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Unusual presentation of disseminated cutaneous leishmaniasis due to *Leishmania major*: Case reports of four Iranian patients

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ABSTRACT

We report four disseminated cutaneous leishmaniasis(DCL) cases referred to leishmaniasis laboratory at the School of Public Health, Tehran University of Medical Sciences with multiple nodular, ulcerative and crusted lesions extended on the face, trunk, and extremities. None of the patients had any complication and historical involvement in their immunological system conditions that suggest as the criteria for DCL. Direct smears of ulcers were positive for *Leishmania* parasite. The parasite was isolated from the active lesions and identified as *Leishmania major (L. major)* using PCR-RFLP assay and sequencing analysis.

1. Introduction

Leishmaniasis is a worldwide protozoal disease caused by several species of the genus Leishmania. Clinically leishmaniasis classified into cutaneous, mucocutaneous and visceral forms[1]. Cutaneous leishmaniasis (CL) is an important and prevalent vector born disease in Iran with about 20 000 new cases annually. *Leishmania tropica* (*L. tropica*) with about 25% and *L. major* with 75% frequency are the main etiological agents for both Anthroponotic and zoonotic CL that reported in more than 18 out of 31 provinces of Iran[2].

In the recent years there are a few reports of atypical clinical forms of cutaneous leishmaniasis patients[3]. Some studies have implicated *L. tropica* as another agent of visceral leishmaniasis in dogs and humans from the north-west and south of the country[4,5]. Few data are available, however, about host immunological response and parasite destruction when leishmaniasis is associated with immunosuppressant. At present, the majority of cases of HIV-leishmaniasis co-infection reported in the Mediterranean basin were caused by *L. infantum*[6]. In Iran there are some reports that show *L. tropica* is principal agent of VL in HIV infected patients[7].

We report here, 4 disseminated cutaneous leishmaniasis patients with clinical, parasitological and molecular results.

2. Case reports

2.1. Clinical presentation

Patient 1

A 41-year-old man with no particular medical history and systemic symptoms referred from the physician to the leishmaniasis lab, department of Medical Parasitology

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and Mycology, School of Public Health, Tehran University of Medical Sciences (TUMS) in November 2009. He was a military crew and his mission was in Dehloran from Ilam province located in the west of Iran near the Iraq border. Upon questioning, the patient described the appearance in the past six months (May 2009) with a number of papulo-nodular cutaneous lesions on his hands and legs. After a while, various skin ulcerative lesions almost disseminated in his entire body. Physical examination showed that the patient was good in general condition, without fever but for the disseminated lesions and hence disability, he lived a very anxious and stressed life. In physical examination, most of cutaneous lesions were papulonodular, infiltrated without any typical form. The lesions were painless. The lesions disseminated to entire body, with the exception of the palms (Figure 1).

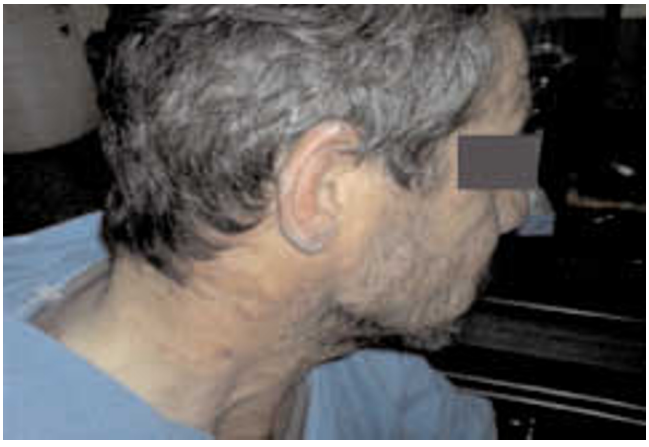

Figure 1. Patient 1 from Dehloran in the west of Iran (2009).

Patient 2

A 59-year-old man with no particular medical history and systemic symptoms referred to the laboratory in November 2010. He was a bank staff and his mission was in Damghan, Semnan Province located in the center of Iran. In Recent10 years Damghan has been reported as an important of ZCL focus in Iran. Upon questioning, the patient described the historical appearance in the past six to eight months (April 2009) with a number cutaneous lesions located on his legs. After a while, various lesions disseminated almost entire legs. The initial lesions became ulcerated. Clinical examination showed that the patient was good in general condition. In examination most of cutaneous lesions, looks like papulonodular reddish-brown and were infiltrated without any typical form. In this case the patient was systemically treated by meglumine antimoniate (Glucantime®) for at least 4 treatment courses. Each treatment courses were at least 3 weeks. Scars appeared and remained 6 months after the last injection (Figure 2).

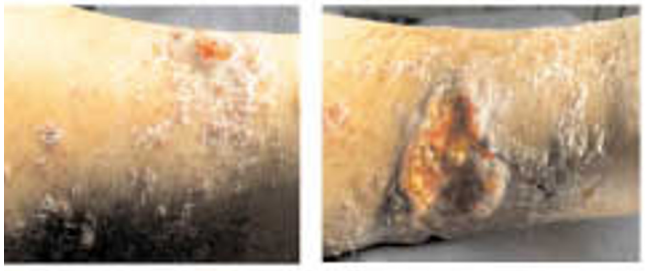

Figure 2. Patient 2 from Damghan in central part of Iran (2010).

Patient 3

The third case was a 46 year-old man whose job was a worker. He lived in Bojnoord city, northern Khorassan Province-in the northeast of Iran. He was referred to a medical center in 2009. He was infected by Leishmania parasite in 1987 meanwhile, he was a volunteer fighter during Iran-Iraq war in Fakkeh (one of the ZCL focuses located in the Iran-Iraq boundary). He was also exposed to the side-effects of a chemical bomb during the war. After being infected to CL, lesions expanded all over his hand, probably because of the side-effects of the chemical bombs. The lesions also dominated palms and nails which are rare in such cases (Figure 3). He received 4 courses of 20 daily of systemically treatment with Glucantime, but no favorable responses were observed after 12 years because the ulcers were active with numerous amastigote forms of *Leishmania* sp.

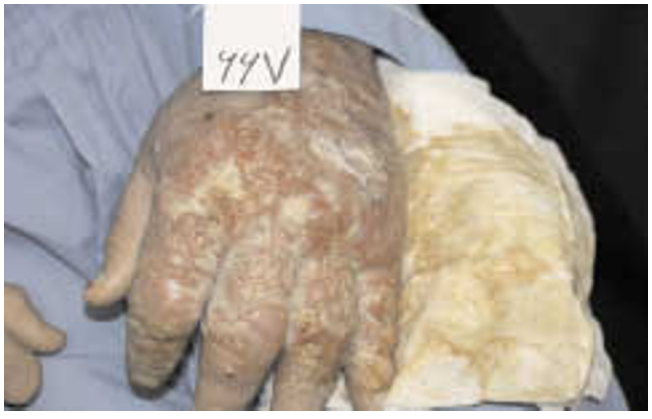

Figure 3. Patient 3 from Bojnoord, in the northeast of Iran (2009).

Patient 4

A 40 year-old woman, housewife, presented with a One year history of skin lesions all over her body especially on her arms from 2003. She was infected in Esfahan, another important ZCL focus in the central of Iran. The patient described the appearance of multiple lesions on her body including hands, chest, and backs. The patient received multiple courses of systematic treatment with Glucantime® but not only the lesions show any healing but also they were

extended to new site of her bodies.

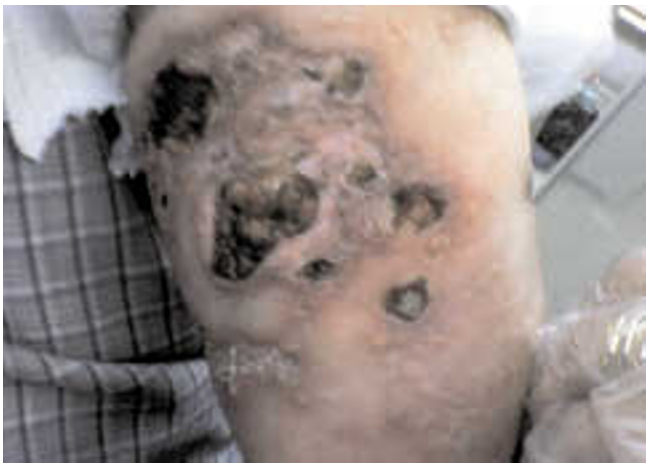

Figure 4. Patient 4 from Esfahan, centeral part of Iran(2003).

2.2. Laboratory presentation

Smears were prepared from scrapings of the popular or nodule lesions on different parts of bodies. Smears prepared from fluid materials of some of the skin lesions, stained with Giemsa 10%(Labtron Co, Iran)and demonstrated numerous *Leishmania* amastigote forms of *Leishmania* spp by light microscope with high magnification (1 000×) in all of the patients. Also in aseptically condition, skin lesion materials were cultured in special medium such as RPMI1640 (Gibco) plus 10% FBS (Gibco)[8]. Promastigote forms appeared after about 1 to 2 weeks post inoculation but unfortunately in third cases, the bacterial contamination was very high and we missed this isolate. In cases 1, 2 and 4, DNA was extracted from the cultured promastigotes and in third case from the prepared direct stained slides[9]. To identify the causative agent of the diseases in above mentioned cases PCR-RFLP was performed. DNA extraction was conducted using the kit (Roche, Germany) .The ITS1 region amplification was performed with 35 cycles, each of 30″ at 94 ℃, 30 ″at 49 ℃, and 45″ at 72 ℃ in a thermo cycler (Peqlab) using the primers LITSR (5′-CTGGATCATTTTCCGATG-3′) and L5.8S (5′-TGATACCACTTATCGCACTT- 3′) . The PCR products were digested with HAEIII or BSUR1 (Fermentas, Germany) as the fast digestion restriction enzyme. Digestion products were separated by 3% agarose gels in TAE buffer and visualized after staining by ethidium bromide[8,10].

Reference stocks: After electrophoresis the PCR pattern of samples was evaluated with the pattern of three Iranian *Leishmania* stock species submitted to the GenBank database with accession numbers, EF653267 for *L. tropica*, *L. major* EF653269, and *L. infantum* EF653268.The PCR-RFLP identified the species of all cases as *L. major*.

The PCR product of two first patients were sequenced using the forward primer according to the manufacturer′s instructions (Bioneer, Korea). The two sequences documented as Accession numbers JN860713 and JN860714.

3. Discussion

Leishmaniasis includes a wide range of clinical signs. From cutaneous leishmaniasis to mucocutaneous, disseminated form to lethal visceral leishmaniasis form[11]. According to the taxonomical studies, different *Leishmania* complex and species are responsible for such various aspects of the disease[12,13]. However beside *Leishmania* various species, reservoirs, vectors, and ecological focus conditions play an important role. According to A.L. Bañuls, *Leishmania* parasites have clonal division. This kind of division and some factors such as geographical barriers and distance hinder genetic mixing take part in hybrid species production[14,15]. In recent decades there have been incredible advances in molecular studies either in diagnosis and taxonomical aspects. These advances enabled us to observe new complex cases. There have been some reported cases of CL by *Leishmania* infantum[16], VL by both *L. tropica*[4,7] and *L. major*[17] and also some DCL cases caused by *L. tropica*[18,19]. Some of these reports pointed out immunology disorders in some cases. But none of the cases reported in this current study had any immunosuppressive in their medical documents and probably we should not underestimate the potential genomic characteristics of *Leishmania* parasites. In recent years multiplicity of internal and external trips and migrations from endemic to non-endemic areas and vice versa, and such factors has leaded to a change in distribution of the parasite[20,21]. This could lead to the production of new hybrids and pathogenicity signs[22,23]. It seems that the number of such cases is increasing. According to these kinds of evidences and achieved reports on different clinical signs which is caused by mutation in Leishamania parasite pathogenicity, there must be an emphasize on molecular epidemiology in endemic areas. We suggest DNA based molecular methods for *Leishmania* identification. The true identification of the causative agent of CL using the molecular methods is essential for treatment, control and prevention of the disease.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Desjeux P. The increase in risk factors for leishmaniasis worldwide. *Trans R Soc Trop Med Hyg* 2001; **95**: 239-243.

[2] Shirzadi MR. Neglected Tropical Diseases Innovative and Intensified Disease Management Leishmaniasis Control. WHO Headquarters report of the consultative meeting on cutaneous leishmaniasis. 2007-April.

[3] Asadi-Kani Z, Qeisari M, Azizaddini SH, Taheri A, Sarlak M. Unusual presentations of cutaneous leishmaniasis in two Iranian patients. *Iranian J Dermatol* 2011; **13**(3).

[4] Alborzi A, Rasouli M, Shamsizadeh A. *Leishmania tropica*-isolated patient with visceral leishmaniasis in southern Iran. *Am J Trop Med Hyg* 2006; **74**(2): 306-307.

[5] Hajjaran H, Mohebali M, Zarei Z, Edrissian GhH. *L. tropica:* Another etiological agent of canine visceral leishmaniasis in Iran. *Iranian J Publ Health* 2007; **36**(1): 85-88.

[6] Alvar J, Canavate C, Gutierrez-Solar B, Jimenez M, Laguna F, Lopez-Velez R, et al. *Leishmania* and human immunodeficiency virus coinfection: the first 10 years. *Clin Microbiol Rev* 1997; **10**(2): 298-319.

[7] Jafari S, Hajiabdolbaghi M, Mohebali M, Hajjaran H, Hashemian H. Disseminated leishmaniasis caused by *Leishmania tropica* in HIV-positive patients in the Islamic Republic of Iran. *East Mediterr Health J* 2010; **16**(3): 340-343.

[8] Hajjaran H, Vasigheh F, Mohebali M, Rezaei S, Mamishi S, Charedar S. Direct diagnosis of *Leishmania* species on serosity materials punctured from cutaneous leishmaniasis patients using PCR-RFLP. *J Clin Lab Anal* 2011; **25**(1): 20-24.

[9] Kazemi-Rad E, Mohebali M, Rezaei S, Mamishi S. Diagnosis and characterization of *Leishmania* species in Giemsa stained slides by PCR-RFLP. *Iranian J Publ Health* 2008; **37**: 54-60.

[10] Schonian G, Nasereddin A, Dinse N, Schweynoch C, Schallig HD, Presber W, et al. PCR diagnosis and characterization of *Leishmania* in local and imported clinical samples. *Diagn Microbiol Infect Dis* 2003; **47**(1): 349-358.

[11] Postigo JA. Leishmaniasis in the World Health Organization Eastern Mediterranean Region. *Int J Antimicrob Agents* 2010; **36**(Suppl 1): S62-S65.

[12] Joseph SC. A comprehensive clinical evaluation of 20,000 Persian Gulf War veterans. Comprehensive Clinical Evaluation Program Evaluation Team. *Mil Med* 1997; **162**(3): 149-155.

[13] Iowa State University. *Leishmaniasis* [Online]. Available from: http://www.cfsph.iastate.edu/Factsheets/pdfs/leishmaniasis.pdf. [Accessed on Oct, 2009]

[14] Schonian G, Kuhls K, Mauricio IL. Molecular approaches for a better understanding of the epidemiology and population genetics of Leishmania. *Parasitology* 2010; **138**(4): 405-425.

[15] Bañuls AL, Hide MPF. *Leishmania* and the leishmaniases: a parasite genetic update and advances in taxonomy, epidemiology and pathogenicity in humans. *Adv Parasitol* 2007; **64**(1): 1-109.

[16] Santos-Oliveira JR, Da-Cruz AM, Pires LH, Cupolillo E, Kuhls K, Giacoia-Gripp CB, et al. Atypical lesions as a sign of cutaneous dissemination of visceral leishmaniasis in a human immunodeficiency virus-positive patient simultaneously infected by two viscerotropic *Leishmania* species. *Am J Trop Med Hyg* 2011; **85**(1): 55-59.

[17] Ensembl Protists. *Leishmania major*. [Online]. Available from:http://protists.ensembl.org/Leishmania_major/Info/Index. [Accessed on Oct 16th, 2012].

[18] Kimutai A, Kamau Ngure P KTW, Muita Gicheru M, Bonareri Nyamwamu L. Leishmaniasis in northern and western Africa: A review. *Afr J Infect Dis* 2009; **3**(1): 14-25.

[19] Dahroug MAA, Almeida ABPF, Sousa VRF, Dutra V, Guimarães LD, Soares CE, et al. The first case report of *Leishmania* (leishmania) chagasi in Panthera leo in Brazil. *Asian Pac J Trop Biomed* 2011; **1**(3): 249-250.

[20] Kassiri H, Sharifinia N, Jalilian M, Shemshad K. Epidemiological aspects of cutaneous leishmaniasis in Ilam province, west of Iran (2000-2007). *Asian Pac J Trop Dis* 2012; **2**(Suppl 1): S382-S386.

[21] Zemanova E, Jirku M, Mauricio IL, Miles MA, Lukes J. Genetic polymorphism within the *Leishmania donovani* complex: correlation with geographic origin. *Am J Trop Med Hyg* 2004; **70**(6): 613-617.

[22] Shiee MR, Hajjaran H, Mohebali M, Doroodgar A, Saadat MH, Teimouri A, et al. A molecular and parasitological survey on cutaneous leishmaniasis patients from historical city of Kashan in Isfahan province, center of Iran. *Asian Pac J Trop Dis* 2012; **2**(6): 421-425.

[23] Ravel C, Cortes S, Pratlong F, Morio F, Dedet JP, Campino L. First report of genetic hybrids between two very divergent *Leishmania* species: *Leishmania infantum* and *Leishmania major*. *Int J Parasitol* 2006; **36**(13): 1383-1388.