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Interferon-Gamma and Interleukin 17 Patterns in Patients Infected With *L. Major*: Early Dermal Lesions

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ABSTRACT

Cutaneous leishmaniasis (CL) is consider from the global neglected health diseases, which are an endemic in many countries. It caused by obligatory intracellular protozoanreturn to genus *Leishmania* and invades phagocytic cells. The current study is aimed to measure interferon-gamma and interleukin 17 levels in serum of patients with *L. major*. It was conductedin Dermatology Department/ Al-Hussein Teaching Hospital inThi-Qarprovince, south of Iraqfor the period from the beginning of January to the end of December 2020. This study included 110 patients haveinfected withcutaneous leishmaniasis, which they wereclinically diagnosed. Furthermore, the samples were taken from early dermal lesions (symptomsappeared on less than 4 weeks) in order to done molecular examination with Nested-PCR technique, and then peripheral blood collected for a measure of interferon-gamma (IFN-γ)and interleukin 17 (IL-17) concentration levels in patients' sera by ELISA assay. The findings of DNA amplification of kinetoplast minicircle DNA gene (kDNA) showed*L. major* in 31 (32.6%) Nested-PCR products, which generated at 560 bp. The results showedan insignificant decreasingin IFN-γ level, while IL-17 level was increased in early cutaneous lesionsamongthe patients infected with *L. major*. Generally, the study confirmed that *Leishmania* parasites inhibit and/or modulate signaling pathways of some cytokines in the host cell, which effect on other immune cells in order its own survival

Keywords: Cutaneous lesion, IFN-γ, IL-17, Nested-PCR, kDNA, *L. major*.

INTRODUCTION

Leishmaniasis are vector-borne diseases, which caused by intra-cellular protozoan of genus *Leishmania* and infect human and other mammals. There are approximately 20 species are confirmed as a pathogenic (1). *Leishmania* spp. are obligatorily infect phagocytic cells and cause three main forms (dermal cutaneous leishmaniasis, muco-cutaneous leishmaniasis and visceral leishmaniasis). Moreover, the form and severity of the infection basically depend on *Leishmania* species and host immunological response (2).Generally, cutaneous leishmaniasis is the most common leishmaniasis, which caused a papule usually chronicandpainless, often at a site of infected sandfly bite (3). Dermal lesion appears after an incubation period my reach to several months, which often heal spontaneously to leave a depressed, depigmented scar and permanent immunity (4).

Once entry of *Leishmania* parasites inside the host cell, they avoid the immune defenses for a survival. Indeed, *Leishmania* spp. have different strategies to affect both innate and adaptive immunity in order its own survival (5). *Leishmania* spp. have a similar scenario of other intra-cellular microbes, whereapoptotic neutrophil is worked as a Trojan horse in order to a silent entry of *Leishmania*parasites

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inside macrophage (6). Neutrophils reduce as result to developingfast lesion and increased *Leishmania* number within the first week formthe infection ofBALB/c mice (7).

The healing of the lesion occurs after an adaptive cellular response that possible to fight the infection (8). However, immune response against leishmaniasis mostly mediated by T cells, that play an essential role in generating response to intra-cellular parasites. T cell responses are directly related to *Leishmania* infection(9). On experimental level, Both Th1 and Th2 responses can be distinguished by cytokines secreted: Th1 cells produce activators of cell-mediated immunity such as: IFN-γ, whereas Th2 cells secrete cytokines: such as IL-4, that promote antibody responses (10).

Macrophages activate by IFN-γ produced $CD4^+$ T cells which contribute to controlling parasite growth, while $CD8^+$ T cells are associated with pathogenesis of parasite(11).IFN-γ not only activates macrophages to kill *Leishmania* parasites but also induce the differentiation of T cells to Th1 cells(12).In fact, high expression of IFN-γ and IL-12 induce a protective immunity against the *Leishmania* parasites and prevent spread of the disease (13). However, IFN-γ induces the activation of microbicidal mechanisms of macrophage. It stimulates the production of nitric oxide (NO) and enhances reactive oxygen species (ROS), that are play a vital role in killing intra-cellular amastigotes(14)(15).

IL-17 is mostly produced by Th17 cells, also produces form other cells such as CD8+ T cells and neutrophils (16). It plays an essential role in the protection of thehostagainst bacterial, parasitic and fungal infection(17). (18) mentioned that IL-17A increases the pathogenesis with *L. major* infection due recruitment of neutrophils at the infection site. Moreover, excessive production of IL-17 may cause tissue destruction, severe inflammation and recruiting neutrophils (19). Recently, elevated levels of IL-17 have been observed in patients infected with CL and MCL, there is suggestion which IL-17 may also has a proinflammatory role in the infection and may be a target for immunotherapy (18).

To understand immune response interactions of patients with *L. major* in early stages of the infection, this study is aimed to cleara relationship betweenIFN-γ and IL-17in early dermal lesions.

MATERIALS AND METHODS

Study design and patient population

This study was carried out at Dermatology Department, Al-Hussein Teaching Hospital in Thi-Qar province, south Iraqfor the period from thebeginning of January to the end of December 2020. It was included 110 patients suffering from CL, after their diagnosed clinically by dermatologists. However, the samples were collected only from early dermal lesions (the symptom date was less than one month) as the following: 0.2 ml of normal saline was injected at the edge of lesion, then withdraw again. Next, the fluid was put into a plain tube for molecular identification. After that, 3 ml of the peripheral bloodsamples were collected form each patient before a treatment, also from normal individuals live the same local area. The blood samples were separated with centrifuge at 3000 rpm. Finally, the serum was put into a plain tube and stored at -20°C till cytokines level measuring withsandwich ELISA assay.

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Ethical approval

Ethical agreementwas obtained from University Committee. Participation in the study is voluntary, the written consent to all patients was taken before starting the study and they had freedom to withdraw from the study.

DNA extraction

For genomic DNA extraction form a fluid, the extraction kit (gSYAN DNA kit/ Geneaid, Taiwan) was used as per produced company instructions. In addition, a Nanodrop spectrophotometer was used to detect DNA concentration and DNA purity, then kept at -20˚C even using in PCR technique.

DNA amplification

A Nested-PCR technique was used to amplify kDNA for identification of *Leishmania* spp., according to Izadi*et al.*(20), with some modification of PCR assay. However, the amplification of *Leishmania*kDNA was included two steps (primers were produced by Macrogen Company, Korea): the first run passed with external primers (CSB2XF: 5'-CGA GTA GCA GAA ACT CCC GTT CA-3' and CSB1XR: 5'-ATT TTT CGC GAT TTT CGC AGA ACG-3'). Then the product of first-run passed with second-run usinginternal primers (13Z: 5'-ACT GGG GGT TGG TGT AAA ATA G-3' and LiR: 5'-TCG CAG AAC GCC CCT-3'). A master mix was provided by AccuPower® PCR PreMix kit. Bioneer, Korea. A Nested-PCR master mix of the first-run was included 5µL of DNA templet, 1µL of each forward and reverse(external) primers, and 13µL of nuclease free water and placed into standard-PCR tubes.Moreover, PCR thermal conditions werean initial denaturation (95°C for 5 minutes), then 30 cycles: denaturation (95°C for 30 seconds), annealing (55°C for 30 seconds)and extension (72°C for one minute), followed by final extension (72°C for 5 minutes). The master mix of the second-run was composed form 3µL of first-run product, 1µL of each forward and reverse (internal) primers, and 15µL of nuclease free water and then putted in standard-PCR tubes with thermal conditions for nested-PCR reaction. The second-run products were passed with electrophoresis (1% agarose gel with 3µL of ethidium bromide). In each well, 10µl of PCR product was added within comb wells. In addition, 5µl of 100bp ladder in first well. The tray was fixed into the electrophoresis room and filled with a TBE buffer. Finally, the electric current was 100 v and 80 mA for one hour, and PCR product bands were visualized by an ultraviolet transilluminator.

Human cytokine level measurements

To determine cytokine (IFN-γ and IL-17) levels in the serum of patients, the samples were prepared depending on company instruction manual (Elabscience, China).

All reagents and samples were brought up to room temperaturebefore using (Al-Ubaydi,2018). The standard solutions and samples (100µL) were added for each micro-plate well, covered with sealer and then incubated for 90 minutesat 37°C, thenthe liquid into each well was removed. Directly, 100µL of biotinylated detection Ab was added to per well, covered by the plate sealer, and incubated for one hourat 37°C. The wells were washed three times. After that, 100µL of HRP conjugate solution was added to per well, covered with the plate sealer, and then incubated for 30 minutesat 37°C. Next, 90µL of substrate reagent was added into each well, covered by a new plate sealer, and then incubated at 37°C for 15 minutes. Stop solution (50µL) was added into each well. The color of wells was turned to yellow directly. Finally, a microplate ELISA reader set at 450 nm to determine the visualdensity (OD value) of per well at once.

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Statistical analysis

An independent samples T test was used in the current study. The data analyzed with SPSS statistical package software (V.25). A significant level was set at P ≤0.05 (Al-Ubaydi,2018).

RESULTS

Agarose gel electrophoresis results showed 95 (86.4%) positive of mitochondrial genome of *Leishmania* spp. (kDNA). There weretwo species co-existing (*L. tropica* and *L. major*) havedistinguished in the study area,*L. major*showed in 31 (32.6%) samples. Nested-PCR product of *L. major* was generated at 560 bp.

The findings of serum IFN-γ level of *L. major*-infected patientswere not showed a significant difference (P>0.05) between infected patients with early lesion and healthy individuals (n=25), which was the mean values 4.607 ± 0.96pg/ml, whereasIFN-γ concentration level ofhealthy group was the mean values 5.114 ± 1.01pg/ml, and t. Value was 1.904, while P. Value was 0.062.

According to statistical analysis of IL-17 level in sera of the groups. There was a significant increasing of IL-17 concentration level in infected with *L. major* at comparison with healthy individuals. Serum IL-17 level values showed 240.5± 12.14. In contrast, IL-17 level in the patients was elevated, where it was mean values 374.3 ± 43.7 pg/ml, whilst t. Value was 16.28 and P. Value was 0.00 (Figure 1).

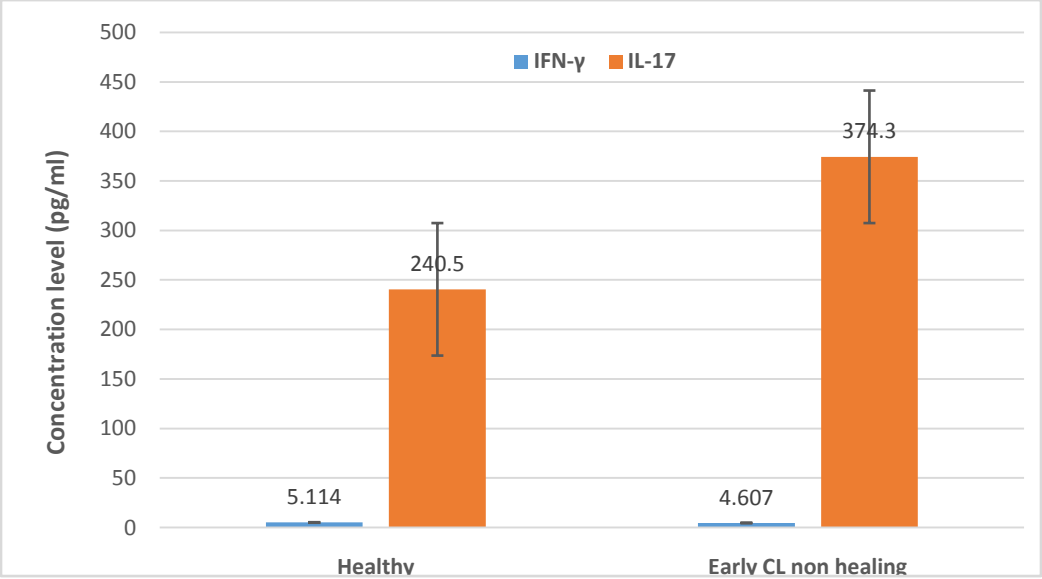

Figure 1: Cytokine levels in early lesion patients with *L. major* and healthy individuals.

DISCUSSION

Traditional methods such as parasite culture or direct smear do not distinguish causative species of dermal cutaneous leishmaniasis(21). Although, molecular techniques were used determine *Leishmania* genus, species or intra-species.The kinetoplast minicircles DNA considers most common method to discriminate *Leishmania* species (22). In addition, an identification and determination of *Leishmania* spp. are an essential, because *Leishmania* infections are administered with different treatment protocols (23). Generally, this study showed both *L. tropica* (67.4%) and *L. major* (32.6%) were present in local

area, which diagnosed depending on kDNA amplification. This finding is close toMezher(24)in Al-Muthanna province;Seaad*et al*. (25) in Al-Qadissyia province and Mohammadiha *et al.* (26)in Khorasan‑Razavi province/ Iran, whereas it was far fromAl-Hassani(27) inAl-Qadissyia province; Al-Tamemy and Al-Qurashi(28)in Wasit province andAmro *et al.* (29) in Libya.

Immunologically, Cytokines are performing important functions in the immune response, this make them as a bridge between both innate and adaptive immune responses (30). However, *Leishmania* spp. infect immune phagocytes, such as neutrophil, dendritic cell (DC) and especially macrophage (31). Further, virulence factors of *Leishmania*spp. inhibit or modify signaling pathways of the host cell (cytokine and chemokine) that inhibit the productionof lymphocytes and/ or other immune cells(32). However, Th1 cell immune responses mediate protection against *Leishmania* parasites, whereas Th2 cells activation is associated with disease development and persistence (33). IFN-γ activates protective immune response against CL. IFN-γ acts as a necessary cytokine to kills *Leishmania* and produces protective immunity of CL in human and murine (10), also it secretes mainly by activated Th1-type and NK cells as a response to IL-12 signaling(13). *L. major* is down-regulating NK cell proliferation and IFN-γ production for establishment of early infection (34). (35) mentioned that *Leishmania* parasites can inhibit DC-Th1 polarizing functions through them evolved strategies. In this study, IFN-γ level in *L. major*-infected sera were not showed a significant difference compared to healthy group. The current finding was consistent with Souza *et al.*(36);Zijlstra *et al.*(37)andMahmoodi *et al.*(38), whereas was inconsistent with Galgamuwa *et al.*(13).

On the other hand,proinflammatory IL-17 participates in promoting inflammatory response, also shares in tissue repair (39). However, IL-17 functions are not sufficiently understood in mammals and most actual researches are focusing at the functions of IL-17 and its regulation in pathological conditions (infectious and autoimmune diseases)(30). In some studies, IL-17A is an important cytokine for IFN-γ production, whereas there are other reports have referred to increase IFN-γ production with the absence of IL-17 (40). In the current study, IL-17 level recorded a significant high in patients of early dermal lesion. This finding was consistent with Kostka *et al.* (41); Teixeira *et al.* (42) andFlaih *et al.*(43).

CONCLUSION

Cutaneous leishmaniasis is still consider one of the neglected health problems, which causes social, psychological, ecological, and economic effects. Furthermore, we need to understand an immune response network, and their interactions. The study showsIFN-γ decreased level and elevated IL-17 in early lesion of *L. major*-infected patients. This is adjacent with previous studies, which showed *Leishmania* parasites inhibit and/or modulate cytokines signaling pathways of the host cell or other immune cells in order to survival, and also IL-17 recruits neutrophils at the infection site in early infection specially.

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CONFLICT OF INTEREST

The authors declare:No conflicts of interests about a publication of this article.

REFERENCES

1. Mesa LE, Manrique R, Muskus C, Robledo SM. Test accuracy of polymerase chain reaction methods against conventional diagnostic techniques for Cutaneous Leishmaniasis (CL) in patients with clinical or epidemiological suspicion of CL : Systematic review and meta- analysis. PLoS Negl Trop Dis. 2020;41(1):1–15.

2. Walker DM, Oghumu S, Gupta G, Mcgwire BS, Drew ME, Satoskar AR. Mechanisms of cellular invasion by intracellular parasites. Cell Mol Life Sci. 2014;71(7):1245–63.

3. Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-velez R, Weina P, et al. Diagnosis and Treatment of Leishmaniasis : Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH). Clin Infect Dis. 2016;63:202–64.

4. Bilgic-Temel A, Murrell DF, Uzun S. Cutaneous leishmaniasis: A neglected disfiguring disease for women. Intern J Women's Dermatol [Internet]. 2019;5(3):158–65. Available from: https://doi.org/10.1016/j.ijwd.2019.01.002

5. Rossi M, Fasel N. How to master the host immune system ? Leishmania parasites have the solutions ! Intern Immunol. 2017;30(3):103–11.

6. Tasew G, Gadisa E, Abera A, Zewude A, Chanyalew M, Aseffa A, et al. In vitro permissiveness of bovine neutrophils and monocyte derived macrophages to Leishmania donovani of Ethiopian isolate. Parasit Vectors [Internet]. 2016;9(218):1–9. Available from: http://dx.doi.org/10.1186/s13071-016-1441-5

7. Sousa LMA, Carneiro MBH, Resende ME, Martins LS, Dos Santos LM, Vaz LG, et al. Neutrophils have a protective role during early stages of Leishmania amazonensis infection in BALB/c mice. Parasite Immunol. 2014;36(1):13–31.

8. Silva-almeida M, Acácio B, Pereira S, Ribeiro-guimarães ML, Alves CR. Proteinases as virulence factors in Leishmania spp . infection in mammals. Parasit Vectors. 2012;5(160):1–10.

9. Sharma U, Singh S. Immunobiology of Leishmaniasis. Indian J Exp Biol. 2009;47(2009):412–23.

10. Maroufi Y, Ghaffarifar F, Dalimi A, Sharifi Z. Interferon-Gamma and Interlukin-4 Patterns in BALB / c Mice Suffering From Cutaneous Leishmaniasis Treated With Cantharidin. Jundishapur J Microbiol. 2014;7(6):7–10.

11. Saldanha MG, Queiroz A, Machado PRL, Carvalho LP, Scott P, Filho EMC, et al. Characterization of the Histopathologic Features in Patients in the Early and Late Phases of Cutaneous Leishmaniasis. Am J Trop Med Hyg. 2017;96(3):645–52.

12. Novais FO, Carvalho LP, Graff JW, Beiting DP, Ruthel G, Roos DS, et al. Cytotoxic T Cells Mediate Pathology and Metastasis in Cutaneous Leishmaniasis. PLoS Pathog. 2013;9(7):1–11.

13. Galgamuwa LS, Sumanasena B, Iddawela D, Wickramasinghe S, Yatawara L. Assessment of intralesional cytokine profile of cutaneous leishmaniasis caused by Leishmania donovani in Sri Lanka. BMC Microbiol. 2019;19(14):1–11.

14. Rodrigues V, Cordeiro-da-silva A, Laforge M, Silvestre R, Estaquier J. Regulation of immunity during visceral Leishmania infection. Parasit Vectors [Internet]. 2016;9(118):1–13. Available

from: http://dx.doi.org/10.1186/s13071-016-1412-x

15. Scott P, Novais FO. Cutaneous leishmaniasis : immune responses in protection and pathogenesis. Nat Immunol. 2016;16:281–592.

16. Gonçalves-de-Albuquerque SC, Pessoa-e-Silva R, Trajano-Silva LAM, Goes TC, Morais RCS., Oliveira CNC, et al. The equivocal Role of Th17 Cells and Neutrophils on immunopathogenesis of Leishmaniasis. Front Immunol. 2017;8(1437):1–11.

17. Banerjee A, Bhattacharya P, Joshi AB, Ismail N, Dey R, Nakhasi HL. Role of pro-inflammatory cytokine IL-17 in Leishmania pathogenesis and in protective immunity by Leishmania vaccines. Cellu Immunol [Internet]. 2016;309:37–41. Available from: http://dx.doi.org/10.1016/j.cellimm.2016.07.004

18. Gonzalez-lombana C, Gimblet C, Bacellar O, Oliveira WW, Passos S, Carvalho LP, et al. IL-17 Mediates Immunopathology in the Absence of IL-10 Following Leishmania major Infection. PLoS Pathog. 2013;9(3):1–14.

19. Andargie TE, Ejara ED. Pro- and Anti-inflammatory Cytokines in Visceral Leishmaniasis Journal of Cell Science & Therapy. J Cell Scie Ther. 2015;6(3):2–8.

20. Izadi S, Mirhendi H, Jalalizand N, Khodadadi H, Mohebali M, Nekoeian S, et al. Molecular Epidemiological Survey of Cutaneous Leishmaniasis in Two Highly Endemic Metropolises of Iran , Application of FTA Cards for DNA Extraction From Giemsa-Stained Slides. Jundishapur J Microbiol. 2016;9(2):1–7.

21. Kheirandish F, Sharaf AC, Kazemi B, Mehdi M., Sarlak A, Tarahi MJ, et al. Identifcation of Leishmania species using PCR assay on giemsa-stained slides prepared from cutaneous leishmaniasis patients. Iran J Parasitol. 2013;8(3).

22. Flaih MH, Al-Abady FA, Hussein KR. Phylogenetic analysis of kinetoplast DNA: kDNA of Leishmania tropica in Thi- Qar province, Iraq. medRxiv Prepr. 2020;1–13.

23. Salloum T, Khalifeh I, Tokajian S. Detection , molecular typing and phylogenetic analysis of Leishmania isolated from cases of leishmaniasis among Syrian refugees in Lebanon. Para Epidemiol Cont [Internet]. 2016;1(2):159–68. Available from: http://dx.doi.org/10.1016/j.parepi.2016.02.002

24. Mezher JO. Study of comparision between traditional methods and molecular diagnosis of cutaneous leishmaniasis in Al-Muthanna Province. Coll. Scien. Uni. Muthanna; 2017.

25. Seaad AA, Abdulah SR, Kelef SF. Epidemiological prevalence of cutaneous Leishmaniasis In immigration people in Al-Qadissyia province. Al-Anbar J Veteri Scien. 2017;10(1):77–83.

26. Mohammadiha A, Dalimi A, Mahmoodi MR, Parian M, Pirestani M, Mohebali M. The PCR-RFLP-Based Detection and Identification of the Leishmania Species Causing Human Cutaneous Leishmaniasis in the Khorasan-Razavi Province, Northeast of Iran. J Arthropod-Borne Dis. 2017;11(3):383–92.

27. Al-Hassani MKKT. Epidemiological, Molecular and Morphological Identification of cutaneous leishmaniasis and, It's insect vectors in Eastern Al-Hamzah district,AlQadisiya province. Coll. Educat. AL-Qadisiya Univ.; 2016.

28. Al-Tamemy AKA, Al-Qurashi NNS. Identification and Sequences of Two Leishmania Species

Isolated From Human Cutaneous Lesion in Iraq. IOSR-JPBS. 2017;12(4):53–7.

29. Amro A, Gashout A, Al-Dwibe H, Alam MZ, Annajar B, Hamarsheh O, et al. First molecular epidemiological study of cutaneous leishmaniasis in Libya. PLoS Negl Trop Dis. 2012;6(6):1–6.

30. González-Fernández C, Chaves-Pozo E, Cuesta A. Identification and Regulation of Interleukin-17 (IL-17) Family Ligands in the Teleost Fish European Sea Bass. Int J Mol Sci. 2020;21(2439):1–15.

31. Meira CS, Gedamu L. Protective or Detrimental ? Understanding the Role of Host Immunity in Leishmaniasis. Microorganisms. 2019;7(695):1–25.

32. Gupta G, Oghumu S, Satoskar AR. Mechanisms of Immune Evasion in Leishmaniasis Gaurav. Adv Appl Microbiol. 2013;82:155–184.

33. Abu Musa M, Nakamura R, Hena A, Varikuti S, Nakhasi HL, Goto Y, et al. Lymphocytes influence Leishmania major pathogenesis in a strain-dependent manner. PLoS Negl Trop Dis. 2019;13(11):1–15.

34. Dayakar A, Chandrasekaran S, Kuchipudi S V., Kalangi SK. Cytokines : Key Determinants of Resistance or Disease Progression in Visceral Leishmaniasis : Opportunities for Novel Diagnostics and Immunotherapy. Front Immunol. 2019;10(670):1–23.

35. Moll H, Scharner A, Kampgen E. Increased interleukin 4 (IL-4) receptor expression and IL-4-induced decrease in IL-12 production by Langerhans cells infected with Leishmania major. Infect Immun. 2002;70:1627–1630.

36. Souza VL, Souza JA, Silva TMC, Veras PST, Freitas LAR. Different Leishmania species determine distinct profiles of immune and histopathological responses in CBA mice. Microbes Infect. 2000;2(15):1807–15.

37. Zijlstra EE, Musa AM, Khalil EAG, El Hassan IM and, El-Hassan AM. Post-kala-azar dermal leishmaniasis. Lancet Infect Dis. 2003;3(2):87–98.

38. Mahmoodi M, Rajabalian S, Fekri A, Esfandiarpour I. Evaluation of In Vitro Production of IFN-γ , IL-10 , IL-12 and IL-13 by Blood Cells in Patients with Cutaneous Leishmaniasis Lesions. Iran J ALLE, ASTH IMMUNOL. 2005;4(1):15–21.

39. Pitta MGR, Romano A, Cabantous S, Henri S, Hammad A, Kouriba B, et al. IL-17 and IL-22 are associated with protection against human kala azar caused by Leishmania donovani Find the latest version : IL-17 and IL-22 are associated with protection against human kala azar caused by Leishmania donovani. J Clin Investig. 2009;119(8):2379–87.

40. Terrazas C, Varikuti S, Kimble J, Moretti E, Boyaka PN, Satoskar AR. IL-17A promotes susceptibility during experimental visceral leishmaniasis caused by Leishmania donovani. FASEB J. 2016;30(3):1135–43.

41. Kostka SL, Dinges S, Griewank K, Iwakura Y, Udey MC, Stebut E. IL-17 Promotes Progression of Cutaneous Leishmaniasis in Susceptible Mice. J Immunol [Internet]. 2009 Mar 1;182(5):3039–46. Available from: http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.0713598

42. Teixeira CR, Santos CS, Prates DB, Santos RT, Araújo-Santos T, Souza-Neto SM, et al. Lutzomyia longipalpis Saliva Drives Interleukin-17-Induced Neutrophil Recruitment Favoring

Leishmania infantum Infection. Front Microbiol. 2018;9(881):1–10.

43. Flaih MH, Al-Abady FA, Hussein KR. Detection of pro-inflammatory interlukin 17 in patients with L. tropica in Thi-Qar province, Iraq. Periódico tchê química. 2020;17(36):949–57.

44. Qasim, Maytham T., and Hussein Khudair Al-Mayali. "Investigate the relation between Baicalin effect and Gene expression of LH, FSH, Testosterone in male rats treated with Gemcitabine drug." Research Journal of Pharmacy and Technology 12.9 (2019): 4135-4141.

45. Qasim, Maytham T., and Hussein Khudair Al-Mayali. "The immunological and protective role of Baicalin in male rats treated with chemotherapy (Gemcitabine)." Journal of Physics: Conference Series. Vol. 1234. No. 1. IOP Publishing, 2019.

46. Tahmasebi S, Qasim MT, Krivenkova MV, et al. The effects of Oxygen-Ozone therapy on regulatory T-cell responses in multiple sclerosis patients [published online ahead of print, 2021 Mar 16]. Cell Biol Int. 2021;10.1002/cbin.11589. doi:10.1002/cbin.11589.

47. Oudah, S. K., Al-Salih, R. M. H., Gusar, S. H., & Roomi, A. B. Study of the role of polyphenolic extract of capparis spinosa L. leaves as acute toxicity and antibacterial agent. Plant Archives, 19(2), (2019):3821-3829.