OPEN ACCESS

Saudi Journal of Pathology and Microbiology

Abbreviated Key Title: Saudi J Pathol Microbiol
ISSN 2518-3362 (Print) |ISSN 2518-3370 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Levels of Interleukin -40 in Patients Serum with Visceral Leishmaniasis

Khalil Ismail Abid Mohammed[1]*, Suha A. AL-Fakhar[1], Ameen Abdul Hasan AL-Alwany[2], Wifaq M. Ali[1], Yasamin Al-Qassab[1], Tharwa H. Hasan Al-Tai[1], Jinan M Mousa[1]

[1]Clinical Communicable Disease Research Unit /College of Medicine, University of Baghdad, Baghdad, Iraq
[2]Department of Internal Medicine, College of Medicine, University of Baghdad, Baghdad, Iraq

DOI: https://doi.org/10.36348/sjpm.2025.v10i04.002 | **Received:** 18.05.2025 | **Accepted:** 25.06.2025 | **Published:** 04.07.2025

***Corresponding author:** Khalil Ismail Abid Mohammed
Clinical Communicable Disease Research Unit /College of Medicine, University of Baghdad, Baghdad, Iraq
Email- drkhalilmohammed1967@gmail.com

Abstract

The study was performed on 31 blood samples collected from children (14 males and 17 females) from Different governorate they were suspected to infection with visceral leishmaniasis were they visited the central public health laboratories in Baghdad their ages ranged from (6 month – 9 years). All data were collected to each patients which included (name, ages and clinical sign and symptoms. All sera were tested by Immunofluorescent Antibodies Assay (IFAT) while interleukin -40 detected by using Enzyme Linked Immunosorbent Assay. The results Indicated that (19) patients with fever, (22) patients with Hepatosplenomegally and (9) patients with different sign and Symptoms. only (8) out of 31 patients produced specific antibodies to visceral leishmaniasis by IFAT Assay. Also, the results indicated increase significantly the level of interleukin -40 in patients in comparison with healthy control.

Keywords: Visceral Leishmaniasis, Immunoflouresense Antibodies, Interleukin-40, Hepatosplenomegally.

INTRODUCTION

Leishmaniasis, a parasitic disease that affects humans and animals, is mainly transmitted fromone host to another through the bite of an infected Phlebotomine sand fly vector. Over 20 species of Leishmania have been reported to cause various forms of leishmaniasis, including cutaneous, mucocutaneous, and visceral forms [1,2]. Visceral leishmaniasis (VL), also known as kalaazar, is caused by L. donovani, and L. infantum [3, 4]. Fever, anorexia, anemia, and weight loss accompanied by an enlarged liver and spleen are the major clinical signs and symptoms of VL, and the disease can be fatal if left untreated [2]. Neutrophils and macrophages are both infected by Leishmania parasites, macrophages serve as the major infected cells that perpetuate the infection in the host because they live longer than neutrophils [5-7] Leishmania parasites are able to promote their survival in macrophages. One mechanism by which they do so is by suppressing Interleukin 12 (IL-12) production in infected macrophages [8, 9]. IL-12 is a key cytokine that induces the T-helper 1 (Th1) response which is essential for parasite elimination in infected macrophages [10-12]. It has been shown that the outcome of Leishmania infection in macrophages depends on their activation status [9,13-15]. Classical activation of macrophages is mediated by Interferon gamma (IFN-γ), an effector cytokine secreted by CD4+ T helper type 1 (Th1) cells, CD8+ T cells, and natural killer (NK) cells [16]. Upon IFN-γ stimulation, macrophages produce inducible nitric oxide synthase (iNOS), which converts L-arginine to nitric oxide (NO), a critical effector molecule for killing of intracellular amastigotes [16]. iNOS production is dependent on NF-κB transcription [17] and is important in facilitating the clearance of parasites. Gregory *et al*.reported that Leishmania parasites inhibit the production of NO by suppressing the production and/or activation of iNOS via Leishmania protease (gp63)-mediated cleavage of p65 subunit of NF- κB [17]. Also, Leishmania parasites inhibit NO production by increasing the expression of arginase which competitively cleaves L-arginine into ornithine [18]. Ornithine favors the proliferation of Leishmania parasites in macrophages [19]. Wei *et al*. showed that the normally resistant (C57BL/6) mice that lost the capacity to synthesize iNOS became susceptible to L. major infection, although they maintained a strong IFN-γ type response [20]. An iNOS inhibitor, N(G)-monomethyl-L-arginine, suppressed IFN-γ mediated parasite clearance

in both mice and human macrophages in vitro [21]. A Resistance to infection by Leishmania parasites is mediated by interferon gamma (IFN-γ) that stimulates macrophages to produce nitric oxide (NO) which is essential for leishmanicidal activity [22]. IFN-γ also inhibits the production of cytokines such as IL-4, and IL-10 associated with Th 2 response. Increased expression of IL-4 and IL-10 was found to be linked with failed healing and disease progression [23] IL-4 plays a major role in the non-healing response observed following Leishmania infection by down regulating the expression of protective Th1 associated cytokines(IL-12 and IFN-γ) and by inhibiting NO production [24].Several studies have shown that Th1 response was prominent in the healing form while Th2 was the prominent response in non-healing forms of CL [25,26]. These observations suggest that the balance between Th1 andTh2 cytokine profiles may decide the development of visceral or cutaneous disease: a prominent Th1 response leads to the cutaneous form, while a predominant Th2 response leads to visceral disease. IL-11 is a member of theIL-6 family and is produced by bone marrow Stromal cells [27]. It promotes differentiation of progenitor B cells. It decreases Th1 cell differentiation and inhibits the production of pro inflammatory cytokines including TNF-α, IL-1β and IL-12p40 (28) while enhancingTh2 responses [29]. In this research the levels of interleukin -40 was detected.

MATERIALS AND METHODS

A Total of 31 patients (14 males,17 females) their age range was (6 months – 14 years). The blood samples were collected in the Central Public Health in Baghdad Governorate. The patients were from different governorates in the Iraq. The sera was collected from the blood samples and were stored in freeze -20C° utile used to detect L. donovanii by use IFAT Indirect immunofluorescent assay kit to test IgG antibodies against L. donovanii (Vircell microbiologists, Spain).

Assay procedure for detection of IgG Abs of L.donovanni IFAT (Vircell microbiologists, Spain):

1. All reagents were bring to room temperature before use, and allow the slides were allowed to reach room temperature before opening.
2. A 1/40 and 1/80 dilutions of serum samples were prepared by adding 10µl of sample to 390µl of PBS2
3. (1/40 dilution). twofold dilutions were made with50µl of PBS (1/80 dilution). The control sera 3 and 4were not diluted.
4. A 20µl of 1/40 and 1/80 dilutions were applied in two slide wells. The same with the positive 3 and negative 4 controls were applied.
5. The slide was incubated in a humid chamber for 30minutes at 37ºC.
6. The slide 1 briefly was rinsed with a gentle stream of PBS 2 (directing PBS was avoid at wells) and was immersed for ten minutes in PBS. The washed slide was dipped briefly in distilled water.
7. The slide 1was allowed to air dry.
8. A 20µl of anti-human IgG FITC conjugate solution 5Gwas added to each well. (No dilution was required).
9. The steps 4, 5 and 6 were repeated.
10. A small drop of mounting medium 6 was added to each well and carefully was covered with a cover slip.
11. The slide was read as soon as possible in a fluorescence microscope at 400x magnification. If this is not possible, it was stored in the dark at 2-8ºCup no more than 24 hours, until observation.
12. If the rescreening testing dilutions are positive, further analyze with up to 1/2048 dilutions were needed.

Detection of Interleukin -40 Levels

The levels of Interleukin -40 (IL-40) for Leishmania patients and healthy control matched with their ages estimated by ELISA according to manual procedure of Sunlong Biotech, China).

Statistical Analysis

The results were analyzed using statistical system SPSS version -18 (T-testing).

RESULTS

Gender

Distribution of visceral Leishmaniasis patients according to their gender, were studied, among them 14 were males and 17 were females. (Table-1)

Table 1: Distribution of visceral Leishmaniasis patients according to their gender

Gender	No.	%
Males	14	45.20
Females	17	54.80
Total	31	100

Geographical Distribution

Twenty (20) patients with visceral leishmaniasis were from Baghdad and the rest (11) of the patients were from different areas of middle and southern of Iraq. (Table-2)

Table 2: Distribution of visceral Leishmaniasis patients according to their Governorate

Governorate	No.	%
Baghdad	20	64.51
Different area	11	35.49
Total	31	100

Signs and Symptoms

Nineteen (19) patients with fever, (22) cases with Hepatosplenomegally, and the rest of the patients had different signs and symptoms (Table-3)

Table-3: Distribution of visceral Leishmaniasis patients according to their Signs and Symptoms

Sign & symptoms	No.	%
Fever	19	61.29
Hepato splenomegaly	22	70.96
Other different symptoms	9	29.03

IFAT Detection Test

A Eight of Visceral leishmaniasis patients had positive results of IFAT used for detection IgG antibodies to L. donovani while there were 23 of patients had negative results to IFAT. (Table-4**)**

Table 4: Distribution of visceral Leishmaniasis patients according to IFAT

	NO.		%	
	+ve	-ve	+ve	-ve
Baghdad	6	14	19.35	45.16
Different area	2	9	6.45	29.04
Total	**8**	**23**	**25.80**	**74.20**

Interleukin-40 Levels

The levels of the IL-40 increased significantly ($p \leq 0.05$) in patients with visceral leishmaniasis in comparison to the healthy individual (Table-5).

Table 5: Levels of IL-40 (ng/L) in visceral Leishmaniasis patients in comparison with Healthy control

Groups	IL-40
Patients (VL+)	* 9.63 ± 4.60
Healthy control (VL-)	5.70 ± 1.53
* ($p \leq 0.05$) significant	

DISCUSSION

Visceral leishmaniasis, commonly known as Kalaazar, is caused by *Leishmania donovani* and *Leishmania infantum*. These Leishmania species infect macrophages throughout the viscera, and parasites are typically found in the spleen, liver, and bone marrow [30]. the results of the study Indicated that were 20 patients from Baghdad [6] of them with Visceral Leishmaniasis detected by IFAT, and 14 of them without VL. While other 11 patients two of them With visceral Leishmaniasis detected by (IFAT) were from different areas of middle and southern of Iraq, the results were conflicted with that reported [31] arapid urbanization leads to the settlement of rural populations in makeshift surrounding without proper sanitation and housing conditions ,leading to a greater exposure to human or animal carriers and a rise in vector population, resulting in increasing the infection , In the present study the IFAT used to detect IgG Antibodies of *L. donovani* , It was found that there were 8(25.8%) patients from 31 patients with positive results of IFAT used to detect IgG of L. donovani Antibodies and this result may be due to the low number of positive cases with visceral leischmaniasis tested .In a general , Visceral leishmaniasis is ranked second in mortality and fourth in morbidity among tropical diseases, with 20,000 to 40,000 deaths per year and over 2 million (disability-adjusted life years) lost symptoms include Hepatosplenomegally , high fever, pancytopenia, and hypergammaglobulinemia, and the disease is almost always fatal if untreated. Complications of visceral leishmaniasis are explained in part by immune complex pathology, particularly nephritis [32,33]. The Result indicated an increased level of IL-40 in Visceral Leishmaniasis patients (Table-5) in comparison with healthy control probably contributes to inflammatory response in patient .In a general, Visceral Leishmaniasis induce the secretion of TGF- ß and IL-10 which play a vital role in the induced immunopathology or prolongation of persistence, by suppressing Th1 responses [34].TGF- ß potentiates the expression of IL-40 by activated B cells [35]. The ability of cytokines like IL-10 and TGF-ß to potentiate IL-40 production by B cells suggests thatoptimalIL-40 expression by B cells may require a specific differentiation mechanism to achieve optimal production [36].

CONCLUSION

The results Indicated that (19) patients with fever, (22) patients with Hepatosplenomegally and (9) patients with different sign and Symptoms. only (8) out of 31 patients produced specific antibodies to visceral leishmaniasis by IFAT Assay. Also, the result indicated increase significantly the level of interleukin -40 in patients in comparison with healthy control

Conflict of Interests: The authors declare that they have no conflict of interest.

REFERENCES

1. Torres-Guerrero, E.; Quintanilla-Cedillo, M.R.; uizEsmenjaud, J.; Arenas, R. Leishmaniasis: A Review. Available online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5464238/ (accessed on 14 July 2020).
2. WHO. Leishmaniasis. Available online: http://www.who.int/gho/neglected_diseases/leishmaniasis/en/(accessed on 5 February 2020).
3. Ready, P.D. Epidemiology of Visceral Leishmaniasis. Available online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4014360/ (accessed on 28 February 2019).
4. Thakur, L.; Singh, K.K.; Shanker, V.; Negi, A.; Jain, A.; Matlashewski, G.; Jain, M. Atypical leishmaniasis:A global perspective with emphasis on the Indian subcontinent. PLoS Negl. Trop. Dis. 2018, 9, e0006659
5. Liew, F.Y.; Millott, S.; Parkinson, C.; Palmer, R.M.; Moncada, S. Macrophage Killing of Leishmania ParasiteIn Vivo Is Mediated by Nitric Oxide from L-Arginine. 1990. Available online: https://www.jimmunol.org/content/144/12/4794 (accessed on 21 June 2019).
6. Soulat, D.; Bogdan, C. Function of Macrophage and Parasite Phosphatases in Leishmaniasis. Front

Immunol 2017. Available online: https://www.frontiersin.org/articles/10.3389/fimmu.2017.01838/full (accessed on14 July 2020).
7. Tomiotto-Pellissier, F.; Bortoleti, B.T.d.S.; Assolini, J.P.; Gonçalves, M.D.; Carloto, A.C.M.;Miranda-Sapla, M.M.; Conchon-Costa, I.; Bordignon, J.; Pavanelli, W.R. Macrophage Polarization inLeishmaniasis: Broadening Horizons. 2018. Available online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6220043/ (accessed on 14 July 2020)
8. Reiner, S.L.; Locksley, R.M. The regulation of immunity to Leishmania major. Annu. Rev. Immunol. 1995,13, 151–177.
9. Jayakumar, A.; Widenmaier, R.; Ma, X.; McDowell, M.A. Transcriptional Inhibition of Interleukin-12 PromoterActivity in Leishmania spp.-Infected Macrophages. Available online: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2665708/ (accessed on 14 July 2020)
10. Costa, S.F.; Gomes, V.O.; dos Santos Maciel, M.O.; Melo, L.M.; Venturin, G.L.; Bragato, J.P.; Rebech, G.T.;de Oliveira Santos, C.; de Oliveira, B.M.N.; de Sá Oliveira, G.G.; *et al*. Combined in vitro IL-12 and IL-15stimulation promotes cellular immune response in dogs with visceral leishmaniasis. PLoS Negl. Trop. Dis.2020, 14, e0008021.
11. Park, A.Y.; Hondowicz, B.D.; Scott, P. IL-12 Is Required to Maintain a Th1 Response during Leishmania major Infection. J. Immunol. 2000, 165, 896–902.
12. Park, A.Y.; Hondowicz, B.; Kopf, M.; Scott, P. The role of IL-12 in maintaining resistance to Leishmania major. J. Immunol. 2002, 168, 5771–5777.
13. Wanderley, J.L.M.; Deolindo, P.; Carlsen, E.; Portugal, A.B.; DaMatta, R.A.; Barcinski, M.A.; Soong, L. CD4+ TCell-Dependent Macrophage Activation Modulates Sustained PS Exposure on Intracellular Amastigotes of Leishmania amazonensis. Front. Cell. Infect. Microbiol. 2019, 9, 105.
14. Hölscher, C.; Arendse, B.; Schwegmann, A.; Myburgh, E.; Brombacher, F. Impairment of AlternativeMacrophage Activation Delays Cutaneous Leishmaniasis in Nonhealing BALB/c Mice. J. Immunol. 2006,176, 1115–1121.
15. Lecoeur, H.; Prina, E.; Rosazza, T.; Kokou, K.; N'Diaye, P.; Aulner, N.; Varet, H.; Bussotti, G.; Xing, Y.;Milon, G.; *et al*. Targeting Macrophage Histone H3 Modification as a Leishmania Strategy to Dampen theNF-κB/NLRP3-Mediated Inflammatory Response. Cell Rep. 2020, 30, 1870–1882.
16. Liew, F.Y.; Li, Y.; Millott, S. Tumour necrosis factor (TNF-alpha) in leishmaniasis. II. TNF-alpha-induced macrophage leishmanicidal activity is mediated by nitric oxide from L-arginine. Immunology 1990, 71, 556–559.
17. Gregory, D.J.; Godbout, M.; Contreras, I.; Forget, G.; Olivier, M. A novel form of NF-kappa B is induced by Leishmania infection: Involvement in macrophage gene expression. Eur. J. Immunol. 2008, 38, 1071–1081
18. Bogdan, C.; Röllinghoff, M. How do Protozoan Parasites Survive inside Macrophages? Parasitol. Today 1999,15, 22–28.
19. Wanasen, N.; Soong, L. L-arginine metabolism and its impact on host immunity against Leishmania infection.Immunol. Res. 2008, 41, 15–25.
20. Wei, X.Q.; Charles, I.G.; Smith, A.; Ure, J.; Feng, G.J.; Huang, F.P.; Xu, D.; Muller, W.; Moncada, S.; Liew, F.Y.Altered immune responses in mice lacking inducible nitric oxide synthase. Nature 1995, 375, 408–411.
21. Gantt, K.R.; Goldman, T.L.; McCormick, M.L.; Miller, M.A.; Jeronimo, S.M.B.; Nascimento, E.T.; Britigan, B.E.;Wilson, M.E. Oxidative Responses of Human and Murine Macrophages During Phagocytosis of Leishmaniachagasi. J. Immunol. 2001, 167, 893–901.
22. Kamhawi, S. The biological and immunomodulatory properties of sand fly saliva and its role in the establishment of Leishmania infections. Microbes Infect. 2000, 2, 1765–1773.
23. Scott, P.; Artis, D.; Uzonna, J.; Zaph, C. The development of effector and memory T cells in cutaneous leishmaniasis: The implications for vaccine development. Immunol. Rev. 2004, 201, 318–338
24. Sunter, J.; Gull, K. Shape, Form, Function and Leishmania Pathogenicity: From Textbook Descriptions to Biological Understanding. Available online: https://royalsocietypublishing.org/doi/10.1098/rsob.170165(accessed on 14 July 2020).
25. Kamhawi, S. The biological and immunomodulatory properties of sand fly saliva and its role in the establishment of Leishmania infections. Microbes Infect. 2000, 2, 1765–1773.
26. Scott, P.; Artis, D.; Uzonna, J.; Zaph, C. The development of effector and memory T cells in cutaneous leishmaniasis: The implications for vaccine development. Immunol. Rev. 2004, 201, 318–338.
27. Keynan, Y.; Larios, O.E.; Wiseman, M.C.; Plourde, M.; Ouellette, M.; Rubinstein, E. Use of oral miltefosine for cutaneous leishmaniasis in Canadian soldiers returning from Afghanistan. Can. J. Infect. Dis. Med. Microbiol. 2008, 19, 394–396.
28. Novais, F.O.; Santiago, R.C.; Báfica, A.; Khouri, R.; Afonso, L.; Borges, V.M.; de Oliveira, C.I. Neutrophils and macrophages cooperate in host resistance against Leishmania braziliensis infection. J. Immunol. 2009,183, 8088–8098.
29. Badaro, R.; Falcoff, E.; Badaro, F.S.; Carvalho, E.M.; Pedral-Sampaio, D.; Barral, A.; Bina, J.C. Treatment of visceral leishmaniasis with

pentavalent antimony and interferon gamma. New Engl. J. Med. 1990, 322, 16–21
30. Kumar R., Nylén S. Immunobiology of visceral Leishmaniasis.Logo of frontimmu Front Immunol. 2012; 3: 251. doi:10.3389/fimmu.2012.00251
31. Salam N., Al-Shaqha WM., Azzi A. Leishmaniasis in the MiddleEast: Incidence and Epidemiology; PLoS Negl Trop Dis. 2014;8(10): e3208. doi: 10.1371/journal.pntd.0003208
32. Mary C., Ange G., Dunan S., Lamouroux D., Quilici M. Characterization of a Circulating Antigen Involved in Immune Complexes in Visceral Leishmaniasis Patients. The American Journal of Tropical Medicine and Hygiene. 1993; 49(4): 492-501
33. Jancar S., Sánchez-Crespo M. Immune complex-mediated tissue injury: a multistep paradigm.; Trends Immunol. 2005; 26(1): 48-55
34. Kupani M, Sharma S, Pandey RK, Kumar R, Sundar S and Mehrotra S. IL-10 and TGF-β Induced Arginase Expression Contributes to Deficient Nitric Oxide Response in Human Visceral Leishmaniasis. *Front. Cell. Infect. Microbiol.* 2021; 10:614165. doi: 10.3389/fcimb.2020.614165
35. Haeberle, H. A., Kubin, M., Bamford, K. B., Garofalo, R., Graham, D. Y., El-Zaatari, F., ... &Ernst, P. B. Differential stimulation ofinterleukin-12 (IL-12) and IL-10 by live and killed Helicobacter pylori in vitro and association of IL-12production with gamma interferon-producing T cells in the human gastric mucosa. Infection and immunity, 1997, 65(10), 4229-4235.
36. Luu, V. P., Vazquez, M. I., & Zlotnik, A. (2014). B cells participate in tolerance and auto immunity through cytokine production. Autoimmunity, 2014, 47(1), 1-12.