

Original article

Role of serodiagnosis in cutaneous leishmaniasis

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Abstract *Background* Cutaneous leishmaniasis caused by different species of *Leishmania* parasite, is endemic in various regions of Pakistan. It is probably the second most prevalent vector-borne disease in the country (after malaria). Diagnosis is mostly made by its clinical presentation, especially in the endemic areas. Sometimes it is aided by slit skin smear examination, histopathological study and parasite culture. Considering the fact that serology has made significant advances in diagnosing various parasitological diseases, this study was carried out to evaluate the role of serological techniques in diagnosis of cutaneous leishmaniasis.

Patients and methods Three serological tests i.e. enzyme-linked immunosorbent assay, immunofluorescent antibody test, indirect hemagglutination test were done in 57 clinically diagnosed cases of cutaneous leishmaniasis.

Results Positive results were seen in 62.4%, 52% and 52% with ELISA, IFAT, and IHA tests respectively.

Conclusion Serological tests can be used as a supporting and screening investigation but not to make the final diagnosis.

Key words

Cutaneous leishmaniasis, serological tests, enzyme-linked-immunosorbent assay (ELISA), immunofluorescent antibody test (IFAT), indirect hemagglutination test (IHT)

Introduction

Leishmaniasis comprises a heterogeneous spectrum of diseases caused by different species of a protozoan parasite *Leishmania*. The spectrum of the disease ranges between a self-healing cutaneous to a more progressive life threatening systemic condition.^{1,2} Leishmaniasis is endemic in many parts of the world including Pakistan. Cutaneous variant of the disease is seen here although there are pockets of the visceral form in the northern part of the country.^{3,4} In view of

the large number of clinical presentations of cutaneous leishmaniasis (CL), a correct diagnosis of the disease becomes important. The clinical picture may be distorted by superadded infections or badly managed treatment and these may cause great difficulty in diagnosis.^{3,5} Moreover, conventional methods of laboratory diagnosis have also certain limitations.^{6,7} In this scenario various immunodiagnostic techniques do help in establishing an accurate diagnosis. Leishmanial parasite contains some antigenic components that evoke both humoral as well as cell-mediated immune responses.⁸ The cell-mediated immune response can be demonstrated by delayed hypersensitivity reactions and

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histopathological picture and to detect humoral response various serological tests have been developed which help in the diagnosis of CL. These include complement fixation test (CFT), indirect hemagglutination test (IHT), direct agglutination test (DAT), gel precipitin test (GPT), gel diffusion and counter current electrophoresis (CEP), immunofluorescent antibody test (IFAT), enzyme linked immunosorbent assay (ELISA), peroxidase antiperoxidase methods (PAP) and monoclonal antibodies.⁸⁻¹¹ This study was focused only to see the humoral response and was aimed to evaluate and compare the effectiveness of some commonly used serological tests namely ELISA, IFAT and PAP.

Materials and methods

Fifty-seven patients of both sexes, belonging to all age groups and having clinically suggestive lesions of cutaneous leishmaniasis, were selected on the basis of following criteria: (i) All these patients were from the known endemic areas or had visited the endemic areas during last six months. (ii) All had skin lesions in the form of either discrete nodules or had non-healing ulcers for the past few weeks. (iii) They had not received treatment with pentavalent antimonial compounds (sodium stibogluconate or meglumine antimonate). (iv) Few of these clinically suspected patients had positive skin slit smears for Leishman-Donovan (LD) bodies. All negative skin slit smears were also included (because negative smear does not rule out the diagnosis of CL). Nine patients were excluded because of the following reasons: (i) Five patients had skin lesions that appeared like the infected lesions of cutaneous leishmaniasis but resolved after a short course of antibiotics. (ii) Three refused to give blood for

serological tests. (iii) One sample was omitted as it turned out to be a case of deep mycosis. Sera of all patients were screened with three different methods to see the presence and the levels of antibodies in the sera of the clinically positive cases. The negative and the positive controls were the same for each test. Different sera dilutions were used and following three methods were used for serological analysis; (a) enzyme-linked immunosorbent assay (ELISA). The dilution used was 1/2. (b) Indirect fluorescent antibody test (IFAT). The sera dilutions used were 1/4 -- 1/8 ---- 1/256. The dilutions below 1/8 were considered negative, up to 1/16 were low levels and up to 1/256 were high levels. (c) Indirect hemagglutination (IHA) test. The commercial kit used for this method was for *Leishmania donovani*. The data was recorded and analyzed in Microsoft Excel programme using frequencies and percentages.

Results

Out of 48 patients, 28 were from Baluchistan (25 males and 3 females) and their ages ranged from 7-43 years. The 20 patients reporting directly to Military Hospital, Rawalpindi were; 9 from different parts of northern areas, 6 from Chakwal area and 5 from Kohat (13 males, 3 females, 4 children) and their ages ranged from 6 months to 35 years. The duration of illness varied between 3-18 months. Clinically the lesions were basically of three types; (i) Wet type (early ulcerative, rural) in 34 patients. (ii) Dry type (late ulcerative, urban) in 15 and (iii) Chronic cutaneous type in 1 patient. Serology was positive in 29 patients with ELISA technique (clinically they were 20

Table 1 Comparative results of various serological tests (n=48)

Sr. No.	Serological tests	No. of positive cases (%)
1	Enzyme-linked immunosorbent assay	29 (60.4)
2	Indirect fluorescent antibody test	25 (52.0)
3	Indirect hemagglutination test	25 (52.0)

wet type, 8 dry and 1 chronic type). IFAT was positive in 25 patients (clinically they were 17 wet type, 7 dry and 1 chronic). IHA also yielded 25 positive results (18 wet type, 6 dry and 1 chronic) [Table 1].

Discussion

For establishing a diagnosis of CL, clinical appearance of the lesions and history of visit to an endemic area are usually sufficient.⁵ These days the diagnosis is generally aided by slit skin smear examination, impression smears, histopathology of the tissue sections and by culture of parasite (where facilities are available).^{6,7} However, unusual clinical presentation, superadded infection or persistence of the disease for a prolonged time, has made scientists to develop more sensitive methods to detect the disease.³ Moreover the conventional diagnostic methods have their own limitations; slit skin smears or impression smears give good results only when the lesions are not secondarily infected and skin biopsies may give inconclusive results.^{6,7} An appropriate screening test was always required for CL. Our study was basically aimed to evaluate the efficacy of serological tests in diagnosis of CL and to compare various available serological tests and no such study was previously done in Pakistan. Patients in our study represented a heterogeneous population group and almost all of them came from the areas where the terrain is mostly mountainous or

hilly. The obvious common factor appeared to be geographical distribution of endemic areas.¹ Clinical profiles of the patients were almost similar as seen in previous studies.^{1,12} A significantly low percentage of females in this series was due to the fact that the study was primarily done on serving soldiers. The results of serology by three different methods were significant and ELISA was found slightly superior to the other two. These serological techniques have a supportive role in diagnosis of CL, but these alone cannot be taken as diagnostic, because serology may remain positive for a long time. Another drawback is that false positive results may be seen with these methods. It may however be used in screening out clinically suspected cases in largely endemic areas or in an epidemic.⁸⁻¹¹

Conclusion

Serological tests are a good addition to diagnostic armamentarium of CL. These can be used to support the diagnosis and for screening purposes in endemic areas but should not be used alone to make final diagnosis.

References

1. Grevelink SA, Lerner EA. Leishmaniasis. *J Am Acad Dermatol* 1996; **34**: 257-72.
2. Lainson R, Shaw JJ. Evolution, classification, and geographical distribution. In: Peters W, Killick-Kendrick R, eds. *The leishmaniasis in biology and medicine*. San Diego: Academic Press; 1987. p. 1-120.
3. Raja KM, Khan AA, Hameed A, Rahman SB. Unusual clinical variants of cutaneous leishmaniasis in Pakistan. *Br J Dermatol* 1998; **139**: 111-3.
4. Mujtaba G, Khalid M. Cutaneous leishmaniasis in Multan, Pakistan. *Int J Dermatol* 1998; **37**: 843-6.
5. Bergan RS. Leishmania, touch preparation as a rapid mean of diagnosis. *J Am Acad Dermatol* 1986; **16**: 1096-1105.

6. Ridley DS. The laboratory diagnosis of tropical diseases: Review. *J Clin Pathol* 1974; **27**: 435-44.
7. Sharquie KE, Hassen AS, Hassan SA, Al-Hamami IA. Evaluation of diagnosis of cutaneous leishmaniasis by direct smear, culture and histopathology. *Saudi Med J* 2002; **23**: 925-8.
8. Manuel J, Behin R, eds. *Immunology of parasitic infections, 2nd edn.* Sidney: Cotton and Kenneth Swarren; 1982.
9. Voller A, Bidweil D. Antigen detection by Elisa. *Asean J Clin Sci* 1985; **5**: 121-3.
10. Follador I, Araujo C, Bacellar O *et al.* Epidemiologic and immunologic findings for the subclinical form of *Leishmania braziliensis* infection. *Clin Infect Dis* 2002; **34**: 54-8.
11. Ryan JR, Smithyman AM, Rajasekariah GH *et al.* Enzyme-linked immunosorbent assay based on soluble promastigote antigen detects immunoglobulin M (IgM) and IgG antibodies in sera from cases of visceral and cutaneous leishmaniasis. *J Clin Microbiol* 2002; **40**: 1037-43.
12. Ridley DS. A histological classification of cutaneous leishmaniasis and its geographical expression. *Trans R Soc Trop Med Hyg* 1980; **74**: 515-9.