

Cutaneous leishmaniasis at Wasit governorate

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Abstract:

The study included identification of *Leishmania* parasites from cases of cutaneous leishmaniasis, by examination of smears for LD bodies, isolation of parasites by culture in media, and characterisation of the isolated parasites by Isoenzyme analysis.

Out of 100 cases suspected for cutaneous leishmaniasis; 85 were diagnosed on culture for *Leishmania* promastigotes and this method was found to be superior to direct microscopy for amastigotes (LD bodies). The cases included in this study belonged to different areas of Wasit/Iraq. There were 30 cases from Hay, 35 from Badra, and 35 cases from Suwaira. The distribution of infection in different age groups indicated that majority of cases belonged to young and middle aged adults. The infection was detected in both sexes with a predominance in males. The clinical picture of cutaneous lesions was suggestive of both wet and dry types of lesions.

Key words: (Cutaneous leishmaniasis , *Leishmania* , Isoenzyme)

Introduction:

Leishmaniasis, a vector-borne disease caused by obligate intramacrophage protozoa, is characterized by diversity and complexity [1]. A total of about 21 *Leishmania* species have been identified to be pathogenic to human. *Leishmania* are one of several genera within the family *Trypanosomatidae*, and are characterized by the possession of a kinetoplast, a unique form of mitochondrial DNA. Leishmaniasis is endemic in 88 countries of 5 continents with a total of 350 million people at risk and 12 million cases with estimated incidence of 1-1.5 million cases of cutaneous leishmaniasis (CL) and 500,000 cases of visceral leishmaniasis (VL) [1].

Leishmaniasis is caused by different species of *Leishmania*, under kingdom Protista and phylum

Euglenozoa [2]. The parasite *Leishmania* exists at least in two forms [3]:

1-Amastigote form: Amastigotes are ovoid and non flagellated form of *Leishmania*, measuring 3-5 μm in length.

2-Promastigote form: Flagellated form found in the sandfly host.

Worldwide, vector-borne transmission is the most common mode of transmission. Other modes of transmission such as parenteral, congenital, sexual, occupational exposures, and person-to-person transmission could also theoretically occur [4].

Materials and Methods:

Sample collection and culture:-

One hundred samples from patients with skin lesion suspected of cutaneous leishmaniasis (CL) were

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randomly collected from different endemic areas of Wasit province / Iraq. The samples were aspirated from the edges of the skin lesions and cultured on Novy-MacNeal-Nicole (NNN) media. Eighty-five isolates growing in the culture were used for characterization. For isoenzyme analysis, primary isolates were subcultured on (NNN) media, supplemented with 2 mM L-glutamine, 15% fetal bovine serum, 100 U/ml penicillin & 100 µg/ml streptomycin. The second or third subcultures were used for characterization.

***Isoenzyme electrophoresis* [5]:-**

Discontinuous vertical PAGE and cellulose acetate were used for isoenzyme analysis of the isolates. Promastigotes were harvested at the end of logarithmic phase by centrifugation at 3000 ×g at 4°C for 20 min. The supernatants were discarded and the pellets of promastigotes were washed three times by PBS (pH 7.2). The pellets of promastigotes were mixed with equal volumes of a hypotonic aqueous solution of enzyme stabilizer (1mM EDTA, 1 mM @-amino -n- caproic acid, 1 mM dithiothreitol), frozen by placing in vapor phase of liquid nitrogen and thawed at 25 – 30 ° C for three times. Soluble extract of lysed promastigotes was prepared by

centrifugation at 30,000 × g at 4°C for 30 min, and stored at -70°C until use .

Results and Discussions:

Out of 85 cases were diagnosed for cutaneous leishmaniasis on culture of exudates from lesions. Table (1) shows the positive cases of direct microscopy (60%), culture on NNN media (85%) and analysis of isoenzyme (100%) for cutaneous leishmaniasis. These results showed higher sensitivity and specificity of isoenzyme analysis than other methods in identification of *Leishmania* species and with agreement of results were recorded by [6]. Table (2) shows the distribution of cases of cutaneous leishmaniasis in different age groups and divided into wet and dry lesions. It was found that the majority of cases between (13- 60) years and the wet lesion was predominance. The results are similar to other studies carried out in Iraq [7], and in other countries [8]. The isoenzyme profile of 100 cases of cutaneous leishmaniasis recovered in present study showed two distinct patterns [9,10]:-

- 1- Banding pattern identical to that obtained with the reference strain of *Leishmania major*.
- 2- Banding pattern identical to the reference strain of *Leishmania tropica*.

Table(1) Results of microscopy, culture & isoenzyme for *Leishmania*

Total suspected cases of CL	+Ve for LD by direct microscopy	+Ve for culture on NNN media	+Ve for analysis of isoenzyme
100	60(60%)	85(85%)	100(100%)

Table(2) Distribution of cases of Cutaneous Leishmaniasis in different age groups

Age groups	+ve cases	+ve cases with wet lesions	+ve cases with dry lesions
Children (1-12)years	15(15%)	10(10%)	5(5%)
Young adults (13-30)years	35(35%)	20(20%)	15(15%)
Adults (31-60)years	40(40%)	28(28%)	12(12%)
Old (above 60 years)	10(10%)	6(6%)	4(4%)
Total +Ve cases	100(100%)	64(64%)	36(36%)

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الليشمانيا الجلدية في محافظة واسط

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الخلاصة :

تناولت الدراسة تشخيص طفيليات الليشمانيا من عينات مصابة بالليشمانيا الجلدية بأجراء الفحص المباشر للطفيلي وزرع الطفيلي على الوسط الزراعي ثم عزله وتشخيصه باستخدام تقنية التحليل الأنزيمي. من خلال فحص 100 عينة مصابة بالليشمانيا الجلدية، جمعت العينات من مناطق مختلفة ولأعمار والأجناس كافة أذ توزعت على قضاء الحي 30 عينة، وقضاء بدر 35 عينة، وقضاء الصويرة 35 عينة. وتبين وجود 85 عينة موجبة الفحص بين الأعمار الفتية والمتوسطة وتفوق الذكور على الإناث بنسب الأصابة. أوضحت الصورة السريرية للأصابات الجلدية وجود النوعين الرطب والجاف.