Comparison of fine needle aspiration and direct skin smear in the diagnosis of acute cutaneous leishmaniasis: polymerase chain reaction as a reference method

Fakhrozaman Pezeshkpoor, MD [1]
Mohammad Tohidi, MD [1]
Pouran Layegh, MD [1]
Majid Shahabi, PhD [2]

1. *Cutaneous Leishmaniasis Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
2. *Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran*

Corresponding Author:
Pouran Layegh, MD
Cutaneous Leishmaniasis Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
Email: Layeghpo@mums.ac.ir

Conflict of interest: none to declare

Received: 29 Februray 2013
Accepted: 20 June 2013

Background: Accurate diagnosis of cutaneous leishmaniasis would avoid unnecessary treatment and scar formation. Direct smear is the most common method for the diagnosis of this disease but its negative result could not rule out the infection; so, the need for more sensitive methods is obvious. We conducted this study to compare the efficiency of direct skin smear with smears prepared by fine needle aspiration (FNA) using polymerase chain reaction (PCR) as a reference method.

Methods: In this cross-sectional study, which was conducted during two years from May 2008 to May 2010, 33 patients with suspicious acute cutaneous leishmaniasis based on clinical studies were randomly selected. Direct skin smears and FNA smears were taken from each patient and PCR was performed on biopsy samples; the results were then compared.

Results: The rate of positive results in each method was as follows: PCR 81.8%, direct skin smear 60.6% and FNA 42.4%. The sensitivity of the direct skin smear was significantly higher than the FNA method (74.1% versus 51.9%, P< 0.001). There was no lesion with a positive result on FNA and negative results on the direct skin smear and PCR, or a positive result on the direct skin smear and a negative result on PCR.

Conclusion: It is clear that the FNA method is not a favorable method for the diagnosis of acute cutaneous leishmaniasis in comparison with the direct skin smear. Since the number of the leishman bodies in FNA is limited, it is better to use this method as a complementary method along with other methods such as culture.

Keywords: acute cutaneous leishmaniasis, direct skin smear, fine needle aspiration, polymerase chain reaction

Iran J Dermatol 2013; 16: 45-48

INTRODUCTION

Cutaneous leishmaniasis (CL) is an endemic disease in Mashhad, North-East of Iran. Rapid and accurate diagnosis of the disease eliminates the need for unnecessary therapy and prevents scar formation. Unfortunately, direct skin smear, the most common diagnostic method with a maximum sensitivity of 70-75%, cannot detect all positive samples;[1] so, the need for more sensitive, rapid, and inexpensive methods is quite obvious. In a study performed by Elahi et al, 298 patients were analyzed with four diagnostic methods [2]. Their results indicated that the direct skin smear had the highest number of positive results (53.6%), followed by leishmanin cutaneous test (50%), culture (38%), and biopsy (8.6%). Because of its rapidity and simple performance, they believed

that the direct skin smear was still the most preferred method for the diagnosis of CL [3]. On the other hand, polymerase chain reaction (PCR) has a sensitivity of 92-98% but its high cost, lack of standardization, technical problems, and the need for equipped laboratories prevent the use of this method as the gold standard in endemic regions of our country. It is possible to use PCR to confirm equivocal results of leishmaniasis in the future since its specificity is almost 100% [3]. Another diagnostic method is fine needle aspiration cytology (FNA) which provides materials for culture and typing of parasites [1]. However, the sensitivity and specificity of this method has not been determined in our area and the results of previous studies are heterogeneous [4-8]. Therefore, in this study, we aimed to compare FNA with direct skin smear using PCR as the reference method.

PATIENTS AND METHODS

In this cross-sectional study performed between May 2008 and May 2010, thirty three patients who were visited at the dermatology clinics of Qaem and Imam Reza teaching hospitals of Mashhad, Iran, were randomly selected. All patients were tested with FNA, direct skin smear, and PCR as follows:

For the direct skin smear, after the administration of local anesthesia, the ulcer was cleaned from crust and dried with sterile gauze, and its margin and central area was scraped. The FNA material was prepared using 0.1 ml of preservative-free saline injected into the border through the intact skin. The fluid was aspirated while the needle was moved back and forth under the skin.

For both methods of FNA and the direct skin smear, we prepared two slides fixed with methanol and stained by Giemsa. The slides were examined completely before they could be called negative. It was important to see the nucleus and the rod-shaped kinetoplast, which is a mitochondrial structure containing extranuclear DNA, in order to confirm the diagnosis of leishmaniasis.

A 3 mm punch biopsy sample along the active border was obtained and stored at -25°C for PCR. The samples results were collected and the sensitivity and specificity of each method was evaluated based on PCR as the reference method. All patients had skin lesions for no more than one year and had not received any prior anti-leishmania treatment. Children under the age of five were excluded from the study to avoid any possible harm due to multiple sampling. The study was approved by the Research Council Ethics Committee of Mashhad University of Medical Sciences. An informed consent was obtained from each patient or his/her guardian prior to participation in the study.

With a 95% power and a 15% type I error, the sample size was calculated to be 33 patients. Since the sensitivity and specificity of FNA were not known, the same number of patients were tested by this method as well.

Data was analyzed with SPSS software version 16 and Chi-square test with a confidence level of 95%.

RESULTS

Among the 33 samples tested with three different methods, 27 (81.8%) had a positive PCR and 20 (60.6%) exhibited positive results on direct skin smear testing. Only 14 (42.4%) patients were found to be positive in FNA. All cases with a positive FNA result had a positive PCR and/or direct smear. No patient was observed with a positive skin smear and a negative PCR. Table 1 shows the demographic data of the enrolled patients.

According to our findings, the sensitivity of the direct skin smear was significantly higher than FNA (74.1% versus 51.9% respectively, $p< 0.001$). Based on the PCR results, 23 (85.2%) cases were infected with *L. tropica*, 3 (11.1%) with *L.major*, and 1 (3.7%) with *L. infantum* (Table 2). All the infected patients with *L. tropica* had urban leishmaniasis on clinical

Table 1. demographic data of patients

No. of patients	33
Sex	
Male	15
Female	18
Age (Mean ± SD)	30.1±3.16
Type of lesions	
Papuloplaque	19
Nodule	1
Ulcer	13
Duration of lesions (Days)	86±14.7
Location of lesions	
Head & neck	8
Upper extremity	17
Lower extremity	8
Size of lesions (Mean ± SD)	367±109 mm^3

Table 2. Frequency of the lesions clinical features, leishmania species, and results of direct skin smear, FNA and PCR. (+): positive and (-): negative result

Type of lesion	Leishmania Species				PCR results		FNA results		Smear results	
	L. tropica	L. major	L. infantum	Unspecified	+	-	+	-	+	-
Papule	10	1	0	1	11	1	9	3	10	2
Plaque	2	1	0	4	3	4	2	5	3	4
Ulcer	10	1	1	1	12	1	3	10	7	6
Nodule	1	0	0	0	1	0	0	1	0	1

evaluation, whereas the other four patients were affected by rural leishmaniasis with a negative FNA and a positive direct skin smear result. They had acquired their disease in regions other than Mashhad, especially the case with *L.infantum* who was a 32-year-old woman from Shirvan.

Positive direct skin smear results were significantly higher in females in comparison with males (P< 0.027). FNA positive results had a significantly higher prevalence in small ($\leq$ 80 mm^2) lesions than larger ones (P<0.008). Papules and plaques were the most common clinical picture (19 lesions). Although the number of lesions was not quite enough for performing reliable statistical analyses, most positive PCR cases had ulcer (92.3%) whereas the papule was more prominent in the FNA and direct skin smear positive samples (75% and 83.3%, respectively). There was no correlation between the patients' age and lesions' duration with the results of the diagnostic methods.

DISCUSSION

Several studies in which different conventional parasitological methods were evaluated in CL diagnosis showed heterogeneous and sometimes conflicting results [4,5]. Direct skin smear is frequently used as a conventional technique for detecting leishmania parasite in North–East of Iran; where the majority of lesions are due to *L. tropica* and papuloplaque lesions are the most common type of clinical presentation [9,10]. FNA cytology is attracting more attention these days; [11] this method is proved to be a suitable alternative procedure for the diagnosis of CL. It is a simpler, less painful and more cost effective method in comparison to the previous conventional scraping method/biopsy followed by histopathology studies [7,11]. In the current study the sensitivity and specificity of these methods which have not yet been estimated in our area were evaluated, using PCR as a reference method.

To our findings, the sensitivity of direct skin smear is compatible with Maekle et al study (74.1% and 70-75%, respectively) but is much higher than that reported by Mengista et al (37.5%). Moreover, they indicated a lower sensitivity of FNA in comparison to direct skin smear (51.9 % versus 74.1%) which is not consistent with Bari et al results (48.3% versus 33.3%) [1,12,13]. Also the positivity rate of skin smear in our study was higher than similar studies performed in our region by Elahi et al, Javidi et al, and Nozadi et al (60.6% versus 53.6%, 55.4%, and 50.4%, respectively) [2,14,15]. The main explanation for this difference is that we prepared and examined two smears for each sample instead of one. We also used more tissue samples for the smears' preparation. The results of our method is consistent with the previous studies which have shown that the sensitivity of this test is affected by the site from which the scrapings are taken from [16], beside the staining quality and the technician proficiency [17]. Our findings suggest that the FNA method is not a method of choice for the diagnosis of all types of CL at least in our area, except for small papule lesions (size $\leq$ than 80 mm^2) (P<0.008). Although at first glance, it seems that ulcerative lesions of cutaneous leishmaniasis may have a higher parasite load and the FNA results are expected to be more positive, but based on other references the best type of lesions for performing FNA are papules, plaques and nodules which is in agreement with our results [7]. Our proposed hypothesis on the reason that ulcerative lesions have a higher rate of negative results is that it may be due to the leakage of the fluid that has been infiltrated in to the tissue from the crusted lesions' surface which could reduce the probability of desired extracted material for performing FNA testing. However, it should also be taken into consideration that the diagnostic accuracy of skin aspiration is highly dependent on the skill and experience of the

personnel who perform the aspiration, prepare the slides and interpret the results [7].

Taken together, it appears that direct skin smear still remains the method of choice for the diagnosis of cutaneous leishmaniasis in our area. According to our findings patients with a high suspicion of having leishmaniasis whom have a negative FNA, direct skin smear or biopsy result, are highly recommended to be referred to a PCR lab.

Since the main reason for lower sensitivity of the FNA method is the small volume of material and leishman bodies, it looks reasonable to use this method in conjunction with proliferative methods such as PCR or culture as previously proposed by Markle et al [1]. Preparing two slides or more and providing more material is highly suggested in order to increase the sensitivity of direct skin smear. Based on our results, it is clear that the FNA method with the exception of small lesions is not a favorable method for diagnosis of acute cutaneous leishmaniasis in comparison with direct skin smear. Since the numbers of leishman bodies in FNA are small, it is better to use this method as a complementary one beside other methods such as culture.

Acknowledgement

We would like to thank Habib A. Esmaili, PhD; Ahmad R. Taheri, MD; Seyed AA. Shamsian, PhD; Fatemeh Mousavi, MD; Mohammad J. Yazdanpanah, MD; Sorourozaman Famili, MD and Mahnaz Banihashemi, MD for their kind assistance in performing this study. This project was supported by a grant from the Vice Chancellor for Research of Mashhad University of Medical Sciences for the thesis with approval number of 2002.

REFERENCES

1. Markle WH, Makhoul K. Cutaneous leishmaniasis: recognition and treatment. Am Fam Physician 2004;69:1455-60.
2. Vega-Lopez F. Diagnosis of cutaneous Leishmaniasis. Curr Opin In Infect Dis 2003; 16: 97-101.
3. Elahi SR, Fata AM, Berenji F. Comparison between various diagnostic Methods in cutaneous leishmaniasis. Med J Mashhad Univ Med Sci 1995; 38:62-68.
4. Al-Jitawi SA, Farraj SE, Ramahi SA. Conventional scraping versus fine needle aspiration cytology in the diagnosis of cutaneous leishmaniasis. Acta Cytol 1995; 39: 82-4.
5. Navin TR, Arana FE, de Mérida AM, et al. Cutaneous leishmaniasis in Guatemala: comparison of diagnostic methods. Am J Trop Med Hyg 1990;42:36-42.
6. Mashhood AA, Khan IM, Nasir S, et al. Fine needle aspiration cytology versus histopathology in the diagnosis of cutaneous leishmaniasis in Pakistan. J Coll Physicians Surg Pak 2005;15:71-3.
7. Kassi M, Tareen I, Qazi A, Kasi PM. Fine-needle aspiration cytology in the diagnosis of cutaneous leishmaniasis. Ann Saudi Med 2004;24:93-7.
8. Khan I, Yasmin R. Comparison of fine needle aspiration with biopsy in the diagnosis of cutaneous leishmaniasis. J Pak Assoc Dermatol 2007; 17: 154-8.
9. Mahmoodi MR, Mohajery M, Tavakol-Afshari J, et al. Molecular identification of Leishmania species causing cutaneous leishmaniasis in Mashhad, Iran. Jundishapur J Microbiol 2010;3:195-200.
10. Mashayekhi V, Mahmoudi M, Rastin M, et al. Detection of Leishmania DNA in paraffin embedded specimens of chronic lupoid leishmaniasis using polymerase chain reaction. Iran J Dermatol 2012; 15: 69-73.
11. Rajabi P, Jalayer T, Asilian A, Abbas-Zadeh MR. Fine needle aspiration cytology compared with scraping for diagnosis of leishmaniasis. Res Med (Pajouhesh dar Pezeshki) 1999;4:3-6.
12. Mengistu G, Akuffo H, Fehniger TE, et al. Comparison of parasitological and immunological methods in the diagnosis of leishmaniasis in Ethiopia. Trans R Soc Trop Med Hyg 1992; 86:154-7.
13. Bari AU, Azam SH, Ejaz A, Mahmood T. Comparison of various cytodiagnostic tests in the rapid diagnosis of cutaneous leishmaniasis. J Pak Assoc Dermatol 2010; 20: 63-9.
14. Javidi Z, Fata A, Mohajeri M, et al. Study the factors affecting cutaneous leishmaniasis on more than 3250 cases referred to Imam Reza Hospital. Med J Mashhad Univ Med Sci 2001; 44: 111-8.
15. Seyed-Nouzadi M, Fata A, Elahi SR, et al. Investigation of cutaneous leishmaniasis among male students of 2 different regions in Mashhad. Med J Mashhad Univ Med Sci 2001; 44:39-45.
16. Ramírez JR, Agudelo S, Muskus C, et al. Diagnosis of cutaneous leishmaniasis in Colombia: the sampling site within lesions influences the sensitivity of parasitologic diagnosis. J Clin Microbiol 2000; 38:3768-73.
17. Robinson RJ, Agudelo S, Muskus C, et al. The method used to sample ulcers influences the diagnosis of cutaneous leishmaniasis. Trans R Soc Trop Med Hyg 2002; 96 Suppl 1:S169-71.