

Short communication

A Comparative Study of Serum, Urine and Saliva Using rk39 Strip for the Diagnosis of Visceral Leishmaniasis

*Sarita Mohapatra, Jyotish C Samantaray, Arnab Ghosh

Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India

(Received 12 Nov 2013; accepted 1 July 2014)

Abstract

Background: Immunochromatographic based rk39 antibody detection test became popular for the diagnosis of visceral leishmaniasis (VL) because of high sensitivity, rapidity, easy to interpret, and cost effectiveness. However, false positive result after complete cure of the patients is the major limitation with this test. The aim of the study to assess the usefulness of non-invasive samples i.e. urine and saliva by rk39 test for the diagnosis of visceral leishmaniasis in comparison to serum.

Methods: Seventy two clinically suspected VL patients were enrolled in the study among which 61 cases were confirmed as VL and 11 cases were included in the control group. Serum, urine, and saliva samples of all the cases were tested for rk39 dip stick test.

Results: Urine and saliva both were equally sensitive as serum for the diagnosis kala-azar. In the control group, rk39 antibody test was negative in 10 cases out of 11 (91%) with saliva in comparison to 4 cases with serum (36%), thereby found to be more specific.

Conclusion: Saliva sample found to be highly reliable for the diagnosis of VL cases by rk39 test. The test with saliva sample showed less false positive result in comparison to serum sample, thereby can be used as an adjunct with serum sample for the diagnosis of kala-azar in endemic areas.

Keywords: Visceral leishmaniasis, Noninvasive samples, rk39 test

Introduction

Visceral leishmaniasis (VL) is a protozoal disease manifested as prolonged fever, hepatosplenomegaly, anorexia, and weight loss. It shows a spectrum of epidemiological diversity: *Leishmania donovani* is the causative agent of kala-azar in East Africa and Indian subcontinent, whereas *L. infantum* is found in Latin America and the Mediterranean basin (Haldhar et al. 2011). Timely and accurate diagnosis of the condition is needed for proper treatment. Direct detection of amastigote forms in splenic or bone marrow aspirate is taken as the gold standard for diagnosis of VL, but these methods are invasive, carry high degree of risk for the patient and require technical expertise to detect. Recently, rk39 dip stick test has gained popularity be-

cause of high sensitivity, specificity, rapidity, and easy to use (Sundar et al. 1998). This can be used as field test for diagnosis of VL cases in kala-azar endemic areas (Khan et al. 2010). But, the major limitation of the test is false positive result in cured patients due to persistence of antibody. Urine and sputum samples from kala-azar patients proved as useful as serum for the diagnosis of VL by variety of diagnostic tests (Singh et al. 2009). However, the role of saliva is not established in the diagnosis of VL patients.

In this study, we compared serum, urine, and saliva samples of clinically diagnosed VL cases by rk39 dip stick test for the diagnosis of VL and also followed up to evaluate their utility in the prognosis of patients.

Materials and Methods

As per WHO, a VL case is defined as a person from an endemic region with fever for more than 2 weeks, splenomegaly and confirmed positive by either rapid diagnostic test based on rk39 antigen or biopsy (Huda MM et al. 2012).

A total of 72 clinically suspected VL patients were enrolled in the study from 2011 to 2012 within a period of 2 year. Ethical clearance for the study was not required as the samples were referred to the Microbiology Laboratory for the diagnostic purposes as a public health measure. Sixty one out of these 72 patients were diagnosed as VL as per the above definition and included in the test group. Rest 11 cases were included in the control group (n= 11), which consisted of pyrexia of unknown origin (PUO) (n= 1), post kala-azar dermal leishmaniasis (PKDL) (n= 3), past history of kala-azar (n= 2), mycosis fungoidosis (n= 1), malaria (n= 3), and filaria (n= 2). Serum, urine and saliva samples were collected from all patients (cases and controls) and tested for rk39dip stick test. Detailed clinical history of the patients such as fever, hepatosplenomegaly, residence, weight loss, loss of appetite, complete blood count, albumin: globulin ratio, liver function test, and past history of kala-azar were obtained from all patients.

Serum sample was tested for rk39 test (Kalaazar Detect™ Rapid Test, InBios International, USA) as per the instruction of the kit. Urine and saliva samples were tested with rk39 strip test following the similar procedure. Only 11 patients out of 61 VL confirmed cases in the test group could be followed up to 3–4 months after completion of treatment and rk39 test with serum, urine and saliva samples were repeated. All the rk39 test strips were graded from 3+ (equal to or more than control) to 1+ (faint) depending on

the intensity of test band in comparison with control band. Statistical analysis could not be done because of small sample size.

Results

All patients in the test group (n= 61) presented with fever for more than 2 weeks and hepatosplenomegaly during the first visit to the hospital. On examination, features like pancytopenia, anorexia, weight loss, hypoproteinemia, and reversal of albumin: globulin ratio was observed less frequently in comparison to the previous one.

Rk39 dip stick test with serum sample was positive in all the patients (100%) of test group (Table 1). Urine showed a complete concordance with serum exhibiting 100 % positivity in all the patients of the test group, whereas only 51 patients (83.6%) were found positive with saliva sample. Eleven patients from the test group were followed up after the completion of treatment and tested with serum, urine and saliva samples to observe the response of treatment. All the cases in the follow up group showed positive result with serum and urine samples in comparison to 10 cases with saliva.

We graded the intensity of the test band of the rk39 strip of these 11 patients before and after treatment as it indirectly correlates the disease status (Table 2). The test band intensity with serum sample before and after therapy obtained to be same among these patients. The band intensity found to be reduced with the urine and saliva samples in 4 cases after completion of therapy (Table 2). Among the control patients (n= 11), the rk39 test with serum, urine and saliva sample found positive in 7 (63.6%), 2 (18%), and 1 (9%) case respectively (Table 3).

Table 1. Comparison of rk39 test with serum, urine, and saliva samples in kalaazar patients and the control group

	Kalaazar pts. (n= 61)	F/up cases (n= 11)	Control (n= 11)
Serum Positive n (%)	61 (100)	11(100)	4(37)
Urine Positive n (%)	61 (100)	11(100)	2(18)
Saliva Positive n (%)	51 (83.6)	10(91)	1(9)

Table 2. Gradation of test band intensity rk39 strips in the kalaazar patients before and after treatment

Pts. Sl. No.	Serum	Serum F/up	Urine	Urine F/up	Saliva	Saliva F/up
1.	4+	4+	4+	4+	4+	4+
2.	3+	1+	1+	1+	1+	1+
3.	4+	4+	4+	2+	4+	2+
4.	4+	3+	3+	1+	1+	negative
5.	3+	4+	3+	4+	3+	3+
6.	4+	4+	4+	1+	4+	2+
7.	4+	4+	2+	2+	3+	1+
8.	4+	4+	4+	4+	4+	4+
9.	4+	4+	4+	2+	4+	4+
10.	4+	4+	4+	4+	4+	3+
11.	3+	2+	3+	3+	3+	1+

*1+ and 2+: band intensity weaker than control, 3+: band intensity matches with control, 4+: band intensity stronger than control

Table 3. Comparison of rk39 test with serum, urine, and saliva samples in control group

	Serum	Urine	Saliva
PKDL			
1.	4+	Neg	Neg
2.	1+	Neg	Neg
3.	1+	Neg	Neg
Past H/O Kalaazar			
1.	3+	Neg	Neg
2.	1+	1+	Neg
Pyrexia of Unknown Origin	2+	Neg	Neg
Mycosis fungoidosis	3+	2+	2+
Malaria			
1.	Neg	Neg	Neg
2.	Neg	Neg	Neg
Filaria			
1.	Neg	Neg	Neg
2.	Neg	Neg	Neg

Discussion

This study was designed to detect the performance of non-invasive samples for the diagnosis and prognosis of VL patients.

It is known that the test band intensity of the ICT strip correlates with the course of many

diseases (Moody 2002, Sako et al. 2011). The intensity is maximum during the active phase of the disease and gradually fades after treatment. In our study, urine was more useful in comparison to saliva for the diag-

nosis of kala-azar in the active stage of disease. During the first visit, the result of rk39 test with urine sample showed complete concordance with that of the serum sample. However, saliva was positive in 84 % cases. The urine and saliva samples showed similar results as with serum samples in follow up patients. It shows that, urine and saliva do not have much role in the determination of prognosis of VL patients. But, in the control group, the rk39 test with the saliva sample was found negative in majority (10/11, 91%) of cases in comparison to serum (4/11, 36.4 %). The test was also observed to be negative in majority of cases with urine sample.

Conditions such as past history of kala-azar, post kala-azar dermal leishmaniasis showed false positive result with serum sample in the control group. All these conditions found to be negative with saliva. The sensitivity and specificity of rk39 test is reported differently in different literatures. Singh et al. found sputum samples to be highly specific for VL patients (2009). Many literatures discussed about the false positive result of urine sample for the diagnosis of VL (Khan et al. 2010). But, in this study the false positive results by urine sample was much less.

The limitation of the study is that, it is comprised of less number of patients in the control group and also did not include the samples of healthy persons from endemic area. However, the results were encouraging. Non-invasive samples in endemic area can be used as an adjunct to the serum samples to decrease the rate of false positivity. Urine and saliva samples can also be used as part of surveillance in countries where animals behave as reservoir and help to control VL (Taran et al. 2007). The role of non-invasive samples in the prognosis of the patients is also difficult to establish because of small sample size in the follow up group. To overcome these limitations and to determine their role, further studies in field with a large sample size is required.

Conclusion

The present data highlights the usefulness of non-invasive samples such as urine and saliva as the diagnostic tools for the patients of kala-azar particularly in endemic regions. They were equally good with regard to as serum samples tested by rk39 strip. These samples can be collected easily in a non-invasive manner. Hence, rk39dip stick test with non-invasive samples such as urine and saliva may be adapted in routine diagnostics along with serum samples in endemic areas for the diagnosis of VL conditions. This may reduce the rate of false positivity with serum samples.

Acknowledgements

The authors declare that there is no conflict of interests.

References

- Khan MGM, Alam MS, Podder MP, Jamil KM, Haque R, Wagatsuma Y (2010) Evaluation of rk39 strip test using urine for diagnosis of visceral leishmaniasis in an endemic area in Bangladesh. *Parasit Vectors*. 3: 114.
- Haldhar AK, Sen P, Roy S (2011) Use of antimony in the treatment of Leishmaniasis: Current status and future direction. *Mol Biol Int*. Article ID 571242, 23 pages, doi:10.406/2011/571242.
- Huda MM, Hirve S, Siddique NA, Malaviya p, Banjara MR, Das P, Kansal S, Gurgung CK, Naznin E, Rijal S, Arana B, Kroeger A, Mondal D (2012) Active case detection in national visceral leishmaniasis in Bangladesh, India and Nepal: feasibility, performance and cost. *BMC Public Health*.12: 1001.
- Moody A (2002) Rapid diagnostic tests for malarial parasites. *Clin Microb Rev*. 15(1): 66–78.

Sundar S, Reed SG, Singh VP, Kumar PC, Murray HW (1998) Rapid accurate field diagnosis of Indian visceral leishmaniasis. *Lancet*. 351(9102): 563–565.

Singh D, Pandey K, Dsa VN, Das S, Kumar S, Tapno RK, Das P (2009) Novel noninvasive method for diagnosis of visceral leishmaniasis by rk39 testing of sputum samples. *J Clin Microbiol*. 47(8): 2684–2685.

Sako Y, Tappe D, Fukuda K, Kobayashi Y, Itoh S, Frosch M, Gruner B, Kern P,

Ito A (2011) Immunochromatographic Test with Recombinant Em18 Antigen for the Follow-Up Study of Alveolar Echinococcosis *Clin Vaccine Immunol*. 18(8): 1302–1305.

Taran M, Mohebbali M, Modaresi MH, Mamishi S, Mahmaudi M, Mojarad M (2007) Diagnosis of canine visceral leishmaniasis by ELISA using K39 sub recombinant antigen. *Iran J Publ Health*. 36(2): 1–6.