (mean level) was significantly higher in patients with visceral leishmaniasis compared to healthy controls (P< 0.001). The present study confirmed the role of IL-4 in predicting kala-azar, demonstrating that 59 out of 60 patients (98.3%) had values exceeding the cutoff (>1236.55), whereas none of the healthy subjects (0%) had IL-4 levels above this cutoff. The difference is highly significant (P > 0.001). Analysis of the ROC curve indicates that the IL-4 cutoff value is >1236.55, with sensitivity, specificity, PPV, and NPV rates of 98.3%, 100.0%, 100.0%, and 96.8%, respectively. ***Conclusion:*** a significant association between serum IL-4 levels in patients with Kala-azar.

Keywords: Kala-azar, IL-4, ELISA.

INTRODUCTION

Leishmaniasis, a disease transmitted by female sandflies carrying the leishmania parasite, results in various forms, including three primary clinical manifestations and visceral leishmaniasis is one form of them [1]. about 50,000 to 90,000 cases of VL annually around the world, is a potentially fatal disease also called the black fever. The transmission through the bite of infected female sandflies that affects important organs in human, animal body such as liver, bone marrow, spleen, a lymph nodes in humans, Leishmania enters the host cell through the bite of the female infected with the parasite (*Phlebotomus spp*.) during its blood meal from a host [2].

The parasite triggers humoral and cellular immune responses as it flees the host cell. thus, the abnormal immune due to damage of liver tissues, besides other organs, the malnutrition status of patients is associated with irregularities in immunological response in VL [3]. Amastigote stage reproduction in spleen, liver and bone marrow, (MPS) system that leads to disorders in the active organs, such as hyperplasia of Kupffer cells, necrosis thus liver damage perhaps occur because of the invasion effects by macrophages and Kupffer cells [4].

Interleukin-4: engaged in controlling immunity against a variety of pathogens, especially bacteria and parasites, It performs a significant part in transforming naïve T cells into Th2 cells, which generate cytokines like IL-4 to enhance humoral immunity and promote eosinophil activity.[5]. Interleukin-4 reduces the output of inflammatory proteins such as COX-2 and pro-inflammatory chemokines like CCL2 along with inducible nitric oxide synthase (Inos). Excessive inflammation can damage host tissue and exacerbate illness. IL-4's ability to suppress the generation of pro-inflammatory chemokines aids in maintaining a stable immunological response and block the immune reactions could harmful to the healthy tissues from escalating. [5,7]. IL-4 plays a role in converting B cells to IgE antibodies, which aid in combating parasites, these Antibodies attach to particular mast cell and basophil receptors, priming them for an active immune response upon contact with parasite antigens and resulting in the release of mediators like cytokines [8].

2. Equipments and Working of Experiments

2.1. Design of study.

A case-control an investigation was carried out. on the subsequent study cohorts between September 2024 and March 2025 from AL-Diwaniyah General

Hospital, AL-Hamza General Hospital, and Central Public Health Laboratory. Participants in the study are separated into two groups. The first group comprises sixty adult patients diagnosed with Kala-azar. patients were diagnosed with the Rk39 method, which revealed that those infected had a chronic (IgG ve+) infection, and patients were categorized based on various medical criteria. In this study, thirty age-matched, apparently healthy individuals were kindly recruited as a control group for the other groups. We did not include patients with autoimmune disease.

2.2. Sampling Criteria

Using disposable syringes, five milliliters (ml) of blood samples were obtained through vein puncture under aseptic technique, and placed in serum-separating tubes that contain separating gel. They were then centrifuged for five minutes at 2000–3000 rpm to obtain serum for IL-4 measurement via ELISA.

2.3. Immunological study

The technique (ELISA) was approved to investigation the immunological parameter in the study, the concentrations of IL-4 in the serum was estimated using an ELISA kit from Elabscines.

2.4. Statistics Analysis

SPSS version 26 is the statistical program used in the social sciences, was utilized for the description, analysis, and display of the data The means and standard deviations (SD) were used for quantitative variables. For qualitative variables, percentages and frequencies were employed. The means of the two groups To compare the groups, the independent T-test was used. Two quantitative variables were correlated using the Pearson correlation method. P≤0.05 was chosen as the value of significance.

RESULTS

Comparison of tests for liver function (ALT, AST) between who suffering the disease and the healthy group have been conducted, with results shown in Table (1). In patients with Kala-azar, the mean serum levels of Alanine transaminase (ALT) are 36.26 ± 6.07 U/L, while in the healthy group, they are 25.59 ± 1.71 U/L; the mean levels were elevated in patients affected by Kala-azar compared to healthy controls, with a notable difference (P = 0.001). and the mean serum levels of Aspartate aminotransferase (AST) were 41.34 ± 7.56 U/L in patients affected by Kala-azar and 27.57 ± 5.41 U/L in the control group; the mean levels were elevated in individuals affected by Kala-azar compared to healthy controls, and this difference was statistically significant (P = 0.001).

In table (2), The average levels of serum of IL-4 level in *visceral leishmaniasis* patients have significantly higher than those of healthy group (1443.92 ± 112.67) versus (946.13 ± 190.52) and P-value was (P < 0.001). To assess the IL-4 cutoff value and prophesy donovani illness as examination or adjunct for diagnosis tests, an ROC curves analysis were conducted, with the results presented in table (3) and figure (1). The cutoff value for IL-4 was >1236.55-fold, with a sensitivity of 98.3%, specificity of 100.0%, positive predictive value (PPV) of 100.0%, negative predictive value (NPV) of 96.8%, and an area under the curve of 0.998 (0.995-1.000). The study imply the IL-4 is viewed as a good or superior diagnostic marker. Additionally, the relationship between Interleukin-4 (IL-4) and other parameters in patients affected by Kala-azar was displayed in tables (4). The current findings indicatenon-substantial association between IL-4 and all other parameters in patients with Kala-azar.

Table 1: Comparison of tests for liver function (ALT and AST) in patients and healthy group

Groups		Alanine aminotransferase (U/L)	Aspartate aminotransferase (U/L)
Kala-azar patients	**Mean ± SD**	**36.26 ± 6.07**	**41.34** ± 7.56
	Range	24.43-52.70	20.00-65.60
Control	**Mean ± SD**	**25.59** ± 1.71	**27.57** ± 5.41
	Range	22.70-30.10	20.00-40.00
p-value		**0.001** † S	**0.001** † S

n: number of cases; **SD**: standard deviation; †: independent samples t-test; S: significant at P ≤ 0.05.

Table 2: IL-4 level in persons who suffering kala-azar and control group

Groups		Interleukin-4 (IL-4) level
Kala-azar patients	**Mean ± SD**	**1443.92 ± 112.67**
	Range	1165.80-1696.70
Control	**Mean ± SD**	**946.13** ± 190.52
	Range	564.00-1215.30
p-value		**< 0.001** † S

n: number of cases; **SD**: standard deviation; †: independent samples t-test; HS: highly significant at P ≤ 0.001.

Table 3: Sensitivity and specificity of IL-4 (> 1236.55-fold) in VL disease

Interleukin-4	Kala-azar suffering persons *n* = 60	control *n* = 30
> 1236.55	59	0
< 1236.55	1	30
Sensitivity %	98.3 %	
Specificity %	100.0%	
PPV %	100.0 %	
NPV %	96.8%	
AUC (95% CI)	0.998 (0.995- 1.000)	

CI: Confidence interval, AUC: Area under curve.

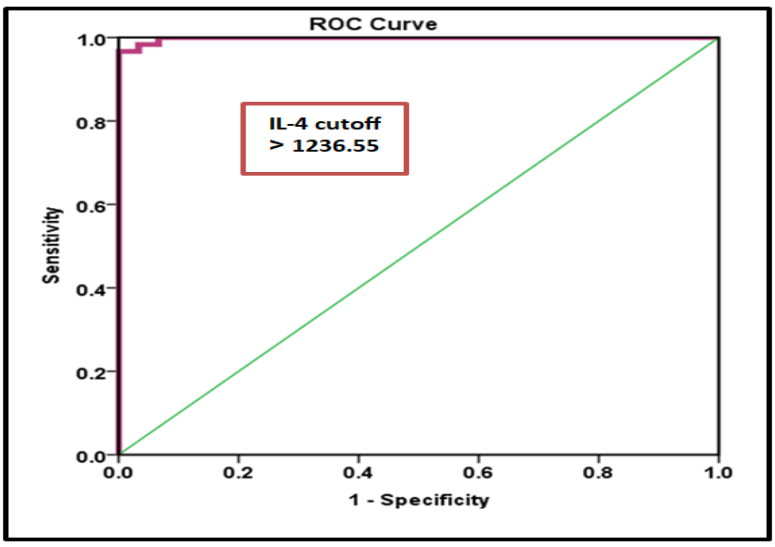

Figure 1: **Analysis IL-4 receiver operator characteristic curve to find a potential diagnostic cutoff value.**

Table 4: Correlation between Interleukin-4 (IL-4) and other parameter in patients with Kala-azar.

Other parameters	IL-4 patients with Kala-azar	
	r	*P*
ALT	0.150	0.253
AST	0.076	0.562

r: Pearson correlation.

DISCUSSION

In the current study, the mean of ALT and AST in serum (36.26 ± 6.07 and 41.34 ± 7.56) was significantly higher in Kala-azar patients than controls (25.59 ± 1.71 and 27.57 ± 5.41) respectively. This could be explained by the concentrations of specific circulating cytokines during the inflammatory processes seen in active VL disease, which may be a cause and effect of severe liver disease [9]. Hepatomegaly and the production of immune complex in VL patients may be the cause of the change in liver function. Changes in liver function may also be caused by the activated macrophage's generation of reactive oxygen species [10].

A clinical report on a patient who presented clinically and biochemically like liver cirrhosis but died of visceral leishmaniasis due to a late diagnosis and treatment with sodium antimony stibogluconate shortly before death described the possibility of confusing visceral leishmaniasis with chronic liver disease. Clinicians frequently misdiagnose patients with altered liver biochemical parameters as having hepatitis. Consequently, the patient's condition might deteriorate [11].

This results consistence with results of Endale *et al.* [12] which showed that the ALT and AST levels was noticeably greater in cases than in controls (P<0.001). and the finding result is resemble to previous study performed in Iraq [13], India [14] they were reported a significantly elevated value of Aspartate aminotransferase and Alanine aminotransferase in VL. Furthermore, Hammam and Hicks,[15] which verified that the mean AST and ALT values were significantly higher (p-value <0.001 and 0.006) among VL patients.

When compared to the control groups average value that appeared to be in good health, the study carried out in Iraq also showed that the ALT levels of VL patients increased significantly ($P < 0.05$) [16]. Furthermore, study done in Italy by Sagnelli *et al.,*[17] supported the result of present study, wherein altered liver function and elevated serum AST and ALT levels were observed. This could be linked to an immune response triggered by parasites in active VL, which causes the release of circulating cytokines and the start of inflammatory processes. It could identify and cause severe liver disease and liver dysfunction [12]. Some studies, in contrast to ours, found no discernible difference between the case and control groups, which contradicted our findings [18]. According to a study done in Iraq, the average ALT and AST values were below normal [19]. Size of samples, design of study, geographical area, age of study participants, severity of the disease, This variation may be caused by the clinical chemistry's examinations reference value as well as variations in study participants' lifestyles.

In present study indicated that serum level of IL-4 in Kala-azar patients was increased significantly as compared to healthy control. It is commonly known that there is an increase in IL-4 production during active VL.[20]. This perhaps as a result of the significant up-regulation of IL-4 manufacture CD4+T cells, which might suggest a successful anti-parasitic reaction [21]. The present results consistence with study done by Barbosa-Júnior *et al.,*[22], who showed the IL-4 is elevated in the serum of VL patients. According to the most recent research on human splenic aspirates, blocking IFN-γ and TNF-α increases synthesis of Interleukin-4, which isn't involved in parasite reproduction. The biological function of Interleukin-4 in human VL target organ is still unknown [23]. Further, It was also noted that antigen-stimulated PBMCs had higher levels of IL-4 (Th2 cytokines) during VL [24].

When a systemic infection occurs and the parasite spreads to the liver, spleen, and other organs, there is an increase in IL-4, a decrease in Th1 T-cell-mediated immunity, and a high titre of circulating antibodies.[25]. The secretion of Interleukin (IL-4) is mainly associated with Th2 immune response [26].

This study confirmed the significance of IL-4 in predicting Kala-azar, which is crucial for recognizing its development or progression. The study shows that 59 out of 60 patients (98.3%) exceeded the cut-off value (>1236.55), while none of the healthy subjects (0%) had IL-4 levels above this cut-off. The ditiniction was highly significant ($P > 0.001$), as illustrated in Table 3 and Figure 1.

Analysis ROC curve shows that the IL-4 cutoff value was >1236.55 with sensitivity, specificity, PPV and NPV levels of 98.3%, 100.0%, 100.0% and 96.8% respectively. The (AUC) of the (ROC) for this cytokine was 0.998 (0.995-1.000), suggesting that these cytokines could predict the severity of Kala-azar. The current findings revealed that there was no significant correlation between TGF-β levels and ALT ($p= 0.253$), AST ($p= 0.562$).

CONCLUSIONS

This study indicates the predominance of visceral leishmaniasis for affected patients, the results in an excessive increase in Interleukin-4 level in serum as a defensive reaction by the host body.

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