

## Original article

# Diagnostic yield of various traditional laboratory investigations in the diagnosis of cutaneous leishmaniasis

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## Abstract

**Background** Leishmaniasis is a tropical protozoal infection endemic in certain areas of Pakistan. The clinical diagnosis of disease can be confirmed by various laboratory investigations like direct microscopy, culture, Leishmanin test and newer serological tests.

**Objective** To determine the diagnostic yield of Leishmanin test, direct microscopy and leishmania culture in the diagnosis of cutaneous leishmaniasis in Pakistan.

**Patients and methods** In this observational study conducted at the Skin Department, PNS Shifa and DESTO Laboratories, Karachi, 40 patients were included. Leishmanin test, direct microscopy and leishmania culture was performed on all these patients.

**Results** Leishmanin skin test was positive in all the 40 patients (100%), direct microscopy of the smear in 28 patients (70%) and *Leishmania* culture in 32 patients (80%).

**Conclusion** Traditional methods of diagnosis have a high yield of positivity in the diagnosis of cutaneous leishmaniasis.

## Key words

Cutaneous leishmaniasis, leishmanin test, direct microscopy, *Leishmania* culture

## Introduction

Cutaneous leishmaniasis is a growing problem in Pakistan. Once the disease was seen only in Balochistan province, but now several cases of this disease have been reported from Multan, Azad Kashmir and North-West Frontier Province,<sup>1</sup> especially in and around Kohat district. The clinical diagnosis of the disease is not much difficult when there is a history of travel to an endemic area and the clinical picture is that of a chronic non-healing ulcer or plaque which has not responded to routine antibiotics. However, on several occasions laboratory diagnosis is required to differentiate the disease from other chronic infections like lupus vulgaris and

deep mycosis. The definitive laboratory diagnosis depends upon the identification of amastigotes in tissue or promastigotes in culture. Anti-leishmania antibodies develop in the serum of patients of cutaneous leishmaniasis, during the early course of the disease. ELISA, immunofluorescent antibody test, indirect hemagglutination test or other assays, detect these antibodies. The leishmanin skin test is based on cell-mediated immunity. It becomes positive when the lesions become crusted.<sup>2</sup>

In Pakistan, where elaborate laboratory techniques are not readily available, novel diagnostic methods like leishmanin test, direct microscopy, leishmania culture and histopathology are still the most useful techniques.

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The purpose of this study was to find the diagnostic yield of leishmanin test, direct microscopy and leishmania culture in the diagnosis of cutaneous leishmaniasis in Pakistan. As the results of this study show, the diagnostic yield of all these investigations is very high.

### **Patients and methods**

The study population was army personnel who suffered from cutaneous leishmaniasis when they visited various endemic areas of Balochistan province. The total number of patients was 40. They were all males. The age ranged from 20-40 years. The duration of illness was from 8 to 12 weeks. The centre of the study was skin department of PNS Shifa, a naval hospital in Karachi. The laboratory investigations were conducted at Defense Science and Technology Organization (DESTO) laboratories, Karachi. This study was conducted primarily to undertake a clinical trial of the effectiveness of allopurinol in comparison with sodium stibogluconate (Pentostam ®) in the treatment of cutaneous leishmaniasis.<sup>3</sup>

The inclusion criteria were mainly clinical. All the patients were adult. They had one or more nodular, ulcerated or crusted plaque over the exposed areas of the body since at least 2 months. Presence of other signs like satellite lesions or sporotrichoid spread further supported the diagnosis. History of contact of the disease in a known endemic area was also one of the inclusion criteria.

Very young and very old patients, patients who did not agree to be included in the trial and those with a doubtful clinical lesion were excluded from the study. Furthermore, patients who had no history of travel to an endemic area and those who

received some definitive treatment for their disease were also excluded from the study.

Leishmanin (Montenegro) test was performed in this study by injecting 0.1 ml of leishmanin (a suspension of phenol-killed cultured promastigotes) on the volar aspect of one of the forearms. 0.1 ml of normal saline was injected in the other forearm as a control. The result was read after 72 hours. The positive reaction showed an induration with some surrounding erythema. It was graded according to the diameter of the induration in accordance with the following criteria<sup>4</sup>: grade-1 when the area of induration was 5-6mm, grade-2 when it was 6-8mm, grade-3 when over 8mm and grade-4 when the induration was associated with blistering.

The procedure adopted for obtaining material for preparing smear for direct microscopy and leishmania culture was saline-aspirate. 1 ml of normal saline was injected at the edge of the lesion and immediately afterwards the injected fluid was extracted. The smear was made from the aspirate and stained with Leishman's stain, and looked for the amastigotes. The leishmania culture was performed, by inoculating the saline aspirate in Nicolle-Novy-McNeal (NNN) medium. It was incubated at 27° C for 2 weeks. After 2 weeks a smear was made from the culture and the slide was stained by Leishman's and looked for promastigotes.

### **Results**

The leishmanin (Montenegro) test was positive in all the 40 patients, i.e. 100% positivity. The test was mostly grade 1 and 2. Direct microscopy of the smear revealed amastigotes in 28 patients (70% positivity). Microscopic examination of the smear prepared from the culture

**Table 1** Comparison of the results of leishmanin test in this study with previous studies

<i>Studies</i>	<i>Positivity</i>
This study	100%
Silveira <i>et al.</i> <sup>6</sup>	51.6%
Pampiglione <i>et al.</i> <sup>7</sup>	80%
Akuffo <i>et al.</i> <sup>8</sup>	92%

**Table 2** Comparison of the results of direct smear examination in this study with previous studies

<i>Studies</i>	<i>Positivity</i>
This study	70%
Robinson <i>et al.</i> <sup>9</sup> (centre)	85%
Robinson <i>et al.</i> <sup>9</sup> (edge)	69%
Ramirez <i>et al.</i> <sup>10</sup> (centre)	90.4%
Ramirez <i>et al.</i> <sup>10</sup> (edge)	78.8%
Rahman <i>et al.</i> <sup>11</sup>	30%

**Table 3** Comparison of the results of leishmania culture in this study with previous studies

<i>Studies</i>	<i>Positivity</i>
This study	80%
Ramirez <i>et al.</i> <sup>10</sup>	91.2%
Romero <i>et al.</i> <sup>12</sup>	67.5%
Salinas <i>et al.</i> <sup>13</sup>	89.8%
Shatry <i>et al.</i> <sup>14</sup>	90%

revealed promastigotes in 32 patients (80% positivity). In 20 patients both direct microscopy and leishmania culture was positive (50%). In 12 patients only leishmania culture was positive, while in 8 patients only the direct microscopy of the smear was positive. It is obvious from the results that the combination of the two techniques increased the yield of laboratory diagnosis to 100%.

## Discussion

For the laboratory diagnosis of cutaneous leishmaniasis, tests based on both immunological and non-immunological methods are employed. In non-immunological methods, direct identification of parasites is done by microscopic examination of the smear

prepared from the lesion, leishmania culture, histopathological examination and animal inoculation. The tests based on immunological methods are further divided into the tests based on cell-mediated immunity and humoral immunity. Among the tests based on cell-mediated immunity, leishmanin (Montenegro) test is most frequently performed. The tests based on humoral immunity include complement fixation test, indirect hemagglutination test, immunofluorescent antibodies, ELISA, gel precipitation test, peroxidase and anti-peroxidase test, DNA probe test and polymerase chain reaction (PCR). PCR has now become the gold standard in the diagnosis of all types of leishmaniasis. It is a valuable method of diagnosis is chronic cases when the parasite load is low. It has a specificity of 100% and sensitivity between 92-98%.<sup>5</sup>

The leishmanin test had 100% positivity in this study. The reasons were two folds. Firstly, all the patients who were included were suffering from cutaneous leishmaniasis at least for the last 8 weeks, having an ample time for the cell-mediated immunity to develop. Secondly, the test was conducted in DESTO laboratories Karachi, where there is a state of art department of Leishmaniasis research. This is the only laboratory in which the leishmanin is prepared in Pakistan. This laboratory has a very high diagnostic yield of Montenegro test, direct microscopy of the smear and leishmania culture.

The results of the leishmanin test in this study is compared with the results of previous studies in **Table 1**. In the study by Silveira *et al.*<sup>6</sup> the yield of the positivity was 51.6%, by Pampiglione *et al.*<sup>7</sup> was 80% and by Akuffo *et al.*<sup>8</sup> was 92%. Silveira *et al.*<sup>6</sup> gave the reason of low

positivity due to the immunoinhibition by the parasite of *L. amazonensis* themselves. In the study by Pampiglione *et al.*<sup>7</sup> the patients who had a negative leishmanin test were those who got infected 20 or more years back.

The result of direct microscopic examination of the smear in this study is compared with the results of the previous studies in **Table 2**. In this study the smears were prepared by saline-aspirate method while in all the other studies they were made by slit-skin method. Furthermore, in the studies by Robinson *et al.*<sup>9</sup> and by Ramirez *et al.*<sup>10</sup> two slit-skin smears were made in each patient, one from the centre and other from the edge of the lesion. The diagnostic yield of the smear from the centre in the study by Robinson *et al.*<sup>9</sup> was 85%, while the one prepared from the edge was 69%. In the study by Ramirez *et al.*<sup>10</sup> the yield of the smear made from the centre was 90.4% and the one from the edge was 78.8%. Hence the results show that the diagnostic yield of the slit-skin smear is better if performed from the centre of the lesion than from the edge, probably due to higher parasite load in the centre of the lesion.<sup>10</sup> Recently the study conducted in skin department, military hospital, Rawalpindi gave the result of slit-skin examination as 30%, which according to authors, was unexpectedly very low.<sup>11</sup>

**Table 3** compares the results of Leishmania culture in this study with the previous studies. Furthermore, it was stressed in two of the previous studies that the yield of positive culture was more if the saline-aspirate was used as the inoculate.<sup>10-12</sup>

## Conclusion

It may be concluded in the light of the results of this study that the traditional methods of the diagnosis of cutaneous leishmaniasis have a very high yield of positivity, provided they are performed in a good laboratory. These methods are still far better than the elaborate laboratory tests like ELISA, indirect hemagglutination test, immunofluorescent antibody test etc. These tests besides being expensive have low diagnostic yield than the traditional methods.<sup>11</sup> Furthermore, combining the direct microscopic examination and leishmania culture in a patient increases the diagnostic yield.<sup>15</sup> Leishmanin test has never been given much importance in the diagnosis of cutaneous leishmaniasis in the past. However, keeping in view the 100% positivity in this study and 80-90% positivity in other studies, its importance in the diagnosis of cutaneous leishmaniasis cannot be ignored. Hence, a negative leishmanin test in a patient with a chronic lesion, which has not been treated, makes the diagnosis of cutaneous leishmaniasis doubtful.

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