International Archives of Medical Research
Volume 6, No. 2, pp.17-20, 2014

ORIGINAL RESEARCH PAPER

Identification of causative species in patients with cuteneous leishmaniasis in Diyarbakır by polymerase chain reaction (pcr)-restriction fragment lenght polymorphism (RFLP)

Nezahat Akpolat [1], İbrahim Halil Yıldırım[2] , Mutalip Çiçek[1], Şebnem Nergiz[1], Özgür Ezin[1], Nida Özcan[1], Mehmet Harman[3]

[1] *Medical Microbiology Department, Medical Faculty of Dicle University, 21280 Diyarbakir/Turkey*
[2] *Genetic Department, Veterinary Medicine Faculty of Dicle University, 21280 Diyarbakir/Turkey*
[3] *Dermatology Department, Medical Faculty of Dicle University, 21280 Diyarbakir/Turkey*

Abstract

Background: *Leishmaniasis is a protozoan disease caused by more than twenty Leishmania species which are transmitted by infected phlebotomine sandflies to humans. Leishmaniasis include multiple clinical syndromes such as visceral , mucosal and cutaneous forms. Cutaneous Leishmaniasis (CL) is the most common and endemic form in southeastern Turkey.*

Purpose: *CL can be caused by L. major, L. tropica, L. mexicana and L. amazonensis. Microscopy remains to be the standard diagnostic method because of its cheapness, ease of application and high specificity . Microscopic examination of smears, parasite culture and serological tests are performed for diagnosis. Recently several molecular methods, especially those based on the Polymerase chain reaction (PCR) have been developed for identification of Leishmania species.*

Method: *In this study, 150 smear samples taken from patients with clinical findings of CL were studied by PCR- RFLP (restriction fragment length polymorphism)*

Results: *Leishmania tropica was detected in 128 smear sample. L. tropica was found to be the most common species causing CL in southeastern Turkey.*

Conclusion: *Identification of Leishmania species is important because different types may require different treatments. Determining the common species in the region may lead to develop treatment protocols.*

Keywords: *Cutaneous Leishmaniasis, Polymerase Chain Reaction (PCR),Restriction Fragment Lenght Polymorphısm (RFLP).*

* Corresponding author: E-mail:nakpolat21@gmail.com, Tel.: +90 412 2488001 (ext. 4096), Fax: +90 412 2488440

Introduction

The Leishmania genus is morphologically similar protozoae. They cause cutaneous, mucocutaneous and visceral Leishmaniasis in human. Leishmaniasis is the third most important vector-borne parasitic disease in the world which is seen in tropical and subtropical regions of especially underdeveloped or developing countries. The World Health Organization (WHO) determined the incidence of the disease as 12 million, while 350 million people were reported to be at risk (1,2,3). Several studies have shown that for CL is endemic in South-eastern Turkey, particularly in Diyarbakır, Şanlıurfa and Adana (4,5,6,7,8). CL can mimic many skin diseases such as acne, boils, eczema, lupus erythematosus, lupus vulgaris, tuberculosis verrucosus, syphilis, leprosy, wounds, psoriasis, and skin tumors. Therefore, the clinical findings are not enough in the diagnosis of CL. Laboratory tests should be performed for a definite diagnosis (3). Microscopy remains to be the standard diagnostic method because because of its cheapness, ease of application and high specificity . Microscopical demonstration of Leishmania amastigotes in Giemsa-stained lesion biopsy smears is the gold standart for CL diagnosis(9). Due to the morphological similarity of Leishmania species, microscopic examination can only identify Leishmania up to genus level. Culture examination characterize the organisms up to species level but it is time-consuming. Furthermore, contamination remains a big handicap for Leishmania cultures. Since the number of circulating antibodies against CL agents tends to be low, serological tests are rarely used in CL diagnosis. Recently several molecular methods have been developed for identification of Leishmania species. Multiplex PCR, random amplified polymorphic DNA (RAPD), single-strand conformational polymorphism, restriction fragment length polymorphism (RFLP) and DNA sequencing are some of molecular methods used to identify the types of Leishmania(4,,10,11,12). Polimerised chain reaction (PCR) based assays currently constitute the main molecular diagnostic approach for leishmaniasis. PCR-RFLP (restriction fragment length polymorphism analysis) is a widely used conventional PCR assay in leishmaniasis. After cleavage with restriction enzymes, PCR amplicons are resolved by electrophoresis and visualized after ethidium bromide staining (13). In this study, PCR-RFLP method was performed to identify CL agents up to species level.

Materials and Methods

A total of 150 biopsies were performed from CL suspected patients by physicians according to ethical protocols, including obtaining patient consent and approval from Dicle University Medical Faculty Ethics Committee For Noninterventional Studies.

The suspected lesion was cleaned gently with 70% ethanol. After properly drying, the base and margins of lesion were scraped gently with a sterile scalpel blade. At least two slides were prepared with these samples. One was remained unstained while others were fixed with methanol and stained with Giemsa. The Giemsa stained slides were examined under oil immersion to look for amastigotes with nucleus and the rod-shaped kinetoplast. The unstained slides were stored at 0˚C until PCR run.

The materials were scraped gently from the slides with a sterile scalpel blade and transferred to sterile eppendorf tubes. Proteinase K, sodium chloride and Tris-EDTA were added to eppendorf tubes for the lysis process of parasite cell membrane. DNA was obtained after removal of the cell membrane debris by phenol-chloroform and precipitation with pure ethanol. Leishmania kinetoplast DNA primers- 13A(5'- TTG ACC CCC AAC CAC ATT ATA- 3') and 13B (Alpha DNA)(5'- GTG GGG GAG GGG CGT TCT -3')- were used to identify the genus level. Fme-Rme primers -(Alpha DNA) (5'- ACA GAA ACT GAT ACT TAT ATA GCG -3', 5'- TAT TGG TAT GCG AAA

CTT CCG -3')- belonging to mini-exon gene region which is more spesific than the kinetoplast DNA were used for identification up to species level .

Result

Smear samples were performed from 300 patients suspected as CL. The Giemsa stained slides were examined under oil immersion. The unstained slides of 150 sample were determined by PCR-RFLP. Among 150 samples, Leishmania DNA was detected in 128 samples. All of 128 samples have been identified as L. tropica.

Discussion

Cutaneous Leishmaniasis is one of the major public health problems in the world especially in tropical and subtropical regions of underdeveloped or developing countries. South-east of Turkey, particularly Diyarbakır, Şanlıurfa and Adana are endemic areas for CL (14). Zeyrek et al (15) stated the serological methods as not efficient in CL diagnosis. Dilmeç et al (16) investigated the genotypic differences between Leishmania strains isolated from Adana and Şanlıurfa by PCR and stated that the strains were genotypically different. Koltaş S. (17) determined Leishmania species by PCR-RFLP in a thesis study and stated the method as suitable in identifying. In addition, he had detected L. infantum in CL patients without visseral leishmaniasis. In a survey study with Ertem M. et al(6) , 443 persons from two villages around Diyarbakir were examined dermatologically. Scar or lesion was detected from 101 (22.79%) of them and smears were performed from 78 CL suspected ones. Leishmania amastigotes had been observed in 20 (25.6%) Giemsa stained slides. Zeyrek et al. had detected three L. major as a CL agent in their recent study (18).
Identification of Leishmania species is still important because different types may require different treatments. Determining the common species in the region may help the clinician to develop treatment protocols.

Acknowledgements

This research was supported by the Dicle University Scientific Research Projects Coordination Office (DUBAP) .

References

1. Reithinger R, Dujardin JC., Molecular Diagnosis of Leishmaniasis: Current Status and Future Applications. Journal of Clinical Microbiology 2007: 21-25
2. World Health Organization web sayfası (http://www.who.int/tdr/diseases/Leish,Progress 2003-2004,17thProgrammeReport) Erişim Tarihi: 05.12.2007
3. Özbel Y, Töz ÖS, Leishmaniosis, Özcel MA. Tıbbi Parazit Hastalıkları 1. Baskı, İzmir: Mete Basım Matbaacılık Hizmetleri, 2007: 198-230
4.Gangneux JP, Menotti J, Lorenzo F, Sarfati C, Blanche H, Bui H, Pratlong F, Garin YJF, Derouin F. Prospective Value of PCR Amplification and Seqencing for Diagnosis and Typing of Old World Leishmania Infections in an Area of Nonendemicity.Journal of Clinacal Microbiology.2003:1419-1422.
5.Gürel MS, Ulukanlıgil M, Özbilge H, 2002. Cutaneous Leishmaniasis in Sanliurfa: epidemiologic and clinical features of the last four years (1997-2000). Int J Dermatol, 41: 1-6.
6.Ertem M, Aytekin S, Acemoğlu H, Akpolat N. Aytekin N. Diyarbakır Dicle İlçesi Dedeköy ve Durabeyli'de Kutanöz Leishmaniasis Olgularının İncelenmesi.Türkiye Parazitoloji Dergisi. 2004 28 (2): 65-68
7.Uzun S, Yücel A, Özpoyraz M.Cutaneous Leishmaniasis: Evalution of 3074 cases in the Çukurova Region,Turkey. Tibbi Parasitoloji Dergisi. 1997:21:142.
8.ÖzpoyrazM, Memişoğlu HR.Kutanöz Leishmaniasis Türkiyedeki durumu.Arşiv 1996:5:1-5.

9.Bensoussan, E., A. Nasereddin, F. Jonas, L. F. Schnur, and C. L. Jaffe. 2006. Comparison of PCR assays for diagnosis of cutaneous leishmaniasis. J. Clin. Microbiol. 44:1435-1439. [PMC free article][PubMed]

10. Weigle KA, Valderlama L, Arias AL, Santrica C, Saravia NG. Leishmnanin standardization and evalution af safety,dose,storage,longevity of reaction and sensitization. Am Journal Tropica Medical Hyeneg. 1991:44:260-271.

11. Piarroux R, Gambarelli F, Duman H, Fontes M, Dunan S, Mary C, Toga B, Qulici M. Comparison of PCR with Direct Examination of Bone Marrow Aspiration,Myeloulture and Serology for Diagnosis of Visceral Leishmniasis in Immunocompromised Patients. Journal of Clincal Microbiology.1994:746-749.

12. Piarroux R, Gambarelli F, Duman H, Fontes M, Dunan S, Mary C, Toga B, Qulici M. Comparison of PCR with Direct Examination of Bone Marrow Aspiration,Myeloulture and Serology for Diagnosis of Visceral Leishmniasis in Immunocompromised Patients. Journal of Clincal Microbiology.1994:746-749.

13. Demirel R, Erdoğan S. Determination of high risk regions of cutaneous leishmaniasis in Turkey using spatial analysis. Turkiye Parazitol Derg. 2009;33(1):8-14.

14. Singh,S., A.Dey, and R. Sivakumar. 2005. Applications of molecular methods for Leishmaniacontrol. Exp. Rev. Mol. Diagn. 5:251-265**. [**PubMed].

15. Zeyrek F, Korkmaz M, Ozbel Y. Serodiagnosis of anthroponotic cutaneous leishmaniasis (ACL) caused by Leishmania tropica in Sanliurfa Province, Turkey, where ACL Is highly endemic. Clin Vaccine Immunol 2007 Nov 29;14(11):1409-15. Epub 2007 Aug 29

16. Dilmeç F, Matur A, Uzun S, Karakaş M, Memişoğlu HR. Çukurova ve Şanlıurfa Bölgelerinde Deri Lezyonlarından İzole Edilen Leishmania sp. DNA'larının Restriksiyon Endonükleazlarla Karşılaştırılması. Harran Tıp Fakültesi Dergisi. 2004; 1: 21-27.

17. Kutanöz Leyişmanyozlu Hastalarda Etken Türlerin Pcr-Rflp Yöntemi İle Tanımlanması ,Fadime Eroğlu,yüksek lisans tezi. Danışman: Prof. Dr. İ. Soner Koltaş,ADANA-2008

18. Yıldız Z. F, Gurses G, Uluca1 N, Yentur D N, Toprak Ş, Yeşilova Y, Culha5 G. Turkiye Parazitol Derg .2014; 38: 270-4