Original Article

Diagnostic accuracy of rapid immunochromatographic test in the diagnosis of visceral leishmaniasis, taking bone marrow as gold standard

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Objective: To determine the sensitivity, specificity, positive predictive value and negative predictive value of Immuno-chromatographic test (ICT) in patients with visceral Leishmaniasis simultaneously diagnosed by examination of bone marrow aspirate, a gold standard test.

Methodology: This descriptive cross sectional study was conducted at a tertiary care hospital from February 2016 to February 2020 on 55 consecutive patients from endemic areas with clinical suspicion of visceral Leishmaniasis, coming for bone marrow examination. A nonrandom convenience sampling technique after informed written consent. Bone marrow was aspirated from each patient under local anesthesia, followed by 3 ml venous blood sample. The ICT was done by trained personal that was kept blind of the prior diagnosis of visceral Leishmaniasis. The data obtained were analyzed by SPSS-24.

Results: Age group <5 years was commonest among affected with male predominance, followed by 5-10 years & >10 years. The ICT was positive in 49/55 (87.27%) and negative in 6/55 (12.73%). Bone marrow examination revealed LD bodies in 50/55 (90.91%) and no LD bodies in 05(9.09%) patients.

Conclusion: ICT had high sensitivity and positive predictive value, but comparatively low specificity and negative predictive value for visceral Leishmaniasis. It should not be used as a single confirmatory test for visceral leishmaniasis. (Rawal Med J 202;46:274-277).

Keywords: Leishmaniasis, visceral, rapid diagnostic test.

INTRODUCTION

Leishmaniasis is a vector borne disease mainly affecting skin, mucocutaneous tissue and viscera, caused by different species of genus Leishmania. It is a disease of poverty and has been recognized by WHO as a neglected tropical diseases having high mortality rate. The worst affected parts of the world include East Africa, South Asia, South America and Mediterranean region. As much as 200,000 to 500,000 new cases are seen every year and 90% occur in Brazil, Ethiopia, India, Kenya, Somalia and Sudan.[1] Three most common forms of Leishmaniasis are cutaneous Leishmaniasis, visceral Leishmaniasis and mucocutaneous Leishmaniasis having variable prevalence in five main countries including India, Bangla Desh, Nepal, Sudan and Brazil. The disease is prevalent in some parts of Pakistan including Hazara division, adjoining areas of Kashmir, Tribal areas, upper Punjab and part of Baluchistan.[2-6] It has been reported from southeastern part of Iran also.[7]

Microscopic examination of tissue aspirated from spleen, lymph node and bone marrow has been the main stay of diagnosis.[8] The sensitivity of bone marrow test has is equal to splenic aspirate, if appropriate time is spent on microscopic examination of the bone marrow aspirate.[9,10] Rapid diagnostic tests based upon immunochromatographic technique (ICT) have been developed and tested in the last decade with different sensitivity and specificity.[11,12] It has been recommended that more than one diagnostic approaches is more helpful in the diagnosis.[13,14] The present study aimed to determine the sensitivity, specificity, positive predictive value and negative predictive value of ICT, where the diagnosis was confirmed by the examination of bone marrow aspirate, a gold standard for the diagnosis of visceral Leishmaniasis.

METHODOLOGY

A total of 55 consecutive patients coming for bone

marrow examination to the department of pathology, Ayub Medical College, Abbottabad with provisional diagnosis of visceral Leishmaniasis, were enrolled in the study by non-random convenience sampling after an informed written consent. After history, examination was performed for pallor, splenomegaly, hepatomegaly, lymphadenopathy and pigmentation. Patients already diagnosed and treated for visceral Leishmaniasis were excluded from the study. After appropriate testing, febrile patients who were smear positive for malaria or sputum positive for tuberculosis were also excluded from the study.

Then complete blood counts were performed by automated hematology analyzer, erythrocyte sedimentation rate (ESR) by Westergren method and Giemsa stained blood film examined for red cell morphology. Bone marrow was aspirated from posterior iliac spine under 2% lignocain (B&H), slides allowed to dry and stained by Giemsa stain. In children <18 months old, bone marrow was aspirated from upper end of tibia. Bone marrow slides were examined for one hour on the same day by an experienced pathologist for Leishman Donovan (L.D bodies). LD bodies were reported as number of parasite per high power field. Bone marrow examination and ICT was performed on all the patients. For ICT, 3 ml venous blood sample was taken from every patient in plain bottle, allowed to clot at room temperature and serum was extracted for rK39 based ITC.

Statistical Analysis: Statistical analysis was performed using SPSS version 24.

RESULTS

The most commonly affected age group was <5 years (63.64%) with male predominance, followed by 5-10 years (30.91%) and >10 years (05.45%), respectively (Table 1). Bone marrow examination revealed LD bodies in 50(90.91%) patients (true positive) and no LD bodies in 05(9.09%) patients (true negative) (Table 2). The sensitivity and positive predictive value of ICT was high, 96.15% each while its specificity and negative predictive value was lower, 71.42% each (Table 3).

Table 1. Age and gender of patients (n=55).

Age (years)	Male No %	Female No %	M:F	Total No %
<5	20 (36.36)	15 (27.27)	1.2:1	35(63.64)
5-10	10 (18.18)	07 (12.72)		17(30.91)
>10	00	03 (5.45)		03(05.45)

Table 2. Result of bone marrow examination.

Result	No. %
Positive for L.D bodies	50 90.91
Negative for L.D bodies	05 9.09

Table 3. Sensitivity, specificity, positive and negative predictive value of ICT.

True positive (a) = 50		False positive (b) =02
False negative (c) = 02		True negative (d)=05
Sensitivity	a/a+c	96.15%
Specificity	d/b+d	71.42%
Positive predictive value	a/a+b	96.15%
Negative predictive value	d/c+d	71.42 %

Table 4. Result of rapid diagnostic test (ICT).

Result	No	%
Positive	49	87.27
Negative	06	12.73

The ICT was positive in 49 (87.27%) patients out of which 2 patients were negative on bone marrow (i.e. false positive) in age group 5-10 years. The test was negative in 6 (12.73%) out of which 2 patients were bone marrow positive (i.e. false negative) in age group >10 years respectively (Table 4).

DISCUSSION

Tissue based microscopic diagnosis has been and is the most reliable and direct method of diagnosis of visceral Leishmaniasis with high specificity. The sensitivity and specificity of different aspirates varies.[15-18] Other diagnostic techniques include culture of tissue aspirate, molecular diagnosis by polymerase chain reaction (PCR) and enzyme linked immunosorbant assay (ELISA).[19] ICT are also suitable for the diagnosis in field conditions. Newer ICT test have now been developed with better performance.[18]

In the present study, ICT was positive in 49/55 (87.27%) patients with higher sensitivity and positive predictive value (96.15% each), but relatively lower specificity and negative predictive values (71.42% each). The sensitivity of ICT in our study is in accordance with an earlier study.[17] An earlier study from Bangladesh has also reported 100 percent sensitivity for the test.[12] This is higher than that of our study. In the present study, bone marrow aspirate was used as the diagnostic test. The diagnostic accuracy of rk39 based antigen rapid diagnostic test but is not sufficient to reach a conclusive diagnosis and should be combined with another test to improve the final diagnosis.[18]

Accurate diagnosis of visceral leishmaniasis is important especially when the efforts are being done to eliminate this disease.[19] This becomes even more important when cases of asymptomatic visceral leishmaniasis are emerging.[20] In our study, two patients in age group 5-10 years presented with febrile ailment with positive ICT but negative bone marrow examination (false positive). They were screened for malaria and tuberculosis which was negative. Probably they were exposed to subclinical visceral leishmaniasis in the past, developed antibodies due to which ICT became positive. They had no organomegaly or LD bodies on bone marrow examination. They were kept on regular follow up and did not develop disease in next two years. Both of these patients belonged to same endemic area in Azad Jammu and Kashmir.

Cases with subclinical leishmaniasis have been reported.[21] Similarly, two females of age group >10 years had negative ICT but positive bone marrow. One of them was on treatment for inflammatory bowel disease and the other was taking medicines for pulmonary tuberculosis. Some of the causes of negative ICT and positive bone marrow in a patient with symptomatic visceral leishmaniasis include inflammatory bowel disease on treatment, Tuberculosis and low titer of antibodies.[22]

CONCLUSION

Patients enrolled in the present study belonged to far-flung hilly areas and came late for confirmation of diagnosis. This might have been one of the reasons of lower sensitivity of ICT in our study compared to earlier ones. One of the possible causes could have been undocumented partial treatment of patients by local paramedics.

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