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Evaluation of rk39 immunochromatographic test with Urine for diagnosis of *Visceral leishmaniasis*

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Abstract

This study evaluates commercially available rK39 Immunochromatographic (ICT) strips using urine for diagnosis of visceral leishmaniasis (VL). Freshly collected urine and serum samples of 280 parasitologically confirmed VL patients and 66 endemic healthy control (EHC), 48 non-endemic healthy controls (NEHC) and 45 different diseases (DD) were tested with rK39 strips. The sensitivity of rK39 in urine were 96.4% while the specificity was low varying from 66.7% in EHC, 77.08% in NEHC to 62.2% in DD, With serum, sensitivity was 100% whereas the specificity was 100%, 92.4% and 95.55% for respective control group. In its present format, the ICT strips cannot be used for the diagnosis of VL using urine samples.

Keywords

rK39 ICT; Visceral leishmaniasis; Urine; diagnosis

Introduction

Demonstration of parasites in tissue smears remains the gold standard for the diagnosis of visceral leishmaniasis (VL), however, it is invasive, risky and requires technical expertise which is not always available in the endemic regions¹. Serological tests like Direct agglutination Test (DAT) is a highly sensitive and specific test, but expensive antigen (The price of rK39 immunochromatographic rapid test is about 1US\$ compared with US\$ 1.5–2.5 for the DAT-FD²), cumbersome procedures and prolonged incubation are its major drawbacks^{3, 4}. A recombinant antigen comprising of 39 amino acid repeats (rK39) from *Leishmania Chagasi syn infantum* has been used both in the ELISA and immunochromatographic rapid strip test formats for the diagnosis of VL with excellent results^{5, 6, 7}. The rK39 strip test using blood or serum is a rapid, cheap, and highly sensitive and specific test, which does not require expertise and has been used extensively for the field diagnosis of VL. In the Indian subcontinent (India, Bangladesh and Nepal), an elimination programme for visceral leishmaniasis has been launched⁸ and rK39 Immunochromatographic rapid strip test is the principle tool recommended for the

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diagnosis⁸. However, obtaining blood can be difficult in the prevailing field conditions especially in children. Moreover, it has been reported that kidney impairment occur during the acute infection with leishmania and antibodies have been detected in urine in patients with VL^{9, 10, 11}. The high sensitivity and specificity of rk39 serology with strip format and presence of immunoglobulin in the urine encouraged us to use urine, which can be acquired non-invasively and easy to obtain, for the diagnosis of Kala-azar. The aim of this study was to evaluate the sensitivity and specificity of commercially available rK-39 strip test in the urine of patients with VL.

Material and Method

This study was conducted at Muzaffarpur, the field site of Kala-azar Medical Research Centre, Banaras Hindu University between April 2008 to January 2009. Urine and sera from 280 parasitologically confirmed VL subjects and 159 control subjects were tested using ready-to-use Immunochromatographic strips (Kala-azar Detect, In Bios International, Seattle, USA). Immediately after collection of Urine and serum, the samples were stored at -20°C until use. The control group consisted of 48 nonendemic healthy controls (NEHC), 66 endemic healthy control (EHC) who were asymptomatic family members of VL subjects and urine from 45 patients with different diseases (DD) like tuberculosis, Malaria amoebic liver abscess with the patients who had a history of fever... rK-39 strip test was performed as per the protocol of the manufacturer. For urine assay, 100–150 µl of neat urine sample were applied directly to the strip without chase buffer (phosphate buffer saline with bovine serum albumin).

Result

Out of 280 parasitologically confirmed VL subjects, 270 were found to be positive in urine samples (sensitivity 96.4%) whereas all tested positive with serum (sensitivity 100%). The specificity of the test was 66.7%, 77.08% and 62.2% with EHC, NEHC and DD comprising of **samples from the patients suffering with malaria, tuberculosis, amoebic liver abscess**, respectively in urine samples and 92.4%, 100% and 95.55% with sera (Table 1).

Discussion

Urine samples are noninvasive, acceptable to most population especially children and the ease of collecting urine makes it extremely attractive alternative for blood or sera in the diagnosis of VL, in addition a invasive procedure is avoided and the risk of accidental infections circumvented.

Urine has been used for diagnosis of VL by ELISA in Bangladesh using rKRP42 antigen¹². The sensitivity of urine based rKRP42 ELISA were 94% whereas the specificities for EHC, NEHC, malaria, tuberculosis and cutaneous leishmaniasis were reported to be 100%. Direct agglutination test with urine samples has also been used for the diagnosis of VL¹³. In this study, a new DAT, in which anti-human IgG was used as enhancing antibody, has been developed for the diagnosis of visceral leishmaniasis and compared the result with the conventional DAT with serum and their previously reported urine-ELISA. The sensitivity of the new DAT (90.7%) was found almost same as that of the conventional DAT (91%) and the urine-ELISA (93.3%). The specificity of the new DAT reported 96.4% which was nearly identical with that of the urine ELISA (97.3%).

Recently, a similar study has been carried out in Bangladesh¹⁰. In this study, the sensitivity and specificity of rK39 ICT test with urine were reported 95% and 93.3% respectively. In our study, though the sensitivity of rK39 strip test using urine was excellent but specificity was unsatisfactory as nearly 30% of controls tested false positive. It is difficult to explain

marked difference between the specificity reported by Khan et al¹⁰ and this study, as the method used were similar and the geographical region is also the same. This appears to be the first such instance of low specificity using rK39 antigen with anybody fluid. In studies with rK39 Strips using blood or sera, endemic healthy controls showed positivity in 3–32% and only a small proportion of patients with different diseases like tuberculosis or malaria tested positive¹⁴. This positivity in endemic controls is due to subclinical infection. However, none of the non-endemic healthy controls have shown a positive result in any study¹³. Therefore, such high degree of positivity in non-VL controls in urine samples could be due to non-specific binding of urinary proteins with rK39 on the strips. Thus, it is obvious that in the present rK39 strip format, urine cannot be used for diagnosis of VL until the problem of non specific binding could be resolved.

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Table 1

Sensitivity and specificity of Urine vs Serum rK39 Immunochromatographic test

Subjects	n	rK39 ICT test result					
		Urine			Serum		
		Sensitivity	Specificity	95% CI	Sensitivity	Specificity	95% CI
Confirmed VL	280	96.4% (270/280)	-	94–99	100% (280/280)	-	98–100
Non endemic healthy control (NEHC)	48	-	77.08% (36/48)	65–89	-	100% (48/48)	98–100
Endemic healthy control (EHC)	66	-	66.7% (44/66)	55–78	-	92.4% (61/66)	83.4–96.7
Different diseases (DD)	45	-	62.2% (28/45)	48–76	-	95.5% (43/45)	85.2–98.8