

LEISHMANIA SEROLOGY IN THE DIAGNOSIS OF CUTANEOUS LEISHMANIASIS

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ABSTRACT

Background: The gold standard to diagnose cutaneous leishmaniasis is histopathology, but there has always been a need of a rapid, reliable, cheap and convenient laboratory investigation. Serological tests fulfill the above criteria.

Objective: The objective of the study was to determine the sensitivity and specificity of enzyme linked immunosorbent assay (ELISA) in detection of leishmania antibodies, in comparison with the histopathology.

Place and duration of study: The study was conducted in Military Hospital Rawalpindi from 1st November 2010 to 30th June 2011.

Patients and methods: The study population included the patients who were clinically diagnosed with cutaneous leishmaniasis. All of them were biopsied and serum was sent for leishmania serology.

Results: A total of 47 patients were included. They were all adult males. The histopathology was positive in 31/47 patients (65.95%), while the leishmania serology was positive in 36/47 cases (76.59%). The sensitivity was 74.19%, specificity was 18.75%, positive predictive value was 63.88%, negative predictive value was 27% and accuracy was 55%.

Conclusion: In the light of sensitivity analysis, it may be concluded that leishmania serology has moderate sensitivity and low specificity; hence it is not a reliable test for cutaneous leishmaniasis.

Keywords: Cutaneous leishmaniasis, ELISA, histopathology, serology.

INTRODUCTION

Cutaneous leishmaniasis is the most common chronic granulomatous infection in Pakistan. The clinical history, appearance, duration of illness and acquisition of the disease in an endemic area all help in the diagnosis. However there are many patients in whom the diagnosis is not straight forward and the histopathology and smears made from the lesions are negative for leishmania trophozoite (LT) bodies. In addition there are many places in the country where the facilities for histopathology are not available. In both the cases there is a requirement for a cheaper and convenient laboratory test, which could give a proper diagnosis. Leishmania serology by ELISA, is one such test. This test only requires a preformed broad spectrum antigen. Besides being cost effective and convenient to perform, it is quite suitable for diagnosis of cutaneous leishmaniasis¹. This study was planned to

determine the efficiency of this test in comparison with histopathology in the diagnosis of cutaneous leishmaniasis.

MATERIAL AND METHODS

This was a validation study, carried out in Military Hospital (MH) Rawalpindi from 1st November 2010 to 30th June 2011.

The study population was the patients who reported to the skin outpatient department of Military Hospital Rawalpindi, a tertiary care hospital of Pakistan Army. The inclusion criterion was mainly clinical. This was presence of one or more ulcerated plaques on exposed body areas for more than a month and history of acquisition of disease in a known endemic area. Patients who had lesions which were clinically doubtful, or those who received some definitive treatment were excluded from the study.

During the study period 47 patients were included in the study fulfilling the inclusion criteria. The study group comprised of 47 male patients. All the patients were admitted, biopsied and specimen submitted for histopathology. The serum was sent for leishmania serology. Both the tests were done in

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Armed Forces Institute of Pathology, Rawalpindi. The former test was done in the department of histopathology, and later in the department of microbiology.

As already proven, histopathology was taken as the Gold standard. Only those cases were declared positive in which clearly demonstrable LT bodies were seen in H and E sections. Sometimes Giemsa stain was used for confirmation. Those cases in which LT bodies were not seen were declared as negative. Leishmania serology was done by "Leishmania Elisa IgG+IgM" manufactured by Vircell-Spain. Antibody index of <9 is considered as negative, 9-11 is equivocal and >11 is positive.

Data had been analyzed using SPSS version 15. Descriptive statistics were used to describe the data. Diagnostic measures were calculated for leishmania serology, keeping histopathology as gold standard.

RESULTS

A total of 47 cases were included in the study. They were all males aged between 19-36 years and mean \pm SD aged 26.77 \pm 7.87 years. All the patients acquired the infection from North or South Waziristan. The duration of illness was from 1- 4.5 months with mean \pm SD of 2.91 \pm 1.34 months.

As far as the clinical presentation is concerned; 15 patients had a solitary lesion, 18 patients had two lesions, (19%) had 3 lesions, (16%) had 4 lesions and only (4%) patients had 5 lesions. Among the total 100 lesions which we observed in our patients, 57(57%) were ulcerated and 33(33%) presented as crusted nodules. Lower limbs were the most common site of involvement 46(46%) lesions, upper limbs had 39(39%), face 13(13%) and trunk 02(2%) lesions. LT bodies were seen in 31(65.96%) patients and were not detected in 16(34.04%) patients. The leishmania serology was positive in 36 (76.6%) and negative in 11 (23.40%) out of total 47 patients.

The sensitivity and specificity of Leishmania serology was calculated in comparison with the histopathology diagnosis, which is taken as gold standard (Table-1). Sensitivity of leishmania serology was 74.19%, specificity was 18.75%, positive predictive value

was 63.88%, negative predictive value was 27% and accuracy was 55%.

As obvious from the results, sensitivity is moderately high, but specificity is low, showing that Leishmania serology is not a reliable test in the diagnosis.

DISCUSSION

Cutaneous leishmaniasis is caused by different species of Leishmania parasites. The parasites are transmitted to humans, by the bite of Phlebotomus sand flies. Leishmaniasis stands third among the vector-borne diseases in accordance to the global burden of disease. There are around 1.5 to 2 million new cases annually, with up to 350 million people at risk of infection².

World over there is a gradual upsurge of cases of cutaneous leishmaniasis. Such increase can be explained, in part, by improved diagnosis and case notification but is also due to other factors such as inadequate vector control, urbanization, deforestation, armed conflicts in endemic areas, emergence of anti-leishmania drug resistance and inability to complete the treatment due to its cost².

In general, the clinical appearance, chronic nature of the illness and history of travel to an endemic area makes the diagnosis very straight

Table-1: Comparison of leishmania serology and histopathology (n=47).

Leishmania serology	Histopathology positive cases	Histopathology negative cases	Total
Positive cases	23 (a)	13 (b)	36
Negative cases	8 (c)	3 (d)	11
Total	31	16	47

Table-2: Result of ELISA done for cutaneous leishmaniasis in previous studies, in comparison with our study.

S/no	Positive yield by ELISA
1	90.04% ⁶
2	92.5% ⁷
3	50% ⁸
4	60% ⁹
5	78.4% ¹⁰
6	52% ¹¹
7	76.6% (our study)

forward. Sometimes diseases like cutaneous tuberculosis, deep fungal infections, sarcoidosis, and tertiary syphilis come in the differential diagnosis. The clinical variety encountered in cases of cutaneous leishmaniasis, is mainly due to the variety of *Leishmania* species, and there is growing evidence that the therapeutic response is species and perhaps, even strain specific².

As far as the laboratory diagnosis is concerned, parasitological diagnosis remains the gold standard because of its high specificity³ and easy availability. However both histopathology and skin smears depend upon the quality of material, staining, type of microscope and the expertise of the dermatopathologist³. Henceforth, these methods have low sensitivity. More sophisticated techniques like polymerase chain reaction have equally high sensitivity and specificity but are currently very expensive and rarely available for common use⁴.

There are many serological tests available, but the serological assays are not sensitive enough to diagnose all the parasitologically confirmed cases of localized cutaneous leishmaniasis, because the number of circulating antibodies against CL-causing parasites tend to be low². In visceral leishmaniasis the circulating antibodies are significantly higher². The specificity can also be variable. In one of the large studies on this subject, the diagnostic yield of serology in the detected anti-leishmania antibodies was 72% in visceral leishmaniasis, 25.55% in cutaneous, 83% in mucocutaneous and 33% in post-Kalazar dermal leishmaniasis⁵. In the current study, *Leishmania* serology was positive in 36 out of total 47 cases of suspected cutaneous leishmaniasis (76.6%) and negative in 11 patients (23.40%). Table 2 shows the positive yield of ELISA in the diagnosis of cutaneous leishmaniasis in all the previous studies. As obvious from the table, there are only 2 studies which give a positive yield of around 90%.

Otherwise all the other studies have a yield between 50-70%. This yield is similar to our study.

In comparison with histopathology, serology has a reasonably good sensitivity but low specificity in our study. The chi-square test shows no statistical difference between the serology and the gold standard. If we compare the cost; histopathology at AFIP would cost almost 3 times more than leishmania serology.

CONCLUSION

It may be concluded that *Leishmania* serology has moderate sensitivity and low specificity; hence it is not a reliable test for cutaneous leishmaniasis.

REFERENCES

1. Singh S, Shuvakumar R. Recent advances in the diagnosis of Leishmaniasis. *J Post Gr Med* 2003; 49: 55-60
2. Richard R, Jean CD. Molecular Diagnosis of Leishmaniasis: Current Status and Future Applications. *J Clin Micro* 2007; 45: 21-25.
3. Herwaldt BL. Leishmaniasis. *Lancet* 1999; 354: 1191-9.
4. Monroy O, Sanchez TG. Molecular probes and the polymerase chain reaction for detection and typing of *Leishmania* species in Mexico. *Trans R Soc Trop Med Hyg* 2002; 96(S): S 101-4.
5. Kar K. Serodiagnosis of leishmaniasis. *Crit Rev Microbiol.* 1995; 21(2): 123-5.
6. Ibrahim MM, Elias KS, Moh'd SAK, Bisharat Z, Oumeish Y, Oumeish WB. Serodiagnosis of cutaneous leishmaniasis in Jordan using indirect fluorescent antibody test and the enzyme-linked immunosorbent assay. *Acta Tropica* 1995; 59: 163-172.
7. Al Nahhas SA. Serodiagnosis of cutaneous leishmaniasis in the Syrian Arab Republic. *Saudi Med J* 2009; 30(3): 382-6.
8. Rehman S, Bari A. Laboratory profile in patients of cutaneous leishmaniasis from various regions of Pakistan. *J Coll Physicians Surg Pak* 2003; 13: 313-6.
9. Safi SH, Evans DA. A comparison of the direct agglutination test and enzyme-linked immunosorbent assay in the sero-diagnosis of leishmaniasis in the Sudan. *Trans R Soc Trop Med Hyg* 1989; 83: 334-7.
10. Zeyrek FY, 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; 14 (11): 1409-15.
11. Thierry J, Borel E, Courrier PL, Courtois D, Mojon M. Cutaneous South-American Leishmaniasis. Parasitological and serological diagnosis by indirect immunofluorescence and enzyme linked immunoassay(ELISA). *Med Trop* 1991; 51(1): 43-8.