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Characterization of *Leishmania* Species and *L. major* Strains in Different Endemic Areas of Cutaneous Leishmaniasis in Iran

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ABSTRACT

Both zoonotic and anthroponotic cutaneous leishmaniasis (CL) caused by *L. major* and *L. tropica*, respectively, are endemic in different parts of Iran. This study was performed to investigate the new changes in epidemiological pattern of CL, and to identify the species of *Leishmania* and the strains of *L. major* isolated from different endemic areas of Iran. Seventy-two isolates from 245 samples collected from different endemic areas of Iran were characterized by monoclonal antibodies (mAb) specific for *L. major, L. tropica, and L. infantum*. Flow cytometry, isoenzyme analysis and PCR amplification were used for identification. Isoenzyme analysis was carried out by PAGE and cellulose acetate. Eight enzymes including malate dehydrogenase (MDH), malic enzyme (ME), glucose 6-phosphate dehydrogenase (G6PD), glucose phosphate isomerase (GPI), nucleoside hydrolase inosine (NHi), nucleoside hydrolase deoxy inosine (NHd), superoxide dismutase (SOD) and 6-phosphogluconate dehydrogenase (6PGD) were used for isoenzyme analysis. PCR assay was developed using specific primers against kinetoplast DNA. The isolates were compared with reference strains (RS). Data obtained from different methods showed 45 out of 72 isolates were similar to RS of *L. major* and 22 similar to *L. tropica*, and also five samples were identified as *Crithidia*. Isoenzyme migration pattern of *L. major* isolates was indistinguishable from each other in six enzymatic systems but differences were observed in profile of two enzymes of SOD and MDH. The data indicate that *L. major* species are dominant in the studied endemic areas, and different strains of *L. major* are present in different geographic areas of Iran. Moreover, it seems that enzymatic system is more useful approach than others for characterization of *Leishmania* species and *L. major* strains. *Iran. Biomed. J. 7 (2): 43-50, 2003*

Keywords: Leishmania species, monoclonal antibodies (mAb), isoenzyme, PCR

INTRODUCTION

Cutaneous leishmaniasis (CL) is a complex disease with wide spectrum of clinical manifestations. It is prevalent in many parts of the world, specially tropical and sub-tropical countries including Iran [1]. The disease is caused by different species and subspecies of protozoan parasites belong to the *Leishmania* genus. Clinical forms of the disease vary from a simple nodule to non-healing disfiguring lupoid form. The evidence suggests that in CL, in addition to the important role of host immunological response, the outcome of infection and clinical manifestation of disease depend upon the strain of the related parasite [2].

Two species of *Leishmania* are involved in CL infections in Iran. *L. major* is causative agent of zoonotic cutaneous leishmaniasis (ZCL) and *L. tropica* causes anthroponotic CL (ACL) [3]. ZCL is endemic in four major parts of Iran: a) central parts, including Isfahan province [4]; b) north-east and north of Iran, including Khorasan [5] and Semnan (a new focus) provinces; c) south-west of Iran, including Khozestan [6] and Ilam provinces; and d) south of Iran including Fars [7], Bushehr [8] and Hormozgan provinces. Whereas ACL is endemic in many large cities including Tehran, Shiraz, Mashhad, Kerman and small city like Bam.

Early classification of *Leishmania* species was based on the clinical manifestation, epidemiological features and geographical distribution [9]. Albeit, all of these parameters remain important in identification of parasite, but these are insufficient for definitely characterization of the isolates.

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Table 1. Number of identified isolates collected from seven endemic areas of CL in Iran. From 72 isolates, 45 were identified as *L. major*, 22 as *L. tropica* and 5 as *Crithidia luciliae*.

Name of province	Endemic area	Total No. of isolates	No. of isolates identified		
			L. major	*L. tropica*	*C. luciliae*
Isfahan	Isfahan	20	5	15	-
	Kashan	20	20	-	-
Ilam	Dehloran	9	9	-	-
Khuzestan	Dezfool	7	5	-	2
Fars	Shiraz	3	-	-	3
Semnan	Damghan	6	6	-	-
Khorasan	Mashhad	7	-	7	-

There are well-established molecular, biochemical and immunological methods for identification of *Leishmania* parasites in the literature [10-12]. The identification with specific monoclonal antibody (mAb) against different species of *Leishmania* using the immune-fluorescence (IF) method and amplification of kinetoplast DNA (kDNA) by PCR remain invaluable and rapid. However, the isoenzyme analysis has been proved as the most useful taxonomic indicator for *Leishmania* isolates [10]. This method is able to detect inter and intra species diversity among isolates of *Leishmania.*

The aims of this study were based on the following items: a) identification of different species of *Leishmania* in most endemic areas of CL in Iran, including a new focus in north of Iran (Semnan province), b) characterization of different strains of *L. major* in different geographic endemic areas; and c) comparison of different methods for identification of *Leishmania* isolates.

MATERIALS AND METHODS

Sample collection and culture. Two hundred forty-five samples from patients with skin lesion suspected of cutaneous leishmaniasis (CL) were randomly collected from different endemic areas of Isfahan, Ilam, Khouzestan, Fars, Semnan, and Khorasan provinces. Outline geographic details of isolates are shown in Table 1. The samples were aspirated from the edges of the skin lesions and cultured in liquid phase (RPMI 1640) of Novy-MacNeal-Nicole (NNN) media. After regular examination of the overlay, seventy-two isolates growing in the culture were used for characterization. Parasites grown in biphasic medium were used for cryopreservation, inoculation of mice and IF method. For isoenzyme analysis and PCR, primary isolates were subcultured in RPMI 1640 media, supplemented with 2 mM L-glutamine, 15% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin. The second or third subcultures were used for characterization.

Cryopreservation. When sufficient number of parasite was obtained, promastigotes were harvested at logarithmic phase of growth, washed, counted and the pellet was resuspended while adjusting to about 5×10^6 parasites/ml in cryoprotectant medium (RPMI contains 40% FCS and 8% glycerol). Aliquots were frozen gradually at –20°C and then -70°C, and finally in liquid nitrogen.

Inoculation of susceptible mice. Female BALB/c mice (4-6 weeks old) were obtained from the breeding stock maintained at the Pasteur Institute of Iran (Tehran). Isolates were harvested at stationary phase of growth and washed once with PBS (pH 7.2). Then, 2×10^6 parasites were injected subcutaneously into the base tail of three BALB/c mice. The mice were examined weekly for appearance of lesion in the injection site up to six months. After massive ulceration, they were sacrificed and the lymph node (LN) and spleens were cultured and tested for the presence of parasite.

Reference strains (RS). The standard Iranian strains of *L. major* (MRHO/IR/75/ER) was kindly provided by Dr. Javadian, School of Public Health, Tehran University of Medical Sciences, *L. tropica (*MHOM/SU/74/K27), and *L. infantum* (MHOM/ TN/80/IPT1) were used as reference throughout the study. The later two strains were obtained from Dept. of Medical Parasitology, London School of Hygiene and Tropical Medicine.

Also, a *Crithidia luciliae* control was provided kindly by Dr. M. Mohebali, Leishmaniasis Laboratory, School of Public Health, Tehran University of Medical Sciences, Iran.

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Monoclonal antibodies. The following mAb were kindly provided by Dr. F. Modabber, TDR, WHO, Geneva, Switzerland: XLVI-5B8-B3 (T1) specific for *L. major;* IS2-2B4-(A11) specific for *L. tropica;* LXXVIII-2E5-A8 (D2) specific for *L. donovani* complex including *L. infantum*.

Identification of isolates by mAb using IF: Preparation of antigen. Promastigotes were harvested at logarithmic phase of growth, and washed twice with PBS (pH 7.2). The pellet was fixed by 2% formaldehyde. After washing, the pellet was resuspended and adjusted to a final concentration of 3-4 × 10^6/ml PBS. For slide preparation, 10 µl of the parasite suspension containing 3-4 × 10^4 organisms were added to each well on slides previously washed with detergent and alcohol. The slides stored at -20°C until use. For flow cytometry assay 100 µl of suspension was used as antigen.

IF procedure. Indirect IF was used for identification of isolates. The prepared slides were washed and then incubated with mAb (1:100 dilution) in a humid chamber for 30 min at 37°C. After washing, polyvalent fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (DAKO) was added and incubated for additional 30 min. Then, the slides were washed, dried and mounted with 90% glycerol in PBS (pH 8.0) and examined under fluorescence microscope.

IF by flow cytometry. To verify the results of IF, 100 µl of specific mAb (1:100 dilutions) was added to 100 µl of antigens and incubated for 30 min at 37°C. Then, after washing, polyvalent FITC-conjugated anti-mouse IgG was added and incubated for additional 30 min. The parasites were finally analyzed without gating by flow cytometry (FACScan, Becton Dickinson, USA), and Lysis II software.

Extraction and PCR amplification of kDNA. Parasites from a 15-ml mid-logarithmic phase of bulk culture were harvested by centrifugation (700 g for 20 min at 4°C) and washed three times in ice-cold sterile PBS, pH 7.2. The pellet was resuspended in 1 ml sterile cell lysis buffer (125 mM NaCl, 125 mM EDTA, 2.5% w/v SDS, 125 mM Tris, pH 8.0) with 100 µg/ml proteinase K, and incubated at 56°C for 3 hours. The DNA was freed from contaminants in the lysate by phenol/chloroform extraction and ethanol precipitation [13]. PCR was performed using a pair of primers of 5' TCGCAGAACGCCCCT- ACC 3' and 5' AGGGGTTGGTGTAAAATAGG 3' (TIB Molbiol, Germany and CinnaGene, Iran), based on Dr. Smyth *et al.* [14]. DNA (1 µl) was added to a PCR mixture (30 µl) contained 10× PCR buffer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 1 unit of *Taq* DNA polymerase (Roche Diagnostic) and 10 pM of each primer. The following amplification program was used: primary denaturation was performed at 95°C for 3 min, followed by 35 cycles of 93°C for 40 s, 57°C for 30 s and 72°C for 1 min. This was followed by final extension cycle at 72ºC for 10 min. PCR products were analyzed by 2% agarose gel electrophoresis with 8 µl of the reaction mixture. Positive and negative controls were included in all tests.

Isoenzyme electrophoresis. 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 (1 mM EDTA, 1 mM ε-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 [15]. Eight enzymes were used for analysis of isolates including, malate dehydrogenase (MDH), malic enzyme (ME), glucose 6-phosphate dehydrogenase (G6PD), glucose phosphate isomerase (GPI), nucleoside hydrolase inosine (NHi), nucleoside hydrolase deoxy inosine (NHd), superoxide dismutase (SOD) and 6-phospho-gluconate dehydrogenase (6PGD) [16-23].

RESULTS

Susceptibility of BALB/c mice to Leishmania isolates. Active lesions in the site of isolates inoculation were shown in 46 out of 72 groups of mice. Forty-five inoculated groups showed dissemination of infection to other organs and development of visceralized ulceration. Promastigotes were shown in cultures of LN and

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spleen isolated from visceralized mice. One group of mice showed the presence of parasite in LN and not in viscera.

IF results. Of 72 isolates discriminated by IF under microscopic examination, 46 cases (64 %) were similar to RS of *L. major* and 1 isolate (1.4 %) with RS of *L. tropica*. The remaining 25 isolates did not react with any mAb against *L. major*, *L. tropica* and *L. infantum.*

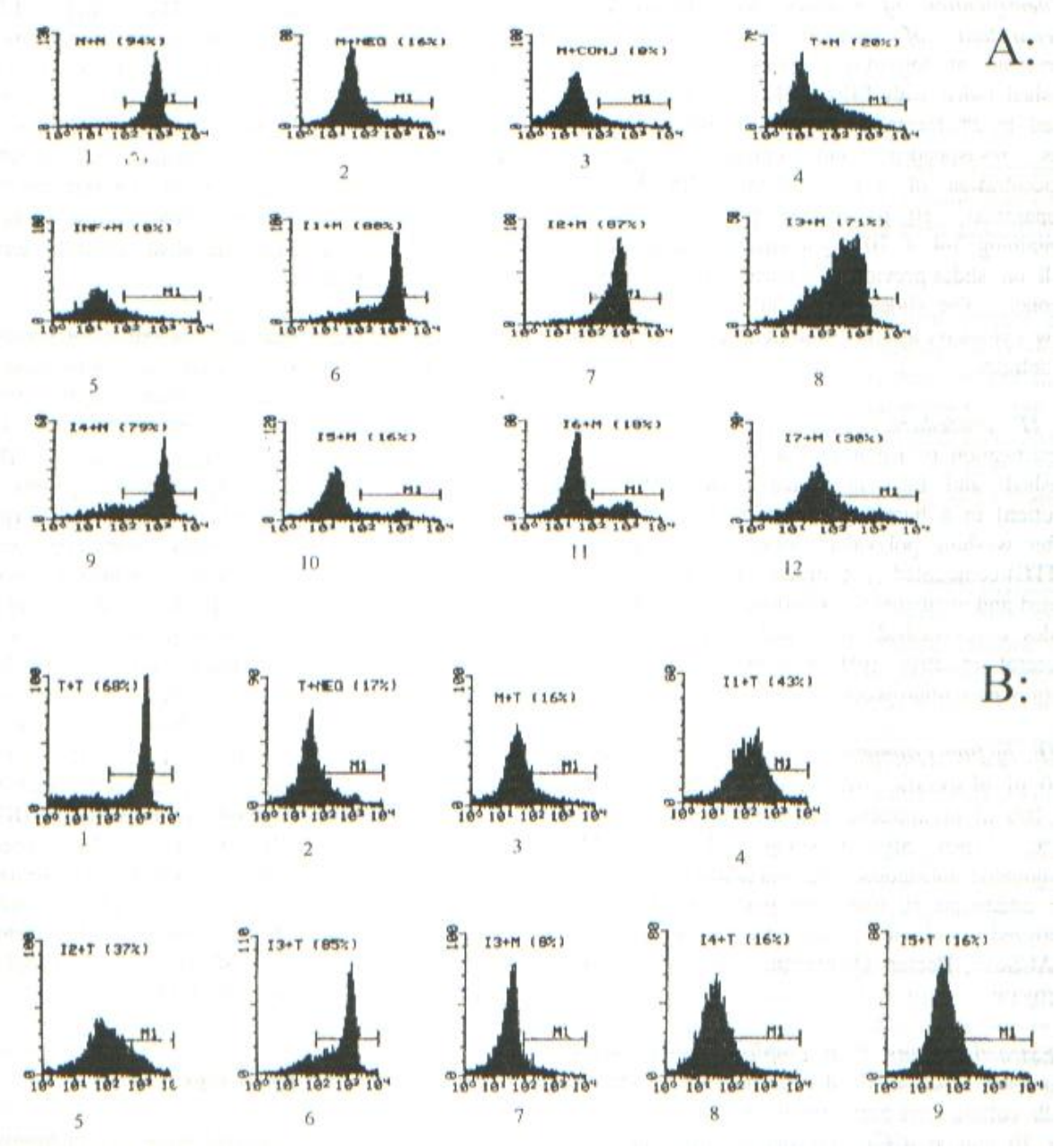


Fig. 1. Analysis of *Leishmania* species using flow cytometry. **(A),** reactivity of anti-*L. major* mAb with reference strain (RS) of *L. major* (No. 1), *L. tropica* (4), *L. infantum* (5), and different isolates (6-12). No. 2 and 3 show reactivity of RS of *L. major* with negative control mAb and FITC-conjugated anti-mouse IgG, respectively. Isolates No. 6, 7, 8 and 9 show 88%, 87%, 71% and 79% reactivity with anti-*L. major* mAb, respectively. These results are almost similar to reactivity of *L. major* RS (94%). **(B)**, reactivity of anti-*L. tropica* mAb with RS of *L. tropica* (No. 1), *L. major* (3), and different isolates (4-9). No. 2 shows reactivity of RS of *L. tropica* with negative control mAb. Isolate No. 6 shows 85% reactivity with anti-*L. tropica* mAb, which is similar to reactivity of *L. tropica* RS (68%).

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Flow cytometry analysis showed that 45 (62%) isolates were positive with anti-*L. major* and 13 isolates (18%) with anti-*L. tropica*, while 7 isolates (10%) reacted with both anti-*L. tropica* (A11) and anti-*L. major* (T1) mAb. Seven isolates (10%) showed no positive reaction with either mAb. The reactivity of *L. major* isolates with anti-*L. major* mAb varied between 47 to 99.5% with the mean of 71 ± 13.3%. However, the reactivity of *L. tropica* with anti-*L. tropica* mAb was between 28.4 to 86% with the mean of 44.2 ± 16%. Some of the histograms were shown in Figure 1.

PCR analysis. By amplification of kDNA, 45 out of 72 isolates were showed similar pattern to RS of *L. major* and 22 isolates to RS of *L. tropica*. The PCR product of *L. major* kDNA was 600 bp and that of *L. tropica* 800 bp (Fig. 2).

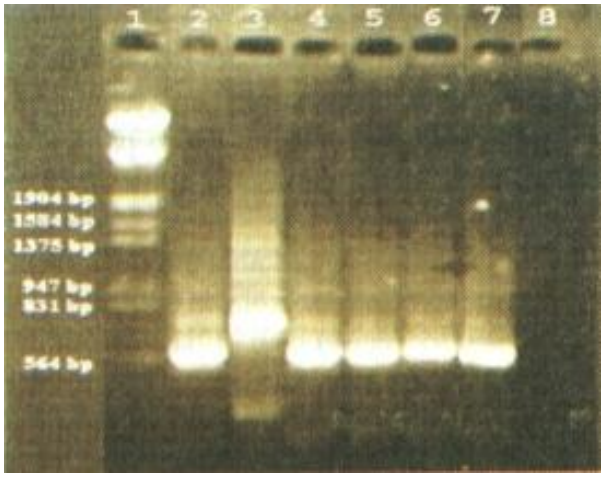


Fig. 2. Gel electrophoresis of kDNA-PCR. Lane 1, molecular weight ladder; Lane 2, reference strain (RS) of *L. major;* Lane 3, RS of *L. tropica;* Lanes 4-7, *L. major* isolates; Lane 8, negative control.

Isoenzyme analysis. After electrophoresis and staining, individual isoenzyme banding pattern (IBP) of each isolate was compared with RS of *Leishmania* concurrently run in PAGE. Comparison of isoenzyme patterns of isolates with standard strains showed that 45 out of 72 isolates have similar IBP to RS of *L. major* and 22 isolates to *L. tropica* species. Five isolates showed similar IBP to RS of *C. luciliae*. The majority of 45 isolates showed similar IBP by seven enzymes of GPI, NHd, NHi, G6PD, ME, 6PGD and MDH (except one isolate which showed additional band in the common pattern of MDH) (Figs. 3 and 4). However, different patterns were seen in SOD enzymatic system. Using SOD enzyme, two different patterns were characterized for *L. major* isolates. Whilst 21 isolates (20 from Kashan and 1 from Isfahan) out of 45 showed two bands with Rf of 0.44 and 0.50, twenty-four isolates showed three bands (Rf of 0.44, 0.47, and 0.51) similar to RS. These isolates were collected from different foci of Khouzestan, Ilam, Isfahan and a new focus of Semnan (Fig. 5).

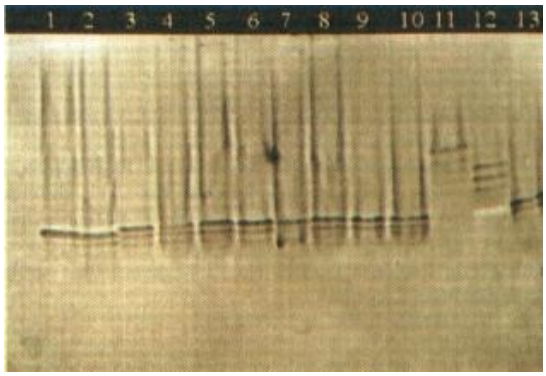


Fig. 3. Electrophoretic patterns obtained for MDH. No. 1, reference strain (RS) of *C. luciliae*; No. 2-9, different isolates; No. 10 and 13, RS of *L. major*; No. 11, RS of *L. infantum*; No. 12, RS of *L tropica*.

DISCUSSION

Characterization of different species of *Leishmania* especially *L. major* variants in various endemic areas of CL, was the major aim of this study. This report has focused for the first time on comparison of IF method using flow cytometry, DNA amplification and isoenzyme analysis for characterization of *Leishmania* species and *L. major* strains. Our findings indicated that out of 72 isolates recovered from 245 samples, 45 belong to *L. major* species and strains. Meanwhile, the species involved in recent outbreak in rural areas of Damghan city (Semnan province) were also identified as *L. major*.

Application of three basic methods used in this study showed a reliable consistency together in identification of *Leishmania* species isolated from different endemic areas. The results showed that *L. major* species are dominant in isolates collected from Isfahan, Kashan, Ilam, Khouzestan and Semnan provinces. On the other hand, total number of isolates collected from Khorasan province and majority of isolates from urban area of Isfahan were characterized as *L. tropica.* These data are in agreement with previous epidemiological studies in Iran [3, 4, 6]. Most of the isolates characterized as *L. major* species had collected from endemic focus around the desert (Isfahan, Kashan and Damghan).

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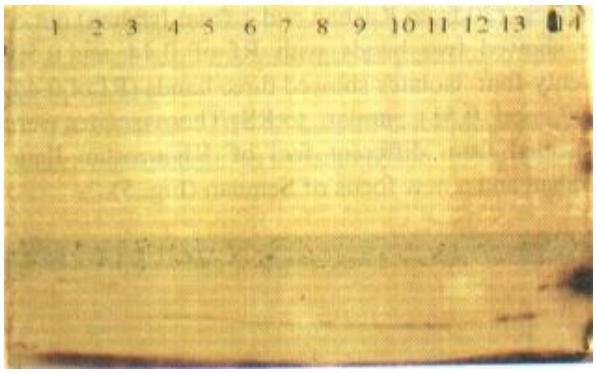


Fig. 4. Electrophoretic patterns obtained for NHd. No. 1, reference strain (RS) of *C. luciliae*; No. 2-11, different isolates; No. 12, RS of *L. major;* No. 13, RS of *L. infantum*; No. 14, RS of *L tropica.* Isolate No. 2 shows identical pattern with *C. luciliae*, and isolates No. 3-11 demonstrate similar pattern to RS of *L. major*.

Isfahan is a famous endemic focus of ZCL recognized during past several decades [4]. However, the focus of Kashan is probably due to transmission of infection from Isfahan focus. Our findings corroborate the result of a previous study in Kashan [24].

L. tropica isolates were found more than *L. major* in Isfahan areas since Isfahan is a well-known endemic area of ZCL, these results seem to be surprising. However, considering the majority of isolates have been collected from urban areas of Isfahan, the results demonstrate a new pattern from ACL focus in the city of Isfahan and a good consistency with other reports implying the presence of *L. tropica* infection in this city [25, 26].



Fig. 5. Isoenzyme patterns (negative image) of different strains of *L. major* isolates in SOD system. No. 3: Reference strain of *L. major*. No. 1, 2 and 4-7, different *L. major* isolates. No. 6 and 7 show double bands, and No. 1, 2, 4 and 5 demonstrate three bands, similar to RS of *L. major*.

No difference was observed in analysis of isolates with 6 enzymatic systems. Variations in isoenzyme banding pattern of *L. major* isolates were clearly shown in MDH and SOD enzymatic systems. In MDH system, one isolate from Ilam province showed a unique pattern with five bands, which was different from banding pattern of the other isolates and RS with four bands. In SOD system, two different banding patterns were observed with two or three bands. Data show that one pattern with two bands belongs to the Isfahan province, which is the ancient focus of ZCL and located in southwest of desert. The second pattern with three bands is seen in other foci, including non-desert areas of Khouzestan, Ilam and a new focus in north of desert. One explanation for these differences may be due to the presence of two distinct strains from *L. major* in different endemic areas of ZCL in Iran. We can speculate that probably the infection has transmitted from Isfahan area to the other foci. During transmission, variations have occurred in the original strain of *L. major* and were displayed in the new pattern. The heterogeneity in *L. major* pattern was in agreement with data reported by other investigators in Sudan [27].

In general, no other remarkable polymorphism was observed among *L. major* isolates (except the above mentioned heterogeneity) and it seems that in most enzymatic systems a marked degree of homogeneity are seen in 45 *L. major* isolates characterized in this study. This finding is consistent with data reported by Le Blancq *et al*. [18], but not by other authors in Sudan [27]. This discrepancy may be due to different strains of *L. major* presented in ZCL foci of Sudan and Iran.

The reason for the presence of *C. luciliae* isolates among the limited number of isolates is not clear, but it may be attributed to a laboratory contamination during a simultaneous work of a student for assessment of anti-DNA (double-stranded) by IF using this protozoon as a substrate.

It is noteworthy that, in the primary study, to distinguish *L. major* isolates from other species of *Leishmania*, all isolates were inoculated to BALB/c mice. Susceptibility of BALB/c mice to *L. major* infection is reported in many studies, and injection of *L. major* isolates to these mice results in generalized and visceralized fatal infection [28, 29]. The results showed exact consistency with data obtained from other methods used in this study.

Although, *L. major* isolates were distinguished precisely by using species-specific mAb, but low reactivity and cross-reactivity were occurred in

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characterization of *L. tropica* by species-specific mAb using IF method. Likewise, analysis using flow cytometry promoted identification of *L. tropica* isolates by mAb, a wide spectrum of intensities and low reactivity were observed for some isolates in comparison to RS. A similar problem has been previously reported in immunoassay analysis [23, 25, 30]. However, in contrast to our observations, there are some reports indicating that species-specific mAb have ability to distinguish *Leishmania* species in most cases of New World [11] and Old World leishmaniasis [31]. Moreover, strong reactivity has been reported using anti-*L. tropica* mAb for identification of a limited number of isolates from different countries in Middle East other than Iran [32]. The explanation for these contradictory results is not clear, but it may be attributed to the possible intraspecies variation of the isolates and the presence of different strains with distinct epitope(s) in Iran.

Data obtained from isoenzyme analysis showed similar patterns in most isolates compared to the RS. Analysis of each enzymatic system confirmed the data obtained by other seven systems. The results of isoenzyme analysis revealed that this method is a more powerful tool for identification of *Leishmania* strains compared to IF test and kDNA amplification. Enzymatic systems used in this study have ability to clearly distinguish three species of *L. major*, *L. tropica* and *L. infantum*, similar to other reports [21, 23].

In conclusion, characterization of isolates collected from different endemic areas of CL showed that *L. major* isolates are distributed in most endemic areas. Likewise, different patterns of *L. major* isolates were shown in some enzymatic systems, which need further investigation. Moreover, this study revealed that among different techniques used for characterization of isolates, isoenzyme and PCR methods are the most powerful and useful tools in characterization of *Leishmania* species. However, isoenzyme analysis, in spite of some drawbacks, is an ideal method for discrimination of *L. major* variants.

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REFERENCES

1. WHO Expert Committee (1990) Control of the leishmaniasis. World Health Organization, Technical Report Series 793. pp. 9-10.
2. McKerrow, J. (1997) Parasitic diseases. In: *Medical Immunology*. (Stites, D.P., Terr, A.I. and Parslow, T.G. eds.) Prentice-Hall International Inc. London, pp. 725-738.
3. Nadim, A. and Seyedi-Rashti, M.A. (1971) A brief review of the epidemiology of various types of leishmaniasis in Iran. *Acta Medica. Iranica. XIV: 99-106.*
4. Nadim, A. and Faghih, M.A. (1968) The epidemiology of CL in Isfahan province of Iran, I, the reservoir, II, the human disease. *Trans. R. Soc. Trop. Med. Hyg. 62: 534-542.*
5. Javadian, E., Nadim, A., Tahvildare-Bidrudi, G. and Assefi, V. (1976) Epidemiology of cutaneous leishmaniasis in Iran B. Khorasan. Part v: Report on a focus of zoonotic cutaneous leishmaniasis in Esferayen. *Bull Soc. Pathol. Exot. Filiales 69: 140-143.*
6. Javadian, E. and Mesghali, A. (1974) Studies on cutaneous leishmaniasis in Khouzestan, Iran. Part I: The leptomonad infection of sandflies. *Bull. Soc. Pathol. Exot. Filiales 67: 513-516.*
7. Moaddeb, A., Gettner, S. and Ardehali, S. (1993) Studies on the causative agent of cutaneous leishmaniasis in Shiraz-Iran. *J. Med. Sci. 18: 28-33.*
8. Hamzavi, V., Mohebali, M., Edrissian, G.H. and Fourozani, A.R. (2000) Epidemiological study of cutaneous leishmaniasis in Dashti and Dashtestani district of Bushehr province. *Iranian J. Publ. Health 29: 177-191.*
9. Chance, M.L. (1979) The identification of *Leishmania.* In: *Problems in the identification of parasites and their vectors.* (Taylor, A.E.R. and Muller, R. eds.), Blackwell Scientific Publications, Oxford. pp. 55-74.
10. Rioux, J.A., Lanotte, G., Serres, E., Pratlong, F., Bastien, P. and Perieres, J. (1990) Taxonomy of

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[DOR: 20.1001.1.1028852.2003.7.2.1.1]

Leishmania. Use of isoenzyme. Suggestions for a new classification. *Ann. parasitol. Hum. Comp. 65 (3): 111-125.*

11. McMahon-pratt, D. and David, J.R. (1981) Monoclonal antibodies that distinguish between New world species of *Leishmania*. *Nature 291: 581-583*.
12. Motazedian, H., Noyes, H. and Maningon, R. (1996) *Leishmania* and Sauroleishmania: the use of random amplified polymorphic DNA for the identification of parasites from vertebrates and invertebrates. *Exp. Parasitol. 83: 150-154*.
13. Kelly, J.M. (1993) Isolation of RNA and DNA from *Leishmania*. In: *Methods in Molecular Biology.* Vol. 21, Human Press, New Jersey, USA. PP: 123-131.
14. Smyth, A.G., Hassan, A., Basu, Q., De Bruijin, D., Adhua, M., Mallik, K. and Barker, D. (1992) Rapid and sensitive detection of *Leishmania* kinetoplast DNA from spleen and blood samples of kala-azar patients. *Parasitol. 105: 183-192*.
15. Evans, D.A. (1989) Handbook on isolation, characterization and cryopreservation of *Leishmania*, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR). Geneva, Switzerland. pp. 15-27.
16. Kreutzer, R.D. and Christensen, H.A. (1980) Characterization of *Leishmania* spp. by isoenzyme electrophoresis. *Am. J. Trop. Med. Hyg. 29: 199-208.*
17. Grimaidi, G., Momen, H. Naiff, R.D., McMahon-Pratt, D. and Barrett, T.V. (1991) Characterization and classification of leishmanial parasites from humans, wild sandflies in the Amazon region of Brazil. *Am. J. Trop. Med. Hyg. 44: 645-661*.
18. Le Blancq, S.M., Schnur, L.F. and Peters, W. (1986) *Leishmania* in the old world: 1. The geographical and hostal distribution of *L. major* zymodemes. *Trans. Roy. Soc. Trop. Med. Hyg. 80: 99-112*.
19. Evans, E.D., Lanham, S.M., Baldwin, C.I. and Peters, W. (1984) The isolation and isoenzyme characterization of *Leishmania braziliensis* ssp. from patients with CL acquired in Belize. *Trans. Roy. Soc. Trop. Med. Hyg. 78: 35-42*.
20. Kreutzer, R.D., Semko, M.E., Hendricks, L.D. and Wright, N. (1983) Identification of *Leishmania* spp. by multiple isoenzyme analysis. *Am. J. Trop. Med. Hyg. 32 (4): 703-715.*
21. Mebrahtu, Y.B., Lawyer, P.G., Pamba, H., Koech, D., Perkins, P.V., Roberts, C.R., Were, J.B. and Hendricks, L.D. (1992) Biochemical characterization and zymodeme classification of *Leishmania* isolates from patients, vector, and reservoir hosts in Kenya. *Am. J. Trop. Med. Hyg. 47 (6): 852-892.*
22. Miles, M.A., Povoa, M.I.M., Souza, A.A., Lainson, R. and Shaw, J.J. (1980) Some methods for enzyme characterization of Latin-American *Leishmania* with particular reference to *Leishmania mexicana amazonensis* and subspecies of *Leishmania hertigi*. *Trans. Roy. Soc. Trop. Med. Hyg. 74: 243-252.*
23. Hatam, G.R., Hosseini, S.M.H. and Ardehali, S. (1999) Isoenzyme studies in characterization of *Leishmania* isolated in Iran. *Irn. J. Med. Sci. 24 (1, 2): 8-13*.
24. Alimohammadian, M.H., Almasi, H., Khabiri, A.R., Hatam, G., Karimi, A., Mahboudi, F., Seyyed Reza Tehrani, S.M. and Amirkhani, A. (1999) Identification of species and characterization of an outbreak of cutaneous leishmaniasis in a new focus of Iran. *Iran. Biomed. J. 3 (1 & 2): 31-39*.
25. Ardehali, S., Moattari, A., Hatam, G.R., Hosseini, S.M.H. and Sharifi, I. (2000) Characterization of *Leishmania* isolated in Iran: 1. Serotyping with species-specific monoclonal antibodies. *Acta Trop. 75 (3): 301-307.*
26. Momeni, A.Z., Amindjavaheri, M. and Ghanadi, F. (1994) Erysipeloid type of leishmaniasis: a clinical study of an unusual presentation of cutaneous leishmaniasis in Iranian women in Isfahan-Iran. *Med. J. IRI. 7: 273-276.*
27. Ibrahim, M.E., Evans, D.A., Theander, T.G., El-Hassan, A.M. and Kharazmi A. (1995) Diversity among *Leishmania* isolates from Sudan: isoenzyme homogeneity of *L. donovani* versus heterogeneity of *L. major. Trans. Roy. Soc. Trop. Med. Hyg. 89: 366-369*.
28. Nasseri, M. and Modabber, F. (1979) Generalized infection and lack of delayed hypersensitivity in BALB/c mice infected with *Leishmania tropica major*. *Infect. Immun. 26: 611- 614.*
29. Sacks, D. and Noben-Trauth, N. (2002) The immunology of susceptibility and resistance to *Leishmania major* in mice. *Nat. Rev. Immunol. 2: 845-858*.
30. Sharifi, I., Ardehali, S., Motazadian, H., Aflatonian, M.R., Fekri, A.R., Ahmadi Mousavi, M.R., Nadim, A., Khamesipour, A., Dowlati, Y. and Modabber, F. (1997) Identification and characterization of *Leishmania* isolates in school children in Bam southeastern Iran. *Irn. J. Med. Sci. 22: 82-88*.
31. De Ibarra, A.A.L., Howard, J.G. and Snary, D. (1982) Monoclonal antibodies to *Leishmania tropica major*: specificities and antigen location. *Parasitol. 85: 523-531*.
32. Jaffe, C.L. and Sarfstein, R. (1987) Species-specific antibodies to *Leishmania tropica* (minor) recognize somatic antigens and exometabolites. *J. Immunol. 139: 1310-1319.*

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