

**Research Article****Value of polymerase chain reaction in comparison with direct microscopy for diagnosis of cutaneous leishmaniasis.****Mohammad Hossein Lohraseb<sup>1</sup>, Fatemeh Khajeh<sup>2</sup>**<sup>1</sup>Assistant Professor, Dept. of dermatology, Fasa University of Medical Sciences, Fasa, Fars, Iran<sup>2</sup>Assistant Professor, Dept. of Pathology, Fasa University of Medical Sciences, Fasa, Fars, Iran**\*Corresponding author**

Fatemeh Khajeh

**Email:** [Mojgankaje@gmail.com](mailto:Mojgankaje@gmail.com)

---

**Abstract:** Cutaneous leishmaniasis (CL) is a major health problem in many countries. Traditional diagnostic tests such as direct microscopy are not always conclusive. purpose of this study is to compare the sensitivity and specificity of these two important diagnostic test: polymerase chain reaction (PCR) assay and direct microscopy for diagnosis of CL. As a prospective study, results of Giemsa stained smear of 100 clinically suspected case of CL, compared with polymerase chain reaction method by use of parasite-specific kinetoplastic DNA sequence. The Results of The sensitivity for CL is 89% in direct microscopy while sensitivity in PCR assay was 97%. In this study the cause of majority of CL (69.69%), was *Leishmania major* by nested PCR method. In Conclusion although the sensitivity of PCR assay for CL is significantly higher in comparison with direct microscopy, but direct microscopy is a valuable diagnostic test for diagnosis CL in our endemic region which may need to be evaluated in negative direct smear but clinically highly suspected patient by PCR.**Keywords:** Cutaneous Leishmaniasis, Direct smear, *Leishmania infantum*, *leishmania major*, PCR.

---

**INTRODUCTION**

Leishmaniasis is the most widely disseminated protozoan infection in the world [1]. Cutaneous leishmaniasis (CL) which is endemic in Iran caused by *leishmania major* [2 – 6]. In Iran the disease is seen in both rural and urban areas. The leishmania parasite is a protozoan organism that transmitted to human by bite of female sandfly of the genera *phlebotomomus* or *Lutzomyia* [3, 7]. Most form of leishmania parasite is zoonotic with the reservoir in rodent. CL is still considered an important health problem in many parts of the world, especially the Mediterranean region, some countries of Africa, and almost all countries of the Middle East, including Iran, CL is a major health problem in endemic areas such as Fars province in Iran. The sand fly bite occurs in all seasons but mostly in summer and the skin manifestation usually appear in the autumn.

Due to traditional wearing in Iran most Cases of CL are seen on face, hand, arms and sometimes on feet.

In ancient Persian books the disease called “Salak” which means about one year and points toward long disease duration. The most commonly used method for diagnosis of CL is direct microscopy of Giemsa stained smear which taken from skin lesion.

The aim of our study is to compare traditional method, direct microscopy of Giemsa stained smear with PCR, as a newer diagnostic method for CL.

**MATERIALS AND METHODS**

One hundred patients with clinically diagnosis of CL who have been referred to Fasa Hamze clinic affiliated to Fasa Medical University(Autumnr 2014) have completed the study. Common diagnosis of CL include, scraping from margins of skin lesion ,methanol fixation, Giemsa staining of the smears and then carefully examined for amastigotes using light microscopy ( magnification X 1000), [9]. The name of patients, age, site of lesion, address and positive or negative microscopic result were recorded.

Then the stained smears used as samples for PCR because these smears easily stored, carried and yield efficient DNA extraction on the basis of previous experiments [10,11]. Extracted DNA, was added to a reaction mixture containing 1.5 μM of MgCl<sub>2</sub> ,200 μM of tag polymerase buffer, 1.5 unit of taq DNA polymerase and 40 pmol of each primer; the primer set characterize as below 110bp. (forward:5-GTGGGGGAGGGGCGTTCT-3) (reverse:5-ATTTTACACCAACCCCGATT-3)

The mixture was amplified in a programmable master cycler gradient eppendorf (thermo cycler )for 5 min at 94°C(1cycle) followed by 30 cycles at 94°C for 30 seconds, 52°C for 30 seconds and 72°C for 5 min(1 cycle) and kept at 4°C.

Then nested PCR performed on this product. The primer sets for nested PCR characterize as below:

CSB1-F:  
5CGAGTAGCAGAACTCCCGTTCA  
CSB1-R:  
5ATTTTTCGCGATTTTCGCAGAACG  
Li-R: 5-TCGCAGAACGCC CCT  
BZ.F: 5-ACTGGGGGTTGGTGTAAAATA

A 10µl sample of final PCR product was subjected to electrophoresis in 1.5% agarose gel. 5 µl of loading buffer was added to the product before electrophoresis and it was visualized under UV light with Ethidium bromide.

The PCR primers were designed for the identification and differentiation of Leishmania, L.Tropica, L.Infantum and L.Major species, and PCR bands at 750 BP, 680 BP and 560 BP of the parasite-specific kinetoplastic DNA sequences was identified for L Major, L Infantum and L Tropica, respectively.

**Statistical analysis**

The results of two diagnostic tests were analyzed by using Mc-Nemar test.

**RESULTS**

In 100 patients, 89 samples were positive microscopically; 97 samples (97%) were diagnosed positive by PCR for leishmaniasis. Of the 97 positive PCR, 69 cases showed banding pattern of leishmania major (69.69%) ,and 28 cases showed leishmania infantum (28.28%). Of 100 clinically diagnosed CL, 39 cases were more than 50 years old and the residual, 61 cases were younger.

**Table-1: The percentage of L .major, L .infantum and negative cases in 100 clinically diagnosed CL .**

	L .infantum	L. major	Negative	Total
< 50 years old	20(33.3%)	38(63.3%)	2(3.3%)	60
> 50 years old	8(20.5%)	31(79.4%)	0(0%)	39
Total	28(28.28%)	69(69.69%)	2(2.02%)	99

**DISCUSSION**

CL is a serious problem plaguing more than 90 countries [8, 18], but, currently more and more people are undertaking adventurous journeys in areas where CL is endemic [5], that increasing physicians encounter with imported tropical diseases presentation [17], so most of the tropical diseases are global problems anyhow.

Nowadays, clinically diagnosed CL by dermatologist needs to be confirmed by laboratory test for physician and patient due to epidemiologic studies, long duration of disease and unpleasant nature of skin lesion. In Iran, although the disease may involves anyone but often has involved children especially in rural region, so the optimal diagnostic test should be cost effective, rapid and preferably performs with once sampling.

Traditionally direct microscopy examination of skin lesions’ margin scraping, which stained by Giemsa method carried out for CL diagnosis; this test can be done in a half an hour; but developments of new diagnostic tests, and some reports of more sensitivity and specificity with newer methods necessitate to compare them with traditional methods.

We evaluated direct microscopic examination in compare with PCR for diagnosis of CL. In addition to above comparison, we characterize leishmania species in positive PCR samples (19 - 21). We have 100 clinically suspected CL patients. The direct microscopic results show 89 positive samples

(89%). one sample with positive smear result was negative by PCR method. Of 97(97%) positive PCR samples 69 (69.69%) and 28 (28.28%) was identified as leishmania Major and Infantum species respectively.

Characterization of leishmania species is important, because different species may require distinct treatment regimen [21, 23]. Furthermore, such information also valuable in epidemiologic studies; since the distribution of leishmania species in human and animal host, as well as in insect vectors, is prerequisite for designing appropriate control measures. In our endemic area of study , Fars province ,Fasa ,L. Major is more prevalent causative species of CL. Based on our studying there is significant difference between direct microscopic examinations of Giemsa stained smear from skin lesions’ margin scraping and PCR method. But in rural and endemic region direct microscopy has adequate sensitivity and specificity that allow to be used as a routine, available practical test in the first line of laboratory diagnostic test. The false positive and negative test in direct microscopy is pitfall of this method which is related to platelet resemblance to leishman body especially in smear with low count of parasite or because of screening by amateur screener.

Although in previous studies has emphasis the high sensitivity and specificity of PCR for leishmania but as overall Giemsa stained smear is more cost effective and rapid in our endemic area, which may be due to high experience of microscopic examiner in endemic areas. Still PCR is used as a gold standard method that allows a precise diagnosis in lesions of

patients that harbor few parasites or in non-endemic region with low experience of microscopic examiner [15].

#### Acknowledgment

We thanks Dr Ahmad Monabati, MD ; Pathologist and associate professor of Shiraz Medical university, Dr Alireza Tavassoli MD ;Pathologist and assistant professor of Fasa Medical university & Miss Behnaz Valibeigi for their technical advice and cooperation .

#### REFERENCES

1. Achado Pinto J, Bravo F, Tying SK; Tropical dermatology. Tropical diseases caused by protozoa. J Am Acad Dermatol. 2009; 60(6): 897-925.
2. Alimohammadian MH, Almasi H, Khabiri AR, Hatam G, Amirkhani A, Lupi O *et al.*; .Identification of species and characteristics of an outbreak of cutaneous leishmaniasis in a new focus of Iran. Iran Biomed J. 1999;3:31–39.
3. Momeni A; Clinical picture of cutaneous leishmaniasis in Isfahan, Iran. Int J Dermatol. 1994; 33: 260–265.
4. Ozbel Y, Turgay N, Ozensoy S, Ozbilgin A, Alkan MZ; Epidemiology, diagnosis and control of leishmaniasis in the Mediterranean region. Ann Trop Med Parasitol. 1995; 89: 89–93.
5. Khoury S, Saliba EK, Oumeish OY, Tawfig MR; Epidemiology of cutaneous leishmaniasis in Jordan. Int J Dermatol. 1996; 35: 566–559.
6. Motazedian H, Karamian M, Noyes HA, Ardehali S; DNA extraction and amplification of leishmania from archived, Giemsa-stained slides, for the diagnosis of cutaneous Leishmaniasis by PCR. Ann Trop Med Parasitol .2002; 96: 31-34.
7. García AL, Parrado R, Rojas E, Delgado R, Dujardin JC, Reithinger R; Leishmaniasis in Bolivia. comprehensive review and current status. Am J Trop Med Hug. 2009; 80(5): 704-11.
8. Nadir A, Seedy RMA; A brief review of the epidemiology of various types of leishmaniasis in Iran. Acta Med Iran. 1971; 8: 99–106.
9. Schönian G, Nasereddin A, Dinse N, Schweynoh C, Schalling HD, Presbe W, Jaffe C; PCR diagnosis and characterization of Leishmania in local and imported clinical samples. Diag Microbial Infect,2003; 47(1): 349-58.
10. Safar Ali Talari, Rezvan Talaei, Gholamreza Shajari, Zarichehr Vakili, Abbas Taghaviardakani; Childhood cutaneous leishmaniasis: report of 117 cases from Iran. Korean J Parasitol. 2006; 44(4): 355–360.
11. Moaddeb A, Gettner S, Ardehali S; Studies on the causative agent of cutaneous leishmaniasis in Shiraz Iran. Iran J Med Sci. 1993; 18: 28–33.
12. Bhutto AM, Soomro FR, Baloch JH, Matsumoto J, Uezato H, Hashiguchi Y *et al.*; Cutaneous leishmaniasis caused by Leishmania (L.) major infection in Sindh province, Pakistan. Acta Trop. 2009.
13. William R. Faber, Linda Oskam, Tom van Gool, Nel C.M. Kroong, Kristine J. Knecht-Junk, Henk Hofwegenf, *et al.*; van der Wal, Piet A. Kager . value of diagnostic techniques for cutaneous leishmaniasis. Journal of the American Academy of Dermatology. 2003; 49( 1): 70-74.
14. Alon Scope, Henry Trau, Gerlind Anders, Aviv Barzilai, Yitzhak Confino, Eli Schwartz; Experience with New World cutaneous leishmaniasis in travelers; Tropical Medicine & Hygiene. 2003; 49(4), 672-678.
15. Herwaldt BL; leishmaniasis. Lancet, 1999; 354: 1191-9.
16. Goldsmith MF; health woes grow in shrinking world. JAMA. 1998; 279: 569.
17. Blum J, desjeux P , Schwartz E, Beck B, Hartz C; Treatment of coetaneous leishmaniasis among travelers. J Antimicrobial Chemotherapy, 2004; 53: 158-66.
18. Al-Jawabreh A, Schönian G, Hamarsheh O, Presber W; Clinical diagnosis of cutaneous Leishmaniasis: A comparison study between standardized graded direct microscopy and ITS1-PCR of Giemsa-stained smears. Acta Trop, 2006; 99(1): 55-61.
19. Al-Jawabreh A, Schnur LF, Nasereddin A, Schwenkenbecher JM, Abdeen Z, Barghuthy F *et al.*; The recent emergence of Leishmania tropica in Jericho (A'riha) and its environs, a classical focus of L. major. Trop Med Int Health, 2004; 9(7): 812-16.
20. E Kazemi-Rad, M Mohebali, H Hajjar, S Rezaei, S.Mamishi; Diagnosis and Characterization of Leishmania Species in Giemsa-Stained slides by PCR-RFLP. Iranian J Publ Health, 2008; 37(1),54-60.
21. El Tai NO, Osam OF, Elfari M, Presber W, Schönian G; Genetic heterogeneity of ribosomal internal transcribed spacer in clinical samples of Leishmania donovani spotted on filter paper as revealed by sing le- strand conformation polymorphisms and sequencing. Trans R Trop Med Hyg,2000; 94(5): 575-79.
22. Berzunza-Cruz M, Bricaire G, Salaiza Suazo N, Pérez-Montfort R, Becker I; PCR for identification of species causing American cutaneous leishmaniasis. Parasitol Res. 2009;104(3): 691-9.
23. Kofler H, Wambacher-Gasser B, Topar G, Weinlich G, Schuler G, Hintner H, *et al.*; Intravenous immunoglobulin treatment in therapy-resistant epidermolysis bullosa acquisita. J Am Acad Dermatology 1997; 36: 331-5.