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Role of rK39 Dipstick Rapid Test in Comparision of Bone Marrow and Splenic Aspiration for The Diagnosis of Kala-Azar .

KEYWORDS: rK39, LD bodies, Leishmaniasis.

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ABSTRACT *Leishmaniasis constitutes a diverse collection of human diseases varying in severity from a spontaneously healing skin ulcer to overwhelming visceral disease. A recently developed nitrocellulose-based dipstick test, rK39, has been widely used for the diagnosis of kala-azar. We compare the performance of the rk39 antigen in relation with demonstration of LD bodies in bone marrow aspirates thus assessing the serodiagnostic potential of the rk39 antigen in diagnosing cases of visceral leishmaniasis in patients from Eastern Bihar and Utter Pradesh in 80patients. study which were undertaken in the Indoor Medical wards of Katihar Medical College Hospital & Rama Medical College, Hapur, India. Our study showed the sensitivity and specificity of the rk39 dipstick test was found to be 95% and 90 % respectively. The positive predictive value (PPV) and the negative predictive value (NVP) were 98.7% and 82.6% respectively.*

INTRODUCTION

Visceral leishmaniasis was first described in 1903, by Leishman and Donovan. Leishmaniasis, a vector- borne disease caused by obligate intra macrophage protozoa, is characterized by diversity and complexity. The disease is transmitted to humans by the bite of the infected female sand fly of the genus Phlebotomus (Old World) or Lutzomyia (New World). The disease predominantly manifests either in a cutaneous form, which causes skin ulcers on the exposed parts of the body, or in a visceral form that is characterized by prolonged fever, splenomegaly, hepatomegaly, substantial weight loss, progressive anemia, and/or pancytopenia . Around 360000 of new cases of VL each year worldwide were estimated and over 60% of these occur in the Northern Indian state of Bihar and bordering regions of Nepal and Bangladesh. In India the disease is found in Bihar, Jharkhand, West Bengal and pockets of Eastern Uttar Pradesh. Amphotericin-B is the first line of drug in the treatment of visceral leishmaniasis, not for resistant cases only. Diagnosis of visceral leishmaniasis (VL) is still a major problem in Eastern India where the disease is endemic in remote rural areas lacking transport, communication and modern health care facilities. Demonstration of parasites, which is the gold standard of diagnosis, cannot be practiced in peripheral centers because of lack of both training and laboratory facilities. Because of difficulties associated with parasite detection, several methods based on non-specific rise in immunoglobulin levels, have been used for diagnosis of V.L but because of their non- specific nature with low sensitivity and specificity, there use have been abandoned. Specific serodiagnostic test for antibody detection have been employed in the diagnosis of Kala-azar. The methods include gel diffusion, complement fixation test. Indirect haemagglutination test, indirect immunofluroscent antibody test (IFAT), countercurrent immunoelectrophoresis and direct agglutination test (DAT). Serologic tests have been developed using the cloned antigen rK39 instead of whole Leishmania parasites. The rK39 antigen, which consists of 39 amino acid repeats of a kinesin like gene found in L. chagasi, a finger prick sample of blood is added to a well and mixed with a drop of buffer, test strip is placed vertically in the well, and the diluted blood rises up the nitrocellulose strip. The entire process takes approximately 20 minutes and results are read visually. These dipstick formats showed high sensitivity and specificity in India, Nepal, Bangladesh, and Brazil. The rk39 dipstick test is a test which can be performed under field conditions as no expert hands or expensive instruments are required. The test is also very easy to interpret and reports can be given within half an hour. The present study was therefore under taken in the Department of Microbiology, Katihar Medical College, Katihar, and Bihar and Rama Medical College, Hapur.

AIMS AND OBJECTIVES

To assess the serodiagnostic potential of the rk39 antigen in diagnosing cases of visceral leishmaniasis in patients from Eastern Bihar and Utter Pradesh. To compare the performance of the rk39 antigen in relation with demonstration of LD bodies in bone marrow and splenic aspirates.

MATERIALS AND METHODS

Total of 80 cases of Leishmaniasis were included into the study. Which was a hospital based study undertaken in the Indoor Medical wards of Katihar Medical College Hospital & Rama Medical College, Hapur, India. Controls included 20 healthy attendants of patients who never suffered from Kala-azar and medical students from Katihar Medical College and Rama medical college Hapur. Patients fulfilling the clinical case definition for suspected kala-azar and showing LD bodies positivity either in Bone marrow or spleenic aspiration were included as case. rk39 Dipstick was obtained commercially from Dia Med- IT LEISH, Switzerland. Was used for rapid diagnosis.

OBSERVATION

General considerations- in this study rk39 dipstick test was performed to detect anti-leishmania antibodies in 80 cases of VL and in 20 healthy controls.

Table 1: rk 39 test results.

rk 39 test	No. of case	Percentage	Male		Female	
			No.	%	No.	%
Positive	76	95.0	53	94.6	23	95.9
Negative	04	05.0	03	05.4	01	04.1
Total	80	100	56	100	24	100

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Our study showing rk39 positivity in the 80 parasitologicaly confirmed cases (presence of LD bodies) in bone marrow aspirates. The rK39 dipstick test showed a positivity of 95% (n=76).

Table 2 :rk39 relation to duration of illness

Duration of illness in weeks	No of patient	
	Positivity (%)	Negativity (%)
01-8	33 (41.25)	04 (05.00)
09-16	32 (40.00)	00
17-24	06 (07.50)	00
25-32	03 (03.75)	00
33-40	01 (01.25)	00
41-48	01 (01.25)	00
>48	00	00

rk39 dipstick positivity in relation to duration of illness. We found in this study that 5% (n=4) of parasitologically confirmed cases tested negative. All these patients had duration of illness ranging from 1 – 8 weeks.

Table 3: rk39 test of healthy controls.

rk39 test	No. of cases	percentage
Positive	1	5.0
Negative	19	95.0

In our study rk39 test was positive in 5.0% of healthy controls.

Table 4 :sensitivity, specificity, positive predictive value and negative predictive value of rk39

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
rk39 dipstick	95%	95%	98.7%	82.6%
Demonstration of LD bodies.	100	-	-	-

Our study shows the sensitivity, specificity, positive predictive value and negative predictive value of rk39 antibody detection. The sensitivity and specificity was 95%, positive predictive value was 98.7% and negative predictive value was 82.6%.

DISCUSSION

rk39 dipstick test was performed in 100 subjects including 80 suspected cases of VL and 20 normal controls. The test was found to be positive in 95% (n=76) of parasitologically confirmed cases of VL. 5% (n=4) of parasitologically confirmed cases tested negative. This was probably due to the fact that all these four cases reported very early to the hospital. Two out of these four cases reported with duration of illness of 2 weeks and the other two had duration of illness of 3 and 4 weeks respectively. As the duration of illness was very short rk39 antibodies were probably not present in the serum or were present in very low titers. Burns et aldemonstrated that rk39 seroreactivity correlated very well with presence of active disease. Sunder et al, in his study comprising of 323 cases of suspected Kala-azar found that all the cases that were positive for LD bodies were also positive rk39 antibodies. In fact in his study rk39 was also detected 4 additional cases that were smear negative for LD bodies. In our study The sensitivity and specificity of the rk39 dipstick test was found to be 95%. The positive predictive value (PPV) and the negative predictive value (NVP) were 98.7% and 82.6% respectively. Bern et al,evaluated rk39 dipstick test in Nepal. He found the sensitivity to be 100%.While the specificity of rk39 dipstick test was 100% .In another study by Sunder et al, the authors found the sensitivity of rk39 antibody detection to be 100% and the overall specificity in both healthy and diseased controls was 93%. Schalling et al,reported the sensitivity and specificity of the rk39 dipstick test to be 85.7% and 82% respectively. This is significantly lower that the sensitivity and specificity of the test as found in the present study. Goswami et al, found the sensitivity and specificity of the rk39 dipstick test to be 100% and 92.2% respectively. Boeleart et al, evaluated rk39 dipstick in the Indian subcontinent they found the sensitivity and specificity of rk39 dipstick to be 95% and 90% respectively and he recommended the use. Mandal et al, found the sensitivity and specificity of rk39 dipstick test be 100% and 87% respectively. Carmen Canavate et al, include 246 patients and confirmed from there study that the sensitivities were 90.5% for rk39. Greg Matlashewski et al, found the sensitivity and specificity of the test performed with blood was 91% and 93% respectively.

CONCLUSIONS

rk39 dipstick test was found to be positive in 76 out of the 80 parasitologically confirmed cases. The test was also positive in 1 out of the 20 controls. The four cases that were negative by the rk39 dipstick test were very early cases with duration of illness ranging from 2-4 weeks. The sensitivity and specificity of the rk39 dipstick test was found to be 95%. The positive and negative predictive values were 98.7% and 82.6%. The rk39 dipstick test proved to be a simple, reliable and cheap test which was easy to perform and gave results within 30 minutes. Leishmania is endemic in Bihar and the disease is more prevalent in the low socio- economic groups who have little access to big hospitals and expert medical care. The rk39 dipstick test can go a long way in reducing the burden of this disease in Bihar. However, further studies as regards the performance of the rk39 dipstick test in smear negative (LD body negative) but clinically suspected cases of leishmaniasis and also in other disease conditions other than leishmaniasis is indicated.

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