DETECÇÃO DE INTERLEUCINA PRÓ-INFLAMATÓRIA 17 EM PACIENTES COM *L. TROPICA* NA PROVÍNCIA THI-QAR, IRAQUE

DETECTION OF PRO- INFLAMMATORY INTERLEUKIN 17 IN PATIENTS WITH *L. TROPICA* IN THI-QAR PROVINCE, IRAQ

تحديد الانترلوكين 17 المحرض للالتهاب في المرضى المصابين بطفيلي *L. TROPICA* في محافظة ذي قار

FLAIH, Mohammed Hassan^{1*}; AL-ABADY, Fadhil Abbas²; HUSSEIN, Khwam Reissan¹

¹ University of Southern Technical, Institute of Al-Nasiriya Technical, Department of Medical Laboratory. Iraq.

² University of Thi-Qar, Faculty of Education for pure Sciences, Department of Biology. Iraq.

*Corresponding author

e-mail: moh.alqurayshi@stu.edu.iq

Received 03 August 2020; received in revised form 28 October 2020; accepted 09 November 2020

RESUMO

A leishmaniose cutânea (LC) é um grande problema de saúde e considerada uma das doenças endêmicas no Iraque. A lesão dérmica ocorre devido a um parasita intracelular obrigatório de *Leishmania* que transmite pela picada do flebotômíneo fêmea infectado. Este estudo tem como objetivo identificar espécies de *Leishmania* na província de Thi-Qar/sul do Iraque, bem como detectar o nível de IL-17 no soro de pacientes infectados com *L. tropica*. O estudo foi realizado em três locais, Al-Hussein Teaching, Al-Suq Al-Shyokh General e Al-Shatrah General Hospitals na província no período entre o início de novembro de 2018 e o final de outubro de 2019. Após o diagnóstico clínico, oitenta das duzentas e quarenta e sete amostras foram selecionadas para exame molecular pela técnica de nested-PCR, onde a borda da lesão foi injetada com soro fisiológico normal e novamente puxada para obter o DNA do parasita. Também é uma medida do nível de concentração de IL-17 no soro dos pacientes com ELISA. Os achados da eletroforese do gene do DNA do minicírculo do cinetoplasto mostraram que 65 amostras eram positivas para leishmaniose cutânea e observaram duas espécies de *Leishmania* spp. na área de estudo, 46 (57,5%) amostras foram *L. tropica* a 750 pb e 19 (23,75%) amostras foram *L. major*. A concentração sérica de IL-17 registrou um aumento significativo entre os pacientes infectados com *L. tropica* em diferentes estágios de infecção, em comparação com as amostras controle. Geralmente, a técnica Nested-PCR é um método preciso para o diagnóstico de amostras clínicas e a determinação molecular de parasitas de *Leishmania*. *L. tropica* é a principal espécie que causou CL na província de Thi-Qar, enquanto *L. major* registrou uma baixa incidência.

Palavras-chave: *Leishmania tropica*, IL-17, leishmaniose cutânea, Nested-PCR, kDNA

ABSTRACT

Cutaneous leishmaniasis (CL) is a widespread health problem and considered one of the endemic diseases in Iraq. The dermal lesion occurs due to an obligate intracellular *Leishmania* parasite, which transmits by the bite of the infected female sandfly. This study aims to identify *Leishmania* species in Thi-Qar province/ South of Iraq and detect IL-17 level in serum of infected patients with *L. tropica*. The study was conducted in three local locations, Al-Hussein Teaching, Al-Suq Al-Shyokh General, and Al-Shatrah General Hospitals in the province for the period from the beginning of November 2018 to the end of October 2019. After clinical diagnosis, eighty out of two hundred forty-seven samples were selected for molecular examination by nested-PCR technique, where the lesion edge was injected by normal saline and pulled again to obtain the parasite DNA. Also, a measure of the IL-17 concentration level in serum of the patients with ELISA. The findings of the electrophoresis of the kinetoplast minicircle DNA gene showed that 65 samples were positive for cutaneous leishmaniasis, and observed two species of *Leishmania* spp. in the study area, 46 (57.5%) samples were *L. tropica* at 750bp and 19 (23.75%) samples were *L. major*. Serum IL-17 concentration recorded a significant increase among patients infected with *L. tropica* at different infection stages than control samples. Generally, the Nested-PCR technique is an accurate method for diagnosing clinical samples and molecular determination of *Leishmania* parasites. *L. tropica* is the dominant specie that caused CL in Thi-Qar province, while *L. major* recorded a low incidence.

Keywords: *Leishmania tropica*, IL-17, cutaneous leishmaniasis, Nested-PCR, kDNA

يعتبر داء اللشمانيا مشكلة صحية واسعة النطاق ويعتبر أحد الأمراض المتوطنة في العراق. الافة الجلدية تحدث بسبب طفيلي اللشمانيا وهو اجباري داخل خلوي الذي يحدث بسبب عضة انثى ذبابة الرمل المصابة. هدفت هذه الدراسة الى تشخيص أنواع طفيلي اللشمانيا في محافظة ذي قار/ جنوب العراق، كذلك قياس مستوى تركيز الانترلوكين 17 في مصل المرضى المصابين بـ *L. tropica*. أجريت الدراسة في ثلاث مواقع هي مستشفى الحسين التعليمي، مستشفى سوق الشيوخ العام ومستشفى الشرطة العام في المحافظة للفترة من بداية شهر تشرين الثاني 2018 الى نهاية شهر تشرين الأول 2019. ثمانين من أصل 247 عينة اختيرت بعد تشخيصهم سريريا، لغرض الفحص الجزيئي بواسطة تقنية Nested-PCR حيث حققت الافة بواسطة المحلول الملحي وسحب مجدول لغرض الحصول على DNA الطفيلي. كذلك قيس مستوى تركيز IL-17 في مصل المرضى بواسطة جهاز الـ ELISA. أظهرت نتائج الترحيل الكهربائي لجين kDNA ان 65 عينة كانت موجبة لداء اللشمانيا الجلدي ولوحظ وجود نوعين من طفيلي اللشمانيا في منطقة الدراسة حيث كانت 46 عينة بنسبة (57.5%) لطفيلي *L. tropica* بطول 750bp و19 عينة بنسبة (23.75%) كانت لـ *L. major*. سجل تركيز الـ IL-17 في المصل زيادة معنوية بين المرضى المصابين بطفيلي *L. tropica* على مراحل إصابة مختلفة مقارنة مع عينة السيطرة. عموما تقنية Nested-PCR هي طريقة مناسبة لتشخيص العينات السريرية والتشخيص الجزيئي لطفيليات اللشمانيا. *L. tropica* هو المسبب الرئيسي للشمانيا الجلدية في محافظة ذي قار، في حين *L. major* سجل حدوث منخفض.

الكلمات المفتاحية: طفيلي *L. tropica*، الانترلوكين 17، داء اللشمانيا الجلدي، تقنية Nested-PCR، kDNA.

1. INTRODUCTION:

Leishmaniasis is caused by flagellated *Leishmania* parasites, an obligate intracellular protozoan, and infects humans and other mammals (Ramírez *et al.*, 2016). It is a vector-borne disease transmits by an infected females sandfly bite, and the disease highly spreads in poor populations and tropical and subtropical countries (Dayakar *et al.*, 2019). However, *Leishmania* parasites can establish and survive inside host cells. They resist and circumvent anti-parasitic immune response pathways (Oghumu *et al.*, 2015). Indeed, the immunological status and the genetic background of the patient, also *Leishmania* species effects severely on the form and type of the CL dermal lesion (Maksouri *et al.*, 2017).

Leishmania spp. have different strategies to affect both innate and adaptive immunity for its survival (Rossi and Fasel, 2017). Generally, *Leishmania spp.* infect immune phagocytes, such as neutrophil, dendritic cell (DC), and especially macrophage. The immunological and cellular mechanisms associated with leishmaniasis infection are not completely clear, and most of the present knowledge about leishmaniasis is based on experimental models (Meira and Gedamu, 2019).

It is necessary to understand immune cell interactions and its secretion. Also, cytokines interplay in host defense or pathogenesis to determine appropriate immunotherapies of leishmaniasis (Dayakar *et al.*, 2019). However, Interleukin-17 has an instrumental role in the protection of the host against several pathogens, such as Gram-positive bacteria, fungi, and parasites (Banerjee *et al.*, 2016). The role of IL-17 in leishmaniasis infections remains poorly

understood, and more studies are required (Gonzalez-lombana *et al.*, 2013). IL-17 is mostly produced by Th17 cells (Andargie and Ejara, 2015). Several modern studies refer to elevated IL-17 with human inflammatory or autoimmune diseases (McGeachy *et al.*, 2019). However, it works on a broad range of cells. It induces other cytokine expression and activates and attracts and recruits neutrophils to inflammation sites (Banerjee *et al.*, 2016). IL-17 plays a vital role in the elimination of the intracellular parasite. Th17 cells are essential in the clearance of *Leishmania* parasites (Gonçalves-de-Albuquerque *et al.*, 2017). IL-17 stimulates other immune cells to produce inflammatory molecules. It affects the neutrophil function at the site of inflammation, reduces apoptosis, and induce the secretion of proinflammatory cytokines, also tissue-damaging molecules (Dayakar *et al.*, 2019). Gonzalez-lombana *et al.* (2013) were observed blocking IFN- γ results a significant high in neutrophil accumulation and IL-17 production in the lesions of mice.

PCR techniques are used to identify *Leishmania* in clinical samples and the detection of causative specie of dermal lesion (Mosleh *et al.*, 2015) (Rodríguez-Brito *et al.*, 2015). The nested-PCR technique is considered a suitable method, has high sensitivity and very resolution identification of *Leishmania* parasites in clinical samples (Al-Tamemy and Al-Qurashi, 2017) (Akhoundi *et al.*, 2017). Moreover, the kinetoplast DNA (kDNA) gene has shown high sensitivity and specificity in detecting (Ranasinghe *et al.*, 2015) (Naseri *et al.*, 2016).

According to the importance and plenty of cutaneous leishmaniasis infections in the province, this study aimed to identify the prevalence of *Leishmania* species and to measure

serum interleukin-17 concentration among infected patients.

2. MATERIALS AND METHODS:

2.1. Study area and patients

The study was conducted in Thi-Qar province, South of Iraq, it included three locations to the samples collection: Al-Hussein Teaching, Al-Suq Al-Shyokh General, and Al-Shatrah General Hospitals for the period from the beginning of November 2018 to the end of October 2019. The hospitals have received patients suffering from Cutaneous leishmaniasis (CL). A total of 247 patients were diagnosed clinically by dermatologists as cutaneous leishmaniasis and did not show any other cutaneous diseases. However, 80 samples were collected randomly from patients with CL (Figure 1). The lesions were injected using normal saline (0.2 ml) in the lesion edge, then pulled again. After that, the fluid was kept in a plain tube for molecular examination. Also, 3 ml of the venous blood was collected from patients before their take treatment, then separated by centrifuge 3000 rpm. The serum was put in a plain tube and stored at -20 °C until use for interleukin-17 level measuring using ELISA assay.

2.2. Ethical approval

Ethical approval was obtained from the Committee of the university. Participation in this study was voluntary, and all patients consented to participate in this study and were free to withdraw at any time from this research (Schroter *et al.*, 2006).

2.3. Genomic DNA extraction

The gSYAN DNA kit (Geneaid, Taiwan) was used for DNA extraction from lesion fluid, according to the protocol of the produced company. DNA concentration was examined using a Nanodrop spectrophotometer and then stored at -20°C until used in PCR amplification (Al-Difaie, 2013).

2.4. Nested-PCR amplification

The kinetoplast minicircle DNA was amplified for identification of *Leishmania* spp, using the Nested-PCR technique, with some modification of PCR assay, according to Izadi *et al.* (2016), which included two steps. DNA of parasite undergone the first run 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 first run product passed with the second run with internal specific primers: 13Z 5'-ACT GGG GGT TGG TGT AAA ATA G-3' and LiR 5'-TCG CAG AAC GCC CCT-3'. PCR-master mix was prepared by (AccuPower® PCR PreMix kit. Bioneer, Korea). Nested PCR primers were provided via the Macrogen Company, Korea. Nested PCR master mix was prepared 5µL of genomic DNA, 10 pmol of each external primer, and 13 µL of PCR water and placed in standard PCR tubes. Thermal conditions of the PCR reaction were included an initial denaturation at 95 °C for five min, followed by 30 cycles at 95 °C for 30 s., 55 °C for 30 s. and 72 °C for one min and finally, final extension 72 °C for five min. A nested PCR master mix of the second run was included 3 µL of first-run product, 10 pmol of each internal primer, and 15 µL of PCR water and placed in standard PCR tubes with thermal conditions for the PCR reaction. The PCR products were passed electrophoresis in 1% agarose gel with 3µL of ethidium bromide. PCR product (10 µl) was added into each comb well and 5 µl of (100bp ladder) in each well. The gel tray was fixed in the electrophoresis chamber and filled by a 1X TBE buffer. The electric current was connected at 100 volts and 80 mA for 1 hr. PCR products were visualized using an ultraviolet transilluminator.

2.5. Human Interleukin-17 level measure

For quantitative determination of IL-17 concentrations in the serum of patients, samples were performed according to company instruction (Elabscience, China). All kit reagents and samples were brought at room temperature before used (Al-Ubaydi, 2018). 100µL of standard, blank, or sample were added per micro ELISA plate well and covered the plate with sealer and incubated for 90 min at 37 °C. The liquid of each well was removed. Immediately 100 µL biotinylated detection Ab working solution was added to each well, covered with the plate sealer, and incubated for 1 hour at 37 °C. All plate wells were washed three times. 100 µL of HRP conjugate working solution was added to each well and covered with the plate sealer, then incubated for 30 minutes at 37 °C. After that, wells were washed five times. Next, 90 µL of substrate solution was added to each well and covered with a new plate sealer, then incubated for about 15 minutes at 37 °C. 50 µL of stop solution was added to each well. The color was turned to yellow immediately. Finally, to determine the optical density (OD value) of each well at once, a microplate reader set at 450 nm.

2.6. Statistical analysis

In the study, an Independent-Samples T test was used to analyze the data using SPSS statistical package software V.17. A significant level was set at ($P \leq 0.05$) (Al-Ubaydi, 2018).

3. RESULTS AND DISCUSSION:

The most common leishmaniasis form is CL and invades the skin epidermis to form a lesion that is usually chronic and painless (Aronson *et al.*, 2016). Currently, there is no vaccine available against leishmaniasis infections. Control of CL is challenging due to the variety of *Leishmania* spp., reservoir hosts, and biological factors (Aflatoonian *et al.*, 2019). CL is often left disfiguring scars, especially on visible body sites, causing also social, psychological, and economic problems (Bilgic-Temel *et al.*, 2019).

Indeed, it is necessary to distinguish between *Leishmania* species for identifying clinically different manifestations of the disease (VL, CL, or MCL) to correct diagnosis, administration of the appropriate treatment, and control of the disease (Mesa *et al.*, 2020). Molecular techniques, mainly PCR-based assays, are nowadays used to detect *Leishmania* spp. in the clinical samples, also to diagnose parasite species, strains, and genotypes (Albuquerque *et al.*, 2017), and they have high sensitivity and specificity for *Leishmania* identification (Izadi *et al.*, 2016) (Soleimanpoor *et al.*, 2016).

3.1. Molecular diagnosis

The Nested-PCR technique for targeting the kDNA gene to detect and characterize *Leishmania* parasites directly in clinical samples was used. Moreover, the kDNA gene has shown high sensitivity and specificity in detecting *Leishmania* (Ranasinghe *et al.*, 2015) (Naseri *et al.*, 2016). A high abundance of kDNA minicircles in each kinetoplast makes an ideal target for the diagnosis of *Leishmania* parasites (Abdolmajid *et al.*, 2015) (Kocher *et al.*, 2017).

The kDNA minicircle amplification results showed that 65 out of 80 were positive for CL and recorded two co-existing species in the local area. The most common species were *L. tropica*, which comprised 46 (57.5%) samples of the total positive samples, while *L. major* 19 (23.75%) showed at a low level. The electrophoresis of the Nested-PCR product of *L. tropica* was generated at 750 bp (Figure 2).

Geographical expansion and global increase of leishmaniasis are still associated with vector population expansion (Cunze *et al.*, 2019). An increase in migration, rapid urbanization, deforestation, and *Leishmania* adaptation with mammalian hosts and additional vectors (Galgamuwa *et al.*, 2018). However, it is necessary to identify and detect *L. major* and *L. tropica* infections because treatment is administered with different protocols (Salloum *et al.*, 2016). This finding is close to Mezher (2017) in Al-Muthanna province; Al-Fahdawi *et al.* (2018) in Al-Ramadi City, and Abdolmajid *et al.* (2015) in Khorasan-Razavi province, Iran. It was inconsistent with Jafer *et al.* (2015) in Kerbala province; Mohammad and Hmood (2018) in Al-Qadisiyah province, and Rahi *et al.* (2019) in Kut City.

3.2. Serum Interleukin-17 level

The results of IL-17 level recorded a significant increase ($P > 0.05$) in 46 infected patients by *L. tropica* compared to the (25) control sample, which was the mean kit values 280 ± 33 pg/ml. In contrast, IL-17 concentration level in the patients was high, where it reached mean values 405 ± 160 pg/ml, and t . The value was 3.83, whereas P Value was 0.00 (Figure 3).

Immunologically, cutaneous leishmaniasis infection accompanies a complex set of interactions, leading to a differentiation of the Th17 cell subset, which is characterized by producing IL-17 (Gabriel *et al.*, 2019). IL-17 induces different proinflammatory cytokines and other chemokines (Maspi *et al.*, 2016) (Khazaei *et al.*, 2019). Generally, the level of IL-17 is high in peripheral blood and tissue of leishmaniasis patients, also associated with the intensity of inflammatory cellular infiltration (Rasouli *et al.*, 2019). Although the role of IL-17 in leishmaniasis is still unclear and differs according to the *Leishmania* species, host, and stage of the infection, its participation in the protective response and disease-promoting during infection is unquestionable (Gonçalves-de-Albuquerque *et al.*, 2017). IL-17 stimulates iNOS activation and several cytokines and chemokines, which lead to the recruitment of leukocytes, especially neutrophils that migration, to create a robust inflammatory infiltrate (Teixeira *et al.*, 2018).

The significant increase of IL-17 level in patients' serums with *L. tropica* compared to the control sample is consistent with Pitta *et al.* (2009) showed that *L. donovani* stimulated differentiation of Th17 cells to produce IL-17, IL-22, and IFN- γ ,

also mention that IL-17 and IL-22 were independent and strongly associated with protection against Kala Azar; Katara *et al.* (2013) have observed significantly higher in plasma IL-17 level of CL samples compared to control sample. Andargie and Ejara (Andargie and Ejara, 2015) concluded that IL-17 contributed significantly to VL pathogenesis during inducing TNF- α and NO production. Teixeira *et al.* (2018) have mentioned that the presence of *L. infantum* leads to an increase of IL-17 production, that associated with neutrophils recruitment that plays an active role at the site of the infection.

4. CONCLUSIONS:

There is little information about identifying and detecting *Leishmania* species responsible for human CL in Thi-Qar province. The study has shown two species that co-existing in the province. The most common causes of dermal lesions have infected with *L. tropica*. The Nested-PCR is a sensitive and accurate method for direct diagnosis and molecular studies of *Leishmania* parasites. The increased level for IL-17 concentration in *L. tropica* patients showed an important role that it plays in the interactions between immune cells at the infections with this parasite.

5. ACKNOWLEDGMENTS:

This study is part of a Ph.D. thesis for Mohammed H. Flaih. The authors thank the staff of dermatology and laboratory departments in the above Hospitals for their kind assistance in the sample collection. This study did not support financially.

6. REFERENCES:

1. Abdolmajid, F., Ghodratollah, S. S., Hushang, R., Mojtaba, M. B., Ali, M. M., and Abdolghayoum, M. (2015). Identification of *Leishmania* species by kinetoplast DNA-polymerase chain reaction for the first time in Khaf district, Khorasan-e-Razavi province, Iran. *Tropical Parasitol.*, 5(1), 50–55. <https://doi.org/10.4103/2229-5070.145587>
2. Al-Difaie, R. S. (2013). Prevalence of Cutaneous Leishmaniasis in AL-Qadissia province and the evaluation of treatment response by pentostam with RT-PCR. *Wasit Uni. Coll. Sci.*
3. Al-Ubaydi, N. A. H. (2018). Epidemiological, Immunological and Physiological Study of type2 Diabetic Patients infected with Toxoplasmosis. *Coll. Edu. pure Sci. Uni. Thi-Qar.*
4. Aflatoonian, M. R., SharifilD, I., Aflatoonian, B., Bamorovat, M., Heshmatkhah, A., Babaei, Z., Almani, P. G. N., Mohammadi, M. A., Salarkia, E., Afshar, A. A., Sharifi, H., Sharifi, F., Khosravi, A., Khatami, M., Arefinia, N., Fekri, A., Farajzadeh, S., Khamesipour, A., Mohebbali, M., Gouya, M. M., Shirzadi, M. R., and Varma, R. S. (2019). Associated-risk determinants for anthroponotic cutaneous leishmaniasis treated with meglumine antimoniate : A cohort study in Iran. *PLoS Negl Trop Dis*, 13(6), 1–18.
5. Akhoundi, M., Downing, T., Votýpka, J., Kuhls, K., Lukes, J., Cannet, A., Ravel, C., Marty, P., Delaunay, P., Kasbari, M., Granouillac, B., Gradoni, L. and Sereno, D. (2017). *Leishmania* infections: Molecular targets and diagnosis. *Molecular Aspects of Medicine*, 57, 1–29. <https://doi.org/10.1016/j.mam.2016.11.012>
6. Al-Fahdawi, H. A., Al-Ani, S. F., and Al-Kubaisi, T. A. (2018). Detection of Cutaneous Leishmaniasis Based on ITS1 Gene by PCR-RFLP Technique. *Egypt. Acad. J. Biolog. Sci.*, 10(2), 1–13.
7. Al-Tamemy, A. K. A., and Al-Qurashi, N. N. S. (2017). Identification and Sequences of Two *Leishmania* Species Isolated From Human Cutaneous Lesion in Iraq. *IOSR-JPBS*, 12(4), 53–57. <https://doi.org/10.9790/3008-1204075357>
8. Albuquerque, A., Campino, L., Cardoso, L., and Cortes, S. (2017). Evaluation of four molecular methods to detect *Leishmania* infection in dogs. *Parasites and Vectors*, 10(57), 1–5. <https://doi.org/10.1186/s13071-017-2002-2>
9. Andargie, T. E., and Ejara, E. D. (2015). Pro- and Anti-inflammatory Cytokines in Visceral Leishmaniasis Journal of Cell Science and Therapy. *J Cell Scie and Ther*, 6(3). <https://doi.org/10.4172/2157-7013.1000206>
10. Aronson, N., Herwaldt, B. L., Libman, M., Pearson, R., Lopez-velez, R., Weina, P.,

- Carvalho, E. M., Ephros, M., Jeronimo, S. and Magill, A. (2016). 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). *Clinic Infect Dis*, 63, 202–264. <https://doi.org/10.1093/cid/ciw670>
11. Banerjee, A., Bhattacharya, P., Joshi, A. B., Ismail, N., Dey, R., and Nakhasi, H. L. (2016). Role of proinflammatory cytokine IL-17 in *Leishmania* pathogenesis and in protective immunity by *Leishmania* vaccines. *Cell Immunol*, 309, 37–41. <https://doi.org/10.1016/j.cellimm.2016.07.004>
 12. Bilgic-Temel, A., Murrell, D. F., and Uzun, S. (2019). Cutaneous leishmaniasis: A neglected disfiguring disease for women. *Internat J Women's Dermatol*, 5(3), 158–165. <https://doi.org/10.1016/j.ijwd.2019.01.002>
 13. Cunze, S., Kochmann, J., Koch, L. K., Hasselmann, K. J. Q., and Klimpel, S. (2019). Leishmaniasis in Eurasia and Africa : geographical distribution of vector species and pathogens. *R. Soc. Open Sci.*, 6, 1–12. Retrieved from <http://dx.doi.org/10.1098/rsos.190334>
 14. Dayakar, A., Chandrasekaran, S., Kuchipudi, S. V., and Kalangi, S. K. (2019). Cytokines : Key Determinants of Resistance or Disease Progression in Visceral Leishmaniasis : Opportunities for Novel Diagnostics and Immunotherapy. *Fronti in Immunol*, 10(670), 1–23. <https://doi.org/10.3389/fimmu.2019.00670>
 15. Gabriel, A., Valério-Bolas, A., Palma-Marques, J., Mourata-Gonçalves, P., Ruas, P., Dias-Guerreiro, T., and Santos-Gomes, G. (2019). Cutaneous Leishmaniasis: The Complexity of Host's Effective Immune Response against a Polymorphic Parasitic Disease. *J Immunol. Res.*, 2019, 1–16. <https://doi.org/10.1155/2019/2603730>
 16. Galgamuwa, L. S., Dharmaratne, S. D., and Iddawela, D. (2018). Leishmaniasis in Sri Lanka : spatial distribution and seasonal variations from 2009 to 2016. *Parasites and Vectors*, 11(60), 1–10. <https://doi.org/10.1186/s13071-018-2647-5>
 17. Gonçalves-de-Albuquerque, S. C., Pessoa-e-Silva, R., Trajano-Silva, L. A. M., Goes, T. C., Moraes, R. C. S. , Oliveira, C. N. C., Lorena, V. M. B., and Paiva-Cavalcanti, M. (2017). The equivocal Role of Th17 Cells and Neutrophils on immunopathogenesis of Leishmaniasis. *Fronti in Immunol*, 8(1437), 1–11. <https://doi.org/10.3389/fimmu.2017.01437>
 18. Gonzalez-lombana, C., Gimblet, C., Bacellar, O., Oliveira, W. W., Passos, S., Carvalho, L. P., Goldschmidt, M., Carvalho, E. M., and Scott, P. (2013). IL-17 Mediates Immunopathology in the Absence of IL-10 Following *Leishmania major* Infection. *PLoS Pathogens*, 9(3), 1–14. <https://doi.org/10.1371/journal.ppat.1003243>
 19. Izadi, S., Mirhendi, H., Jalalizand, N., Khodadadi, H., Mohebbi, M., Nekoeian, S., Jamshidi, A., and Ghatte, M. A. (2016). 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.*, 9(2), 1–7. <https://doi.org/10.5812/jjm.32885>
 20. Jafer, A. M., Al-Kubessy, A. H., and Al-Amar, M. H. (2015). Molecular and Immunological study of Cutaneous *Leishmania* in Karbala city. *J. of Kerbala Univ.*, 13(2), 34–47.
 21. Katara, G. K., Raj, A., Kumar, R., Avishek, K., Kaushal, H., Ansari, N. A., Bumb, R. A., and Salotra, P. (2013). Analysis of localized immune responses reveals presence of Th17 and Treg cells in cutaneous leishmaniasis due to *Leishmania tropica*. *BMC Immunology*, 14(1), 1–9. <https://doi.org/10.1186/1471-2172-14-52>
 22. Khazaei, N., Moghaddas, E., Rezaee, S. A., and Shamsian, S. A. (2019). IL-8 and IL-23 Levels in Peripheral Blood Mononuclear Cells of Patients with Cutaneous Leishmaniasis Caused by *Leishmania major*: A Case-Control Study. *Iran Red Crescent Med J*, 21(6), 1–6. <https://doi.org/10.5812/ircmj.85441>. Research
 23. Kocher, A., Valière, S., Bañuls, A., and Murienne, J. (2017). High-throughput sequencing of kDNA amplicons for the

- analysis of *Leishmania* minicircles and identification of Neotropical species. *Parasitology*, 1–8
24. Maksouri, H., Dang, P. M., Rodrigues, V., Estaquier, J., Riyad, M., and Akarid, K. (2017). Moroccan strains of *Leishmania major* and *Leishmania tropica* differentially impact on nitric oxide production by macrophages. *Parasites and Vectors*, 10(1), 1–10. <https://doi.org/10.1186/s13071-017-2401-4>
 25. Maspi, N., Abdoli, A., and Ghaffarifar, F. (2016). Pro-and anti-inflammatory cytokines in cutaneous leishmaniasis: a review. *Pathogens and Global Health*, 110(6), 247–260. <https://doi.org/10.1080/20477724.2016.1232042>
 26. McGeachy, M. J., Cua, D. J., and Gaffen, S. L. (2019). The IL-17 Family of Cytokines in Health and Disease. *Immunity*, 50(4), 892–906. <https://doi.org/10.1016/j.immuni.2019.03.021>
 27. Meira, C. S., and Gedamu, L. (2019). Protective or Detrimental? Understanding the Role of Host Immunity in Leishmaniasis. *Microorganisms*, 7(695), 1–25.
 28. Mesa, L. E., Manrique, R., Muskus, C., and Robledo, S. M. (2020). 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*, 41(1), 1–15
 29. Mezher, J. O. (2017). *Study of comparison between traditional methods and molecular diagnosis of cutaneous leishmaniasis in Al-Muthanna Province*. Coll. Sci. Uni. Muthanna.
 30. Mohammad, F. I., and Hmood, K. A. (2018). Detection of *Leishmania* species by Nested-PCR and virulence factors GPIPS, GP63 in *L. major* by conventional-PCR. *Biochem. Cell. Arch.*, 18(2), 2255–2259
 31. Mosleh, I. M., Schönan, G., Geith, E., Al-Jawabreh, A., and Natsheh, L. (2015). The Jordanian Mid Jordan Valley is a classic focus of *Leishmania major* as revealed by RFLP of 56 isolates and 173 ITS-1-PCR-positive clinical samples. *Exp. Parasitol.*, 148, 81–85
 32. Naseri, A., Fata, A., Rezai, A., Hedayatimoghadam, M., Berengi, F., Akbarzadeh, O., and Shamsian, S. A. (2016). Molecular identification of *Leishmania* species in Torbat-e Heydarieh, Khorasan Razavi province, Iran. *Int J Med Res and Hea Scie*, 5(6), 87–92
 33. Oghumu, S., Natarajan, G., and Satoskar, A. R. (2015). Pathogenesis of Leishmaniasis in Humans. *Human Emerging and Re-Emerging Infections*, 1, 337–348. <https://doi.org/10.1002/9781118644843.ch18>
 34. Pitta, M. G. R., Romano, A., Cabantous, S., Henri, S., Hammad, A., Kouriba, B., Argiro, L., Kheir, M., Bucheton, B., Mary, C., El-Safi, S. H., and Dessein, A. (2009). 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 Clinic. Investig.*, 119(8), 2379–2387. <https://doi.org/10.1172/JCI38813>. Neutrophils
 35. Rahi, A. A., Jasim, S. E., and Abed, F. A. (2019). Clinical picture of cutaneous leishmaniasis in Wasit, Iraq. *EAS J Orthop Physiother*, 1(3), 29–32
 36. Ramírez, J. D., Hernández, C., León, C. M., Ayala, M. S., Flórez, C., and González, C. (2016). Taxonomy, diversity, temporal and geographical distribution of Cutaneous Leishmaniasis in Colombia: A retrospective study. *Nature Publishing Group*, 1–10. <https://doi.org/10.1038/srep28266>
 37. Ranasinghe, S., Wickremasinghe, R., Hulangamuwa, S., Sirimanna, G., Opathella, N., Maingon, R. D. C., and Chandrasekharan, V. (2015). Polymerase chain reaction detection of *Leishmania* DNA in skin biopsy samples in Sri Lanka where the causative agent of cutaneous leishmaniasis is *Leishmania donovani*. *Mem Inst Oswaldo Cruz*, 110(8), 1017–1023. <https://doi.org/10.1590/0074-02760150286>
 38. Rasouli, M., Moazamian, E., Nasiri, M.,

- Keshavarz, M., and Asaei, S. (2019). Interleukin-17A Genetic Polymorphisms as a Prognostic Markers for Resistance to Visceral Leishmaniasis in the Iranian Population. *Arch Clin Infect Dis*, 14(2). <https://doi.org/10.5812/archcid.57163>. Research
39. Rodríguez-Brito, S., Camacho, E., Mendoza, M., and Niño-Vega, G. A. (2015). Differential identification of *Sporothrix* spp and *Leishmania* spp by conventional PCR and qPCR in multiplex format. *Med. Mycol.*, 53, 22–27
 40. Rossi, M., and Fasel, N. (2017). How to master the host immune system? *Leishmania* parasites have the solutions! *Internat Immunol*, 30(3), 103–111. <https://doi.org/10.1093/intimm/dxx075>
 41. Salloum, T., Khalifeh, I., and Tokajian, S. (2016). Detection, molecular typing and phylogenetic analysis of *Leishmania* isolated from cases of leishmaniasis among Syrian refugees in Lebanon. *Para Epidemiol and Cont*, 1(2), 159–168. <https://doi.org/10.1016/j.parepi.2016.02.002>
 42. Schroter, S., Plowman, R., Hutchings, A., and Gonzalez, A. (2006). Reporting ethics committee approval and patient consent by study design in five general medical journals. *J Medical Ethics*, 32(12), 718–723. <https://doi.org/10.1136/jme.2005.015115>
 43. Soleimanpoor, H., Dabirzadeh, M., and Fooladi, B. (2016). Identification of Species Causing Cutaneous Leishmaniasis by PCR in Chahbahar, Iran. *Med Laborato J*, 10(2), 46–51.
 44. Teixeira, C. R., Santos, C. S., Prates, D. B., Santos, R. T., Araújo-Santos, T., Souza-Neto, S. M., Borges, V. M., Barral-Netto, M., and Brodskyn, C. I. (2018). *Lutzomyia longipalpis* Saliva Drives Interleukin-17-Induced Neutrophil Recruitment Favoring *Leishmania infantum* Infection. *Frontiers in Microbiology*, 9(881), 1–10. <https://doi.org/10.3389/fmicb.2018.00881>

Figure 1: Cross-section of the dermal lesion showed amastigotes inside phagocytes (400X H&E).

Figure 2: Agarose gel (1%) electrophoresis of *Leishmania* species using nested-PCR of kDNA in CL positive samples. Where M: marker (100-2000 bp), lanes 1, 2, and 4-12 show positive at product size 750 bp of *L. tropica* isolated from human skin lesions, while lane 3 shows a negative sample.

Figure 3: Human IL-17 ELISA standard curve