ORIGINAL REPORT

Comparison of Leishmanin Skin Test and Direct Smear for the Diagnosis of Cutaneous Leishmaniasis

Seyyede Neda Hashemi[1], Mehdi Mohebali[2], Parvin Mansouri[1], Amir Bairami[2], Homa Hajjaran[2], Behnaz Akhoundi[2], and Soorour Charehdar[2]

[1] *Department of Dermatology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

[2] *Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

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Abstract- Cutaneous leishmaniasis (CL) is an endemic disease in some parts of Iran and it has high morbidity in some areas of the country. The disease is detected by parasitological examinations including direct microscopic and culture tests. This comparative study aimed to evaluate the relationship between positivity of the leishmanin skin test (LST), microscopically examination and clinical forms of CL for the diagnosis of human cutaneous leishmaniasis. This study was performed on 66 patients suspected to cutaneous leishmaniasis. CL cases evaluated by both microscopical examination and leishmanin skin test. In this study, 1 ml of leishmanin fluid (lot no 121/1, produced in Pasteur institute of Iran) was injected intradermally in forearms of all patients and indurations were measured after 72hours. Induration of 5 mm and higher was considered as positive results. The collected data were statistically analyzed using the SPSS version 13.5. From 66 CL patients who were evaluated in this study, 30 (45.5%) of them had positive microscopically results while 28(42/4%) of them had showed positive leishmanin skin test (≥5mm diameter). From 36 (54.5%) patients who had negative microscopical examination, only 6(16/6%) of them had positive leishmanin skin test. The agreement between two tests was 87.9 % by kappa analysis ($p< 0.01$). In attention to the results of this study, it seems the LST would be used as an alternative diagnosis method when there is a strong clinical doubt to cutaneous leishmaniasis even there is no parasite in direct smear.

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Keywords: Cutaneous leishmaniasis; Leishmanin skin test; Microscopical examination; Human

Introduction

Leishmaniasis is one of the important parasitic diseases, which is endemic in 88 countries where are situated in contents such as Asia, Africa, Europe, northern and southern America. At least 12 million cases of this disease are reported in the world and the incidence of that is about of 1.5 – 2 million cases every year (1). (about 1-1.5 million for cutaneous and about 500,000 for visceral) (2). The incidence rate of leishmaniasis is estimated about 20000 cases per year in Iran (3).

Leishmaniasis is caused by several species of parasites belonging to the genus *Leishmania* (4).

The first cases of this disease were seen in the old world, for example in southern Europe, Middle East, west southern of Asia and Africa. Then it developed to new world where included northern America and Latina America (1). This disease includes cutaneous leishmaniasis (which has two forms: diffuse and local), muco-cutaneous leishmaniasis and visceral leishmaniasis (1). Cutaneous leishmaniasis is divided to urban form and rural form that are caused by *L. tropica* and *L. major*, respectively.

The disease initiates in the form of small and erytematous papule that grows up and makes a nodule. Progressly, the lesion is crusted and then changes in to an ulcer. The ulcer is usually painless and finally it remains as a scar (5).

Currently, LST is indicative of the delayed-type hypersensitivity to *Leishmania* spp, which plays a major role in disease resolution and lesion healing. This test characteristically becomes positive 5-7 weeks after initiation of infection (6). The test is performed by intradermal injection of 0.1 ml of leishmanin solution. After 24-48 hours, the injection site is inspected and induration is measured. The test is usually considered

Corresponding Author: Mehdi Mohebali
Department of Medical Parasitology and Mycology, Tehran University of Medical Sciences, Tehran, Iran
Tel.: +98 21 8951400, 912 3430048; Fax: +98 21 88951392, E-mail: mohebali@tums.ac.ir

positive when induration is higher than 5 mm (7). A Guatemala study of the LST showed that an antigen comprising *L. major* promastigotes gave a sensitivity of 85 percent and specificity of 100 percent (8). Another study has shown that LST-positive leishmaniasis remained positive for 6 months to 3 years (8).

Because of the high sensitivity of LST in cutaneous leishmaniasis, the test has also been frequently applied in diagnosis of the disease (9, 10).

The objective of this study is to evaluate skin test positivity and its relationship to the microscopical detection and clinical form of cutaneous leishmaniasis.

Materials and Methods

This is a descriptive and comparative study. From Jan of 2007 to Feb of 2008, sixty six suspected patients who referred to the leishmaniasis lab. The School of Public Health, Tehran University of Medical Sciences.

For all patients, the questionnaires were filled. The recorded data included age, gender, occupation, address, and duration of disease, clinical form, location of the lesions, number of the lesions, medical history, result of LST and result of direct smear test. Then the lesions were evaluated by both direct parasitological test (microscopical examination) and leishmanin skin test.

Direct smear test

We prepared samples from the border and the base of lesions by sterile vaccinostyle. All prepared smears were fixed by absolute methanol and were stained with Giemsa 10% stain, and then they were seen by light microscope with magnification 1000X. It was considered positive if amastigotes were seen in macrophages or out of the cells. The cytoplasm of amastigots was light blue and nucleus became purple (Figure 1). We divided patients to two groups; group A: patients with positive smears, group B: patients with negative smears.

Leishmanin skin test

We injected 0.1 ml of leishmanin liquid into the alcohol-cleansed volar surface of patients' forearms intradermally. The leishmanin solution was made from *L.major* (MRHO/IR/75/ER) Pasteur institute of Iran, and the lot no was 121/1. After 48-72 hr, the induration was measured along two diameters by the ball pointed pen technique (11). Induration with diameter 5mm or more was considered as a positive test result.

Analysis method

Analysis was conducted using SPSS software version 13.5, with a probability (*P*) value of <0.05 as statistically significant. Chi square test was used to compare the LST and microscopical examination for the diagnosis of human cutaneous leishmaniasis.

Results

In our study, 26 patients were female and 40 patients were male. From 26 females, 10 patients had positive smears and 9 of them had positive LST. From 40 males, 20 patients had positive smears that 19 of them had positive skin test. There were no statistically correlation between gender and positive LST ($P = 0.22$).

The range of patients' age was 6- 73 years (34.23 ± 18.62). Most patients (32.35%) had lesions from 1 month to 3 months (Table 1). There was found statistically correlation between positive skin test and duration of lesions ($P = 0.032$).

The clinical form of lesions included nodule (n=12; 18.2%), vesicular lesion (n=1; 1.5%), postular lesions (n=11; 16.7%), ulcer (n=24; 36.4%) and others (n=18; 27.2%) (Figure 2).

Table 1. Relationship between leishmanin skin test (LST) and duration of lesions in patients with suspected cutaneous leishmaniasis

Lesion duration (month)	LST		SMEAR	
	Positive	Negative	Positive	Negative
<1	3	9	3	9
1-3	11	14	11	14
3 -6	7	0	5	2
6-12	5	2	5	2
12-24	3	4	3	4
≥24	5	3	3	5
total	34	32	30	36

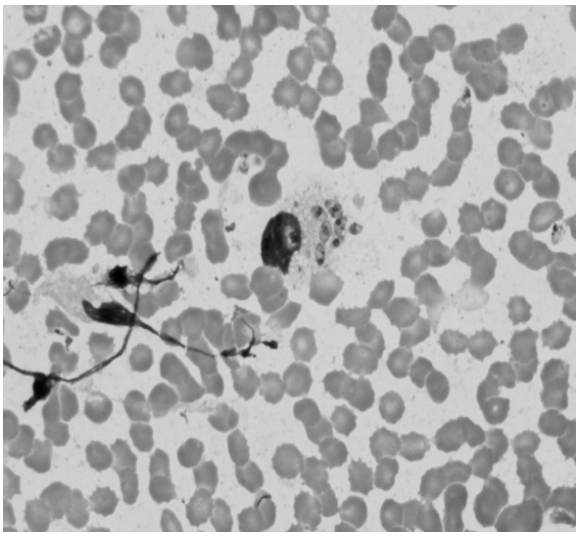

Figure 1. *Leishmania* amastigote forms in a macrophage of CL patient

The lesions were placed on hands, feet, face, and in 5 cases were on other parts of body. Most of lesions (36.3 %) were on hands (Figure 3).

There were no statistically relationship between form of lesions and result of skin test (P = 0.62%), and also there was no statistically correlation between location of lesions and result of leishmanin skin test (P = 0.38%).

The range of number of lesions was 1-10 lesions. 39 patients had 1 lesion, 25 patients had 2-5 lesions and 2 patients had 8-10 lesions (Figure 4). There was no significant relationship between positive LST rate and number of lesions (P = 0.34).

From 66 patients, had suspicious leishmaniasis lesions, 30 patients (45.5%) were positive in direct microscopical examination (group A). 28 patients (93.3%) of them had shown positive leishmanin skin test and 2 patients (6.7%) had negative leishmanin skin test. From 36 patients (54.5%) who had negative smears (group B), 6 patients (16.6%) had positive skin test and 30 patients (83.4%) had negative leishmanin skin test. There was observed statistical differences between two groups (P = 0.002). 3 patients, who had positive LST and negative smears, had positive history of cutaneous leishmaniasis. Four patients with positive leishmaniasis who had negative smear, but their lesions and their histories were like cutaneous leishmaniasis, were re-evaluated and parasites were found, then they were placed in positive smear group (Figure 5).

After analysis, sensitivity of LST was 93.3%. Using a cut off value of 5 mm and higher of LST, the agreement between two tests was 87.9 % by kappa analysis (P< 0.01).

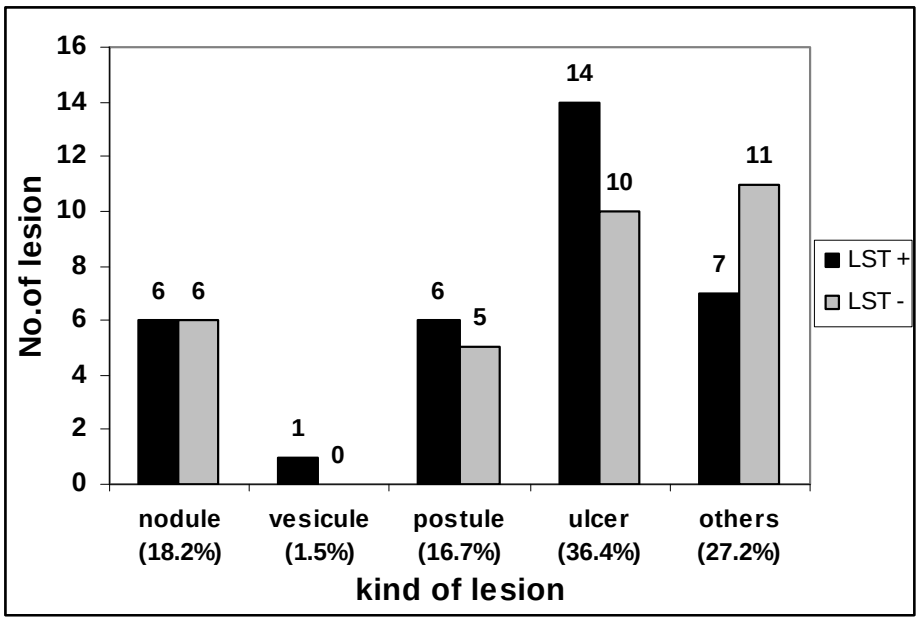

Figure 2. Relationship between leishmanin skin test and clinical form of lesions in patients with suspected cutaneous leishmaniasis

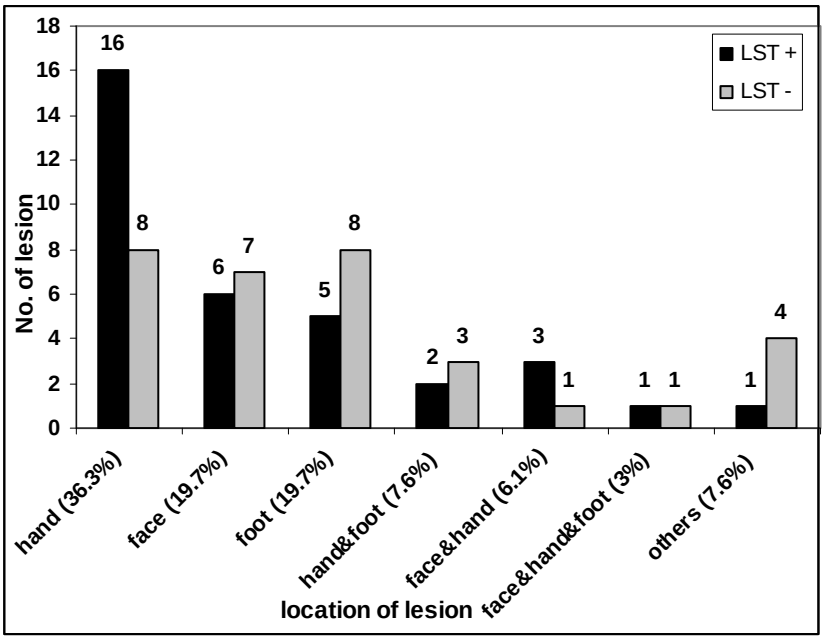

Figure 3. Relationship between leishmanin skin test (LST) and location of lesions in patients with suspected cutaneous leishmaniasis

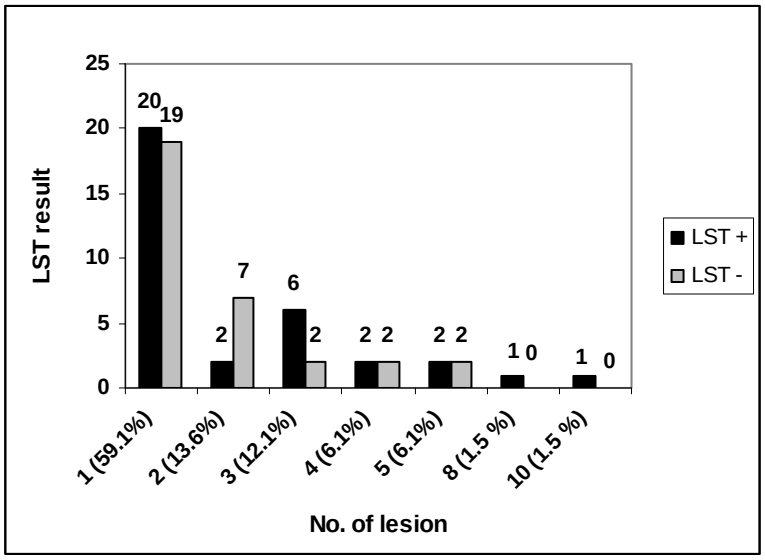

Figure 4. Relationship between leishmanin skin test (LST) and number of lesions in patients with suspected cutaneous leishmaniasis

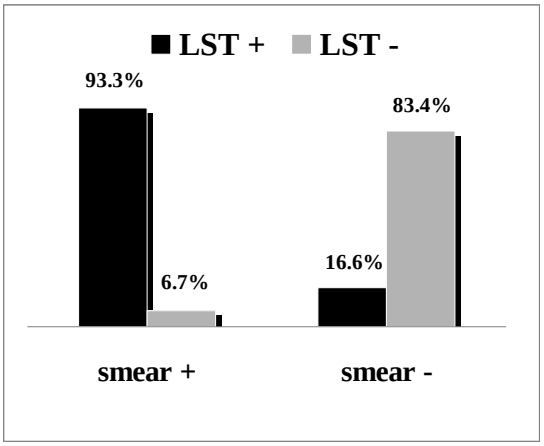

Figure 5. Relationship between leishmanin skin test and direct parasitological test in patients with suspected cutaneous leishmaniasis

Discussion

The routine test which is used to diagnose leishmaniasis is the direct parasitological test. In some studies , clinical methods and LST have used for diagnosis, for example in a study in Colombia, physical examination, historical information and LST were used together to diagnose leishmaniasis in chronic lesions, and sensitivity, specificity and efficiency of them were reported 92%, 70%, 87% . In this study inclusion of LST skin test consistently improved the specificity of two other methods (12).

In our study, like some of the other studies (13, 14), there was not difference between LST in male and in female; otherwise, in some studies there were significant differences. In a study, which Ali Ahmed et al have done in northeast Ethiopia, positive LSTs in males have been more than in females (15), but in another study in north-central Nigeria, LSTs were positive in females more than in males (16). Maybe, these differences have been resulted because of rate of exposure to vectors of disease in one of two genders.

There was a relationship between becoming positive of LST and duration of lesion in our study. In some studies, of course, this result has been reported. A study in Pakistan has evaluated 100 patients with cutaneous leishmaniasis by LST. The LSTs were positive in 78 percents of patients in 2 weeks after diagnosis of disease, but after 6 weeks, they were 98 percents (17). Of course, in another study in Esfahan, Iran, the results of LST were considered in 198 patients with cutaneous leishmaniasis. During of this study, LST was repeated for patients whose LST was negative. From 198 patients 94% had positive LST and only 6% of patients remained negative until the end of study (18).

The results of our study showed there was no significant relationship between the number and the location of skin lesion and the positivity of the leishmanin skin test. This was similar to results of a study in Esfahan, Iran (18). But our result about clinical form of lesions was different to that study. In our study, there was no statistically relationship between clinical form and positive LST rate. However, the significant relationship between positivity of the LST and clinical forms had resulted after repeating LST in that study.

The sensitivity of LST in our study was like in some other studies. For example, in a study in Esfahan, in Iran, the sensitivity of LST has been reported 93.04% in urban forms and 95.8% in rural forms (19). Also it was 84.3% in a study in Peru (20).

In attention to agreement between LST and microscopical test, we could conclude if there was a clinical doubt to cutaneous leishmaniasis but there were found no parasites in the microscopical test and culture, especially in chronic skin lesions, lupoid forms and muco-cutaneous leishmaniasis, the LST would be used as an alternative method of diagnosis.

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