Molecular identification of *Lieshmania* spp. And its Immunological Effects

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**KEY WORDS**  
Interferon gamma  
*Leishmania*  
Phylogenetic tree  
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**Abstract:** Leishmaniasis, a third from the most common vector-borne illnesses, caused by a protozoan involuntary intracellular parasite of the *Leishmania* genus, spread by a bite of sand flies. Leishmaniasis is one of the predominantly neglected tropical health disorder with high incidence around the world. A total of 57 samples passively obtained from immigrants in all parts of Iraq from patients with skin ulcers indicative of Leishmaniasis with age ranging from 5-14 years, attended hospitals and health centers in northern diyala Governorate and more precise research was conducted to ensure Lieshmania's infection from period July to September 2019. The microscopic examination of 57 staining slide to detect Leishmania body, there are 30(52%) positive slides, but the results of PCR directed to DNA was 37(64.9%). The result found in Iraq is L. major more than L. tropica by PCR-sequencing and Serum samples were taken after treatment in the same group of the study and the IFN-γ levels were compared between patients that have been received treatment and patients that have not received any treatment and the result shown high serum level of IFN-γ in untreated patients.

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**INTRODUCTION**  
*Leishmania* SP. It may cause three main types from human infection: Cutaneous, visceral and mucosal leishmaniasis. In the old World, the two main types of leishmaniasis are cutaneous and visceral[1].  

Leishmaniasis is a “vector-borne” disease in which the single-celled parasite *Leishmania* infects cells and is endemic to many lands of the world and manipulate millions of individuals[10]. There are numerous Leishmania species and depending on the infecting species, the disease may have varying clinical signs and consequences. Visceral leishmaniasis, involving internal organs hepatic cells (liver) and spleen, dermal leishmaniasis that skin slough occur and mucocutaneous leishmaniasis where mucous tissue ulcers occur are the major clinical symptoms of leishmaniasis[3]. Serological examination but not appliance choice for cutaneous and mucocutaneous leishmaniasis, since in these cases antibodies cannot always be available while PCR is sufficient for all cases[3].  

There is however, some unanimity as to which aim is better suited for molecular disclosure. We have...
previously used the 18S rRNA gene, an extremely repetitive and highly maintained sequence, as our target for diagnostic testing.

**MATERIALS AND METHODS**

**Samples collection:** A total 57 Samples passively obtained from patients with skin ulcers indicative of Leishmaniasis with age ranging from 5-14 years, they were attending hospital and health centers in North diyala Govern orate from immigrants in all parts of Iraq and more accurate tests were done to ensure the infection of Liesthmania. Within period of July to September of 2019 (Fig. 1). Patients complained for skin lesions in exposed sections of the body mainly in the face, leg and arm and were clinically diagnosed with a special dermatologist as cutaneous leishmaniasis. Samples divided into two partition, small amount to preparing staining smear and the other preserved in deepfreeze (-20°C) for molecular analysis. The primary isolation was made from patients with cutaneous lesions. The puncture site (lesion) was purged with 70% ethanol before a test ambition. A 1 mL syringe containing 0.1-0.2 ml of sterilized saline was embedded intradermal into external fringe of the injury. The syringe was orbited and the tissue liquids were delicately suctioned into the needle while its withdrawal. The aspirated material was inoculated in culture tubes containing five ml of NNN medium. All inoculated tubes were incubated at 25°C. Almost all cultures were incubated and inspected for 15-30 days before the negatives were counted. Patients have been positively diagnosed with CL when active motile promastigotes were seen in culture[4].

**Genomic DNA extraction and PCR program:** DNA was extracted by using ZYMO research kit according to manufacturer’s manual directly from which had showed positive results by serological test. Purified DNA was stored at -20°C until further analysis. In order to target 18s ribosomal RNA gene within the DNA, the following primer pairs was used: The sequence of forward primer “GCGCATGGAGAAAGCTCTAT”. And reverse; “AAAATGGCCAACGCGAAGTTA” total volume of 25 μL was performed as a PCR reaction. (KAPPA, USA) and continue the volume up to 25 μL withDNase/RNase-free water. The PCR program that was applied in the thermocycler devices as seen in Table 1.

Products were finally visualized on an red safe-stained agarose gelEnzyme linked immunosorbent assay was utilized to estimate Human IFN gamma ELISA kit in compliance with the instructions issued by the manufacturer (ab46025) Abcam, USA.

<table>
<thead>
<tr>
<th>Steps</th>
<th>Temperature (°C)</th>
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<td>Denaturation 2</td>
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<td>Extension 1</td>
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<td>Extension 2</td>
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**RESULTS AND DISCUSSION**

The microscopic examination of 57staining slide to detect Leishmania body, there are 30(52%) positive slides but the results of PCR directed to DNA was 37(64.9%). These results compatible with the study conducted by Rezai when analysis of (93) samples taken by direct smears from suspicious patients, (81) of which were decisive for parasitological examination whilst(84) were positive in PCR directed to DNA[5]. The results also agreement with other study by Rehman et al.[6].

The fifty seven samples that tested by molecular test and specifically PCR technique, only 37 samples was positive and the other were negative, the resulted bands of PCR that appear sharp and 450bp in length as shown in Fig. 2.

Twenty μL of PCR products were sent of 18s ribosomal RNA gene to Macrogen company for sequencing in south Korea. The result found in Iraq is L. major more than L. tropica. This results also agreement with other studies in iraq the study conducted by Qader showed that L. major is widely[7] and agreement with several study[8, 9] and the study conducted by Kamil shows Research for sequencing findings using their ABI 3730xl Genetic Analyzer Homology search. The phylogenetic trees of these two species are seen in Fig. 3 from the Molecular Evolutionary Genetics Research (MEGA) software Version 6.0. (3) Such alignments occurred in the 18s ribosomal RNA gene via. the local Twenty μL of PCR products were sent of 18s
Fig. 2: Gel electrophoresis PCR for Leishmania, first round on "1.5% agarose gel stained with red stain. Lane’s M: 100 DNA ladder and lanes 2-15:" showed positive results for PCR products of Leishmania (band 450bp) from examined samples.

Fig. 3: Neighbor-joining tree Leishmania of 18S rRNA gene

Fig. 4: Serum levels of Interferon gamma (pg/mL)

Serum samples have taken in the same study group after treatment and the levels of IFN-γ had compared between patients were receiving treatment and patients not receiving any treatment and the result showed high serum level of IFN-γ in untreated patients. As seen in Fig. 4, Systemic inflammation (inflam.) identified as the cause from clinical manifestations in many intracellular infections including "leishmaniasis"9-11. On the other hand, Leishmania patients show deficiency of IFN-γ development T-cells.
that are specific in vitro to Leishmania antigens and are recovered following successful therapy\textsuperscript{[12, 14]}. Curiously, patients with Leishmania including IL-6, IL-21 and IL-27\textsuperscript{[14, 15]}. 

**CONCLUSION**

Both of them are *L. tropica*, *L. major* are skin leishmaniasis causative operator in Iraq *L*. The major species in Iraq is the dominant Leishmania species. The PCR-sequencing is a highly reliable method for differentiating and identifying species.

**REFERENCES**


