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# Characterization of *Leishmania* species by using Isozyme analysis

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**Abstract :** *A prospective study was done on 96 patients with Cutaneous Leishmaniasis (CL) attending to Al-Karamah Teaching Hospital in Iraq, during a period from November, 2014 to January, 2015. All samples were investigated for Leishmania amastig otes by Giemsa-stained smear were gave positive 68(70.8%); however the Novy Macneal Nicolle (NNN) culture led to the growth of promastigotes in all samples 60 (62.5 %). Also the characterization of the parasites by Isozyme analysis revealed 100% posit ive. Our results showed that the highest number of patients 41 (42.7%) was in age group ( 21-30) years old and 51(53.2%) of CL patients were had wet lesions while 45 (46.8%) had dry lesions, most of them in arm. The statistical analyses were carried out with Mi nitab version.*

***Keywords :*** *Cutaneous leishmaniasis, Isozyme, Culture, Human*

## 1. INTRODUCTION:

Leishmaniosis comprises a variety of syndromes caused by members of the protozoan parasite *Leishmania* (Kinetoplastida: Trypanosomatidae), which are transmitted to mammal hosts by the bite of infected phlebotomine sandflies belonging to the genera *Phlebotomus* (Old World) and *Lutzomyia* (New World) [1].

Various forms of clinical mani-festations of human leishmaniosis have been described and divided into three major clinical entities: visceral leishmaniosis (VL, kala azar), cutaneous leishmaniosis (localised, diffuse or disseminated CL, oriental sore, uta, pian bois, chiclero's ulcer) and mucocutaneous leishmaniosis (MCL, espundia) [2].

*Leishmania major*, *L. tropica* and sometimes *L. infantum* are the causative agents of cutaneous leishmaniasis in Old World . Most of the cases of CL occur in Afghanistan, Algeria, Saudi Arabia, Brazil, Iran, Iraq, Syria, and Sudan [3]. In Iraq, two species are present: *L. tropica*, the agent of anthroponotic cutaneous leishmaniasis (ACL), and *L. major*, the agent of zoonotic cutaneous leishmaniasis (ZCL). Both ACL and ZCL were reported as causative agents of leishmaniasis in Iraq, but ACL is found mainly in suburban areas [4] .

In the last two decades, biochemical techniques, most notably isozyme electrophoresis (IE), provided an effective and reliable tool for characterization of *Leishmania* isolates [5-8]. This work aimed to throw light on prevalence rate of cutaneous leishmaniasis in Iraq and assessment of their epidemiological and clinical aspects.

## 2. MATERIALS & METHODS:

### 2.1 Population study

This study was carried out during the period from October 2014 to February 2015 in Al-Karamah teaching hospital of Kut city, Iraq. A total of 96 skin samples were taken from patients with CL. All patients were divided into four age groups.

### 2.2 Parasitological Examination

Smears were prepared from skin scrapings of the edge of the ulcer, then fixed in methanol and stained with Giemsa and examined under the microscope with a 40 X lens and with a 100 X oil immersion lens. If at least one intra- or extra-cellular amastigote with a distinctive kinetoplast was found the smear was declared positive. When no amastigotes were seen after 15 minutes of inspection, the smear was declared negative. Many of the patient smears were double checked, the observations were in concordance.

### 2.3 Culture

The lesions and the adjacent normal-looking skin around them were cleaned, sterilized with 70% ethanol, and allowed to dry. A small amount of the scraped tissue wasinoculated on the liquid phase of Novy-McNeal-Nicolle (NNN) medium

 

(10% of rabbit blood) medium. The culture was incubated at 25°C and examined for parasite growth by the inverted microscope and also light microscope every 4 days until promastigotes were seen or up to one month before being discarded as negative. The cultures were made at least in duplicates for each case [9].

### 2.4 Isozyme Electrophoresis

The parasite was submitted to Isozyme Electrophoresis on cellulose acetate plates . Running conditions and revelation techniques were derived from Dujardin *et al.*, (1996) [10]. Each sample was mixed with a hypotonic enzyme stabilizer, maintained during 30 min on ice, centrifuged for 2 min at 3500×g and then immediately run for electrophoresis. Each 16 ml aliquot allowed the survey of as many as 12 different enzyme systems, including additional analyses for control and further verifications.

### 2.5 Statistical Analysis :

The suitable statistical method was used in order to analyze and assess the results by using T-test in Minitab version [11].The comparison of significant (P-value) in any test were: S= Significant difference ($P<0.05$), HS= Highly Significant difference ($P<0.01$), and NS= Non Significant difference ($P>0.05$).

## 3. RESULTS & DISCUSSION:

### RESULTS

Table 1. show the prevalence of positive cases of CL by using different diagnostic methods. The highest infection (100%) appeared by using isozyme analysis while the lowest infection ( 62.5% ) appeared by culture on NNN media. The isozyme profile of 96 cases of cutaneous leishmaniasis recovered in the present study showed two distinct patterns [12]:

1- Banding pattern identical to that obtained with the refernce strain of *L. major*.

2- Banding pattern identical to the refernce strain of *L. tropica*.

Regarding age distribution, CL have been reported high prevalence (42.7%) in age group (21-30) years old and the wet lesions more frequently ( 53.2%) than dry lesions (46.8%)( Table 2).

### DISCUSSION:

The diagnosis of CL classically relies on microscopic examination and in vitro cultivation. These classical methods require the presence of a relatively high number of viable or morphologically intact parasites; this may pose a problem particularly in the chronic phase of CL where parasite levels in skin lesions are very low. In contrast, an isozyme approach is both sensitive and specific [13,14]. The present study showed higher sensitivity and specificity of isozyme analysis(100%) than Giemsa-smeared (70.8%) and culture on NNN medium (62.5 %) in identification of *Leishmania* species and with agreement of results were recorded by other researchers[13-15] . The negative samples may have belonged to the lesions that have received antileishmanial treatment or the old lesions with spontaneous healing progress. This is in agreement with previous report [16] , also with Kumar *et al.* who indicated that direct microscopy or parasite culture alone detected respectively 65.5 % and 48.2 % of the positive samples [17] .

The majority of the CL cases (42.7%) in age groups (21-30) years old, which may be due to several factors, such as outdoor activities and sleeping outdoors. The most likely reason is an increase in human- sandfly contact because high activity. These results were in agreement with previous reports indicating more exposure as a result of educational and occupational situations [18,19]. Ulcerative wet type lesions were present in 62%, while the nodule dry type lesions were present in 38% .These observations are in agreement with those reported from Iraq[20] , Iran [21], Colombia[22] , Pakistan[23] , and Afghanistan [24].

### CONCLUSIONS:

This study revealed that Isozyme profile is a reliable method for identification of *Leishmania* species and can applied in epidemiologic investigations in Iraq.

 

**Table 1. Comparison among three methods in diagnosis of CL cases**

| No. of Examined | Giemsa-smear | Culture on NNN | Isozyme analysis |
|---|---|---|---|
| 96 | 68(70.8%) | 60 (62.5 %) | 96(100%) |

**Table 2. Age distribution of CL cases according to the type of ulcer**

| Age groups (Year) | + ve cases | | Total |
|---|---|---|---|
| | Wet | Dry | |
| 10 and less | 8(8.3%) | 6(6.2%) | 14(14.5%) |
| (11-20) | 14(14.6%) | 16(16.7%) | 30(31.3%) |
| (21-30) | 23(23.95%) | 18(18.75%) | 41(42.7%) |
| (31-40) | 6(6.25%) | 5(5.2%) | 11(11.5%) |
| Total | 51(53.2%) | 45(46.8%) | 96(100%) |

| P-value | C.S |
|---|---|
| 0.01 | Significant |

 

## REFERENCES:

[1] Ali Mohammadian M.H., Jones S.L., Darabi H., Riazirad F., Ajdary S., Shabani A., Rezaee M.A., Mohebali M., Hosseini Z. and Modabber F. Assessment of interferon-γ levels and leishmanin skin test results in persons recovered for leishmaniasis. Am. J. Trop. Med. Hyg., 20012; 87 (1), 70–75.

[2] World Health Organization (WHO). Control of the leishmaniases, WHO Technical Report Series 949, WHO, Geneva, Switzerland, 2010; 1–186.

[3] Rasheed, Z.N. Leishmaniasis in Iraq. *Leishmania* and zoonotic disease section. Communicable Disease Control Center CDC/ Baghdad/ Iraq.2004.

[4] Postigo JAR. Leishmaniasis in the World Health Organization Eastern Mediterranean Region. Int. J. Antimicrobial Agents. 2010; 36: 62-65.

[5] Aljeboori, T. I. & Evans, D. A. *Leishmania* spp. in Iraq. Electrophoretic isoenzyme patterns. 1. Visceral leishmaniasis. Transaction of Royal Society of Tropical Medicine and Hygiene,1980; 74, 169-177.

[6] Evans, D. A., Lanham, S. M., Baldwin, C. I. & Peters, W. The isolation and isoenzyme characterization of *Leishmania braziliensis* subsp, from patients with CL acquired in Belize. Trans Roy Soc Trop Med Hyg,1984; 78(1), 35-42.

[7] Evans, D. A. Handbook on Isolation, Characterization and Cryopreservation of *Leishmania*. 1211 Geneva, Switzerland, UNDP / World Bank / WHO Special Programme for Research and Training in Tropical Diseases (TDR).1989.

[8] Shamsuzzaman, S. M., Masato, F., Shamsuzzaman, A. K. M., Shamsuzzaman, S. M., Furuya, M., Shamsuzzaman Choudhury, A. K., Korenaga, M. & Hashiguchi, Y. Characterization of Bangladeshi *Leishmania* isolated from kala-azar patients by isoenzyme electerophoresis. Parasitology International, 2000; 49, 139-145.

[9] Herwaldt, B.L. Leishmaniasis. comprehensive review covering parasitology, epidemiology, clinical manifestations with variations,diagnosis and treatment. Lancet.1999; 354 : 1191-1199.

[10] Dujardin, J.P., Le Pont, F., Cruz, M., Leon, R., Tarrieu, F., Guderian, R., Echeverria, R., Tibayrenc, M., Cryptic speciation in Lutzomyia (Nyssomyia) trapidoi (Fairchild & Hertig) (Diptera: Psychodidae) detected by Multilocus Enzyme Electrophoresis. Am. J. Trop. Med. Hyg.,1996; 54 (1), 42–45.

[11] Meyer Ruth K and David D. Krueger . A Minitab Guide to Statistics (3rd ed.). Upper Saddle River, NJ: Prentice-Hall Publishing.2004.

[12] Markle WH and Makhoul K . Cutaneous leishmaniasis: recognition and treatment, Am. Fam. Physician.2004; 15; 69(6): 1455-460.

[13] Talmi-Frank D, Nasereddin A, Schnur L, Schnian G, T S, Jaffe C, *et al*. Detection and identification of old world *Leishmania* by high resolution melt analysis. PLoS Negl Trop Dis 2010; 4(1): 581.

[14] Pratlong E., Martini A.,Lambert M., Lefebvre M., Dedet J. and Rioux JA. Identification of Leishmaniasis by isoenzyme technique. Medicine et' armies , 1994; 22 : 61-65.

[15] Lee S T, Chiang S C, Singh A K and Liu H Y. Identification of *Leishmania* species by a specific DNA probe that is conserved only in the maxicircle DNA of human-infective *Leishmania* parasites. J Infect Dis., 2002; 172: 891-894.

[16] Fazaeli, A., Fouladi B. and Sharifi I. Emergence of cutaneous leishmaniasis in a border area at south-east of Iran: an epidemiological survey. J Vector Borne Dis., 2009; 46.

[17] Kumar, V. Kishore, K. Palit, A. Keshari, S. Sharma, MC. and Das, VN. Vectorial efficacy of *Ph. argentipes* in Kala-azar endemic foci of Bihar under natural and artificial conditions. J. Comm. Dis., 2001; 33: 102- 9.

[18] Alimoradi S.,Hajjaran H,Mohebali M, Mansouri F. Molecular Identification of Leishmania Species Isolated from Human Cutaneous Leishmaniasis by RAPD-PCR. Iranian J Publ Health, 2009; 38(2): 44-50.

[19] Khalifa E. Sharquie and Rafid A. Najim. Disseminated cutaneous leishmaniasis. Saudi Med J., 2004; 25 (7):951-954.

[20] Korzeniewski K. Health hazards in Iraq, Lekarz Wojskowy.2005; 81(3):176-180.

[21] Talari SA, Shajari G and Talaei R . Clinical finding of cutaneous leishmaniasis as a new focus of Iran. Internet J Infec Dis. 2006 ; 1 (2).

[22] Ramirez JR, Agudelo S, Muskus C, et al. Diagnosis of cutaneous

leishmaniasis in Colombia: the sampling site within lesions influences the sensitivity of parasitologic diagnosis. J Clin Microbiol. 2000 ; 38:3768-3773.

[23] Arfan B and Rahman S . Correlation of clinical, histopathological, and microbiological finding in 60 cases of cutaneous leishmaniasis. IJDVL. 2006; 72:28-32.

[24] Faulde M, Schrader J, Heyl G and Amirih M. Differences in transmission seasons as an epidemiological tool for characterization of anthroponotic and zoonotic cutaneous leishmaniasis in northern Afghanistan.Acta Tropica. 2008; 105:131-138.

 