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ISOENZYME CHARACTERIZATION OF IRANIAN *LEISHMANIA* ISOLATES FROM CUTANEOUS LEISHMANIASIS*

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Abstract – During the period from 1996 to 1997, 45 isolates of *Leishmania* were recovered from patients suspected of cutaneous leishmaniasis (CL) and one from a gerbil. These specimens were received from various parts of Iran. Isoenzyme profiles of these isolates were compared with those of reference strains of *L.tropica*, *L.major* and *L.infantum* using cellulose acetate electrophoresis (CAE) and 10 enzyme systems (MDH,ME, NH1, NH2, PGM, GPI, 6PGD, GOT, SOD and G6PD). The isolate obtained from the gerbil was characterized as *L.major*. From the patients with CL, 27 isolates were characterized as *L.tropica*, 10 as *L.major* and 8 remained undetermined.

Keywords – Cutaneous leishmaniasis, Isoenzyme, Iran

1. INTRODUCTION

Cutaneous leishmaniasis, with diverse clinical manifestations, is prevalent in Iran and remains a major public health problem. Several provinces of Iran are endemic foci for both forms of CL: zoonotic cutaneous leishmaniasis (ZCL), due to *Leishmania major*, is found in many rural foci of Isfahan, Khuzestan and Khorasan provinces, while anthroponotic cutaneous leishmaniasis (ACL), due to *L. tropica*, is endemic in many large and small cities including Shiraz in the south, Bam and Kerman in the southeast, and Mashed in northeastern Iran [1, 2]. In the last two decades, biochemical techniques, most notably isoenzyme electrophoresis (IE), provided an effective and reliable tool for characterization of *Leishmania* isolates [3-6]. In the last few years, Iranian investigators have reported new foci of CL in Iran and used several typing techniques such as IE, ELISA using monoclonal antibodies (Mab) and RAPD-PCR in order to characterize the circulating *Leishmania* species [7, 8]. Because of the wide clinical spectrum of CL in Iran and the appearance of new CL foci, there is a real need for *Leishmania* parasite characterization. Here, we are reporting the isoenzyme typing of 46 isolates of *Leishmania* from different geographical areas of Iran.

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2. MATERIALS AND METHODS

The *Leishmania* parasites were isolated from patients suspected of CL from different endemic foci of leishmaniasis (Table 1); only one was isolated from *Rhombomys opimus* in Isfahan. Biphasic NNN medium, or an enriched modification of Evan's modified Tobies medium were used [4, 9]. Samples of overlay were examined regularly to monitor the growth and check for the presence of bacterial or fungal contaminations. Mass cultivation of the organisms were carried out on RPMI-1640 medium (Sigma, Germany) containing 15% fetal calf serum (Gibco, Germany). The cultures were harvested at the end of the logarithmic phase of growth; the number of organisms were adjusted to 1-1.5 x 10^7/ ml. Appropriate lysate for IE were prepared using Evan's method [5].

Table 1. *Leishmania* isolates from patients suspected of CL and one gerbil from different endemic foci of leishmaniasis in Iran

Geographical origin	Code number of the isolates	Total of isolates
Khuzestan	101*,104,105	3
Tehran	102,103,106	3
Fars	107-111,116,139	7
Kerman	112-115,117-125,138,140, NLI-7,NI-4,LI6,SHB1-1, SHB2-2,HSH-5,MH-3	22
Isfahan	126-134,136**,137	11
Total		46

*The infection probably occurred in Kabul (Afghanistan)
**Isolate from Rhombomys opimus

3. CHARACTERIZATION

Parasites were characterized in Casablanca by IE typing on cellulose acetate on 10 loci: glucose phosphate isomerase (GPI), glucose-6-phosphate dehydrogenase (G6PD), malate dehydrogenase (MDH), malic enzyme (ME), nucleoside hydrolase 1 and 2 (NH1 and 2), phosphoglucomutase (PGM), superoxide dismutase (SOD), 6-phosphogluconate dehydrogenase (6PGD), and aspartate aminotransferase (ASAT=GOT). Staining with a specific substrate and coenzyme for development of the activity of each enzymatic system and specific staining for visualization of the isoenzyme activity on cellulose acetate plates were used. Developing conditions for each enzyme system were adapted from Harris & Hopkinson, Kreutzer & Christensen Lanham *et al.*, Tibayrenc *et al*., and recaped by Ben Abderrazzak *et al*. [10-14]. After electrophoresis and staining, isoenzyme banding patterns (IBP) of each isolate were compared with those of WHO *Leishmania* reference strains concurrently run in the CAE assay (Table 2).

Table 2. Reference strains of *Leishmania* used along with the unknown isolates

International WHO code	Species and zymodemes
1. MHOM / IN / 80 / DD8	*L. donovani* MON-2
2. MHOM / TN /80/IPT1	*L. infantum* MON-1
3. MHOM / SU /73 / 5ASKH	*L. major* MON-4
4. MHOM / SU/ 74 / K27	*L. tropica* MON-60
5. MHOM/IR/95/LEM3119 *	*L. tropica* MON-39
6. MHOM/FR/97/LEM3336 *	*L. infantum* MON-29

*kindly provided by the International Cryobank of *Leishmania* (Montpellier, France)

4. RESULTS

Isoenzyme typing of the 45 human isolates obtained from patients with CL revealed that 10 isolates were *L. major*, while 27 isolates were *L. tropica*. In 8 cases isoenzymic patterns were different from all the reference strains. According to the geographical origin of the isolates, 7 were from Fars province in the South of Iran, of which 5 were *L. major* and 2 were *L. tropica***.** From the 11 human isolates from Isfahan province in the center of Iran, 4 were *L. major* and 7 were *L. tropica*. The isolate from the gerbil was *L. major*. Of the 22 isolates from Kerman province in the south of Iran, 18 were *L. tropica*, 2 were *L.major* and 2 remained undetermined. Finally, isolates from Tehran, the capital of Iran, and Khuzestan in the southwest showed isoenzyme patterns different from the reference strains of *L.infantum*, *L. major* and *L. tropica* and were classified as undetermined species (Table 3)**.** Furthermore, these 6 isolates revealed the same isoenzyme profile for the 10 enzyme systems. In Fig. 1 electrophoretic profiles obtained with soluble extracts of *Leishmania* promastigotes for six enzymatic systems were considered. In this diagram, isoenzymic patterns of undetermined zymodem were compared with MHOM/SU/74/K27, the reference strain of *L.tropica* and MHOM/SU/73/5ASKH, the reference strain of *L.major*. Determined zymodems showed similar profiles in comparison with the reference strain in at least more than 7 enzymatic systems.

Table 3. Geographical origin of the isolates characterized in this survey

Leishmania species Region	L. major	L. tropica	Undetermined species	Total
Fars	5	2	-	7
Isfahan	4	7	-	11
Kerman	2	18	2	22
Khuzestan	-	-	3	3
Tehran	-	-	3	3
Total	11	27	8	46

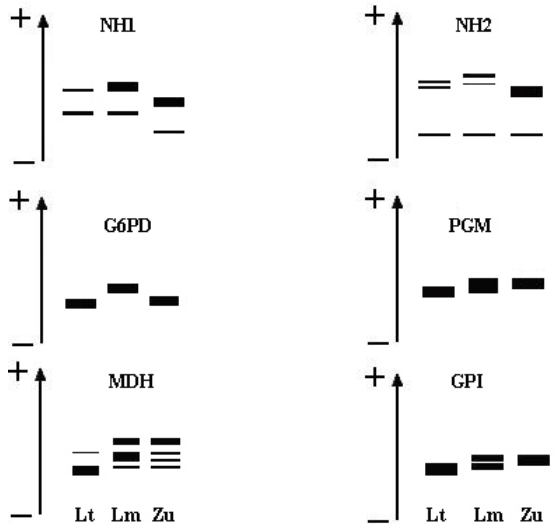

Fig. 1. Electrophoretic profiles obtained with soluble extracts of *Leishmania* promastigotes for six enzymatic systems. Undetermined zymodem(Zu) were compared with MHOM/SU/74/K27, the reference strain of *L.tropica*(L.t) and MHOM/SU/73/5ASKH, the reference strain of *L.major* (L.m). (+) is anode and (-) is cathode. Arrows exhibit the direction of electrophoresis

5. DISCUSSION

The presence of CL due to *L. major* and *L. tropica* was previously reported in Iran on the basis of epidemiological data and clinical manifestations [1] and recently confirmed by IE, PCR and monoclonal antibodies serotyping [7, 8, 15]. Furthermore, dermotropic isolates of *L. infantum* were also recently characterized in Iran [8, 16].

The characterization of 45 human CL isolates showed that in Fars, Kerman and Isfahan the disease is either due to *L. major* or *L. tropica*. Rural areas of Isfahan, Kerman and Fars provinces are known to be the most important foci of ZCL in Iran, while urban areas of Kerman and Fars provinces, and recently the city of Isfahan, are known as foci of ACL [2, 8, 15].This shows that CL foci of *L. major* and *L. tropica* can coexist in the same area, at least in some conditions that remain to be investigated. The massive human migrations may explain this situation. However, up to now the data indicates that CL caused by *L. major* is still a rural disease, while CL caused by *L. tropica* is an urban disease in Iran. In this series of isolates, no CL isolates from any of these Iranian localities were typed as *L. infantum*. On the other hand, 6 isolates from Khuzestan and Tehran and 2 isolates from Kerman remained undetermined. Their isoenzyme patterns were different from the 6 reference strains used (Table 2). According to previous findings, *L. tropica* and rarely *L. infantum* are causative agents of CL in Tehran, and both *L. tropica* and *L. major* are the causative agents of the disease in Khuzestan and Kerman [8, 15]. In previous isoenzyme studies of *Leishmania* isolated in Iran, zymodemes not comparable to the reference strains were also observed. The two undetermined isolates were from patients coming from Afghanistan: one case was a seven-year-old girl and the other was an eighteen-year-old man. The zymodeme patterns of these two isolates were completely different from previous isolates and reference strains studied so far [17]. The presence of such isolates with different IBPs in comparison with reference strains, emphasizes the heterogeneity of causative agents of CL in Iran. It may be suggested that these isolates may play a role in some vaccination and chemotherapy failures in endemic foci. Thus, further investigation on similar isolates in these areas is recommended.

6. CONCLUSION

This study confirms the previous data on the geographical distribution of CL in Iran, and also demonstrates the coexistence of CL caused by *L. major* and *L. tropica* in the same geographical areas. Extensive eco-epidemiological studies could bring complementary information of high interest for setting preventive measures. For this epidemiological purpose, multi-loci enzyme electrophoresis using cellulose acetate support is a valuable technique. However, using this technique, 8 isolates from this series shared a common but different isoenzyme pattern from the reference strains, suggesting the need of using more discriminative complementary techniques in a reference laboratory. This will probably bring some new data in the *Leishmania* species/strains circulating in Iran, which could have an interesting impact on the knowledge about leishmaniasis in Iran, and more widely in the Old World.

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