MOLECULAR IDENTIFICATION AND PHYLOGENETIC OF LEISHMANIOSIS

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ABSTRACT

One of the diseases spread in the world that causes health problems is Leishmania spp., which causes leishmaniasis disease; this parasite is transmitted by a variety of insects (sand fly) and easily transmitted between humans. This study intended to diagnose this parasite through molecular diagnostics as well as identifying mutations and making a phylogeny for this parasite in Iraq. Using PCR analysis, the parasite was diagnosed for DNA sequencing of 10 samples taken from patients. The samples were cultivated on 3N agar, then examined for Leishmania major, which showed positive results. The genotypes of this parasite were determined (kDNA) by the symmetry and compared with the blast analysis.

Key words: Leishmaniasis, sand fly, DNA, primer, electrophoresis and phylogenetic

I. INTRODUCTION

This parasite is transmitted by a bite from a sand fly that feeds on humans and animals, including dogs, and rodents. This disease is very prevalent due to the carrier, and also it remains alive in the infected people for a very long time. This is considered a source of the spread of the disease, especially when they are infected with no asymptomatic(1). Another way to transmit the parasite is by using the personal tools of infected individuals. Most people at risk in this way suffer from an immune system disorder (2,3). There are several forms of this disease, and the symptoms depend on the parasite type, such as the type that affects the skin, so lesions appear on the skin in a scar for long periods. The host body can get rid of this parasite by itself, however, the scarring effect may remain permanent on the skin of the infected person (2,3). The main species of the parasite are: Cutaneous leishmaniasis which cause an open sore at the bite sites, Mucocutaneous leishmaniasis causes both skin and mucosal ulcers with damage to the nose and mouth, and Visceral leishmaniasis or kala-azar (black fever) is the most serious form and is generally fatal if untreated (4-6). The estimated global infection with this disease about 12 million, 1-1.5 million is skin type infection, and 500000 infections of the visceral type (7,8). The parasite is widespread in countries where there is a sand fly that increases the spread of the disease widely, which is found in about 72 developing and 16 developed countries (8,9,10 and 11). In the current study, we investigate the benefits of using PCR as an instrument for the high accuracy diagnosis and detection of Leishmania parasites.

1. Materials and Methods

The samples were collected from 10 patients with clinical signs (skin lesions). The samples taken were cultivated on (3N) agar, and the microscopic examination and PCR technique were performed on them. Promastigotes, microscopic examination, molecular diagnosis, and DNA extraction were performed as per company instructions (Geneaid).

PCR amplification was carried out with the Leishmania kinetoplast DNA (kDNA) primers forward T2 (5’-CGGCTTGCACCATGCGGTG-3’) and reverse B4 (5’-ACATCCCTGCCCACATACGC-3’) according to the manufacturer recommendations.
The reactions were done in a total volume of 25 µl containing 20 µl 1x PCR MIX, 0.3 µl of Taq-DNA polymerase, and 5 µl of the DNA was added to the mixture. The PCR amplification was done in a DNA thermocycler (Eppendorf) using 1 cycle of 95°C for 180 sec, 63°C for 30 sec, and 72°C for 60 sec, followed by 35 cycles of 93°C for 40 sec, 63°C for 40 sec, and 72°C for 60 sec. Each experiment included a positive and negative control. The presence of amplification products was confirmed with 2% agarose gel electrophoresis analysis and visualized by ethidium bromide staining (0.5 µg/ml) (11).

3. Results and Discussion

Microscopy is the most commonly used to detect Leishmania, but it lacks accuracy in determining the type of parasite (8). Therefore, scientists tended to use a more accurate examination in diagnosis, which is PCR (10, 11 and 14). The PCR technique is one of the best and most accurate techniques used at present to diagnose pathogens, due to the sensitivity, short time, and accuracy of the diagnosis. It is accomplished by extracting the genetic material in a certain way and amplifying the allocated part of this sample, and then several copies of this region are shown to detect the parasite by electrophoresis (12-14).

In the current study, we investigated the benefits of using the PCR as an instrument for high accuracy in the diagnosis and detection of Leishmania parasites. So, the DNA of the parasite was extracted and then analyzed using the electrophoresis technique to determine the infected sample ones. To reveal the gene sequence of detected Leishmania, the conventional PCR assay on 620 bp was used.

The PCR analysis of Leishmania major using specific primer T2 showed a specific band (KDNA) at 620 bp on the agarose gel (Fig. 1). From the obtained sequencing, it is found that the samples showed the genotype corresponds to Leishmanial.

![Figure 1. Amplification of 620 bp by Agarose gel (2%) electrophoresis fragments of 18 RNA geneof Leishmania major. Lane M showed a PCR marker.](image1)

![Figure 2. DNA sequence of Leishmania major spp.](image2)
The phylogenetic tree of strain found in Iraq looks similar to that recorded in UK and India, but it is totally different from that one in Senegal (Fig. 3).

Figure 3. Phylogeny of Leishmania major by the program Mega v.6 (620 based on the 18S KDNA gene; Iraq strain Leishmania sp.).

II. CONCLUSION

The aim of the current study is to diagnose the Leishmania parasite through molecular diagnostics, identifying mutations and making a phylogeny for this parasite in Iraq. The parasite was diagnosed using PCR analysis for 10 samples and the DNA sequencing was determined as well. The genotypes of this parasite were determined (kDNA) by the symmetry and compared to the blast analysis. All the examined samples showed the Leishmania major.

4. Ethical Considerations

Permission for the study and ethical approval was obtained from the Dentistry Department Kut University College – Iraq and College of Health and Medical Techniques / AL-Bayan University Iraq.

5. Conflict of interest

No conflict of interests.

REFERENCE

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