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Novel Noninvasive Method for Diagnosis of Visceral Leishmaniasis by rK39 Testing of Sputum Samples▽

Visceral leishmaniasis (VL) is a major public health problem in the eastern states in India, namely, Bihar, West Bengal, Jharkhand, and Eastern Uttar Pradesh. Bihar alone accounts for >90% of the total number of cases of kala-azar reported in India. The disease is predominantly found in poor and malnourished people (9). The development of a simple, noninvasive, cheap, and reliable diagnostic tool has been suggested as a prerequisite to controlling the disease (3, 4).

Ideally, diagnosis of kala-azar is done by direct demonstration of the parasite in splenic or bone marrow aspirates under the microscope (1, 5). Detection of the parasite in splenic aspirate is sensitive, but splenic aspiration is invasive and painful and carries the risk of serious or fatal hemorrhage, whereas bone marrow aspiration is safer and relatively easy, but detection of the parasite in bone marrow aspirate is less sensitive (60 to 85%), and both methods of detection demand technical support. In vitro cultivation of parasites is expensive and time consuming and requires expertise, thus severely restricting its use in routine diagnosis. Serological tests, though good, have their own limitations, viz., they cannot differentiate between present and past infection, asymptomatic cases versus clinical cures, etc. Sensitivity and specificity also vary from test to test, and some serological tests are not user friendly and not suitable for field conditions (2).

In the last few years, a serum immunoglobulin G (IgG)-based detection system using rK39 antigen has been found to be useful in diagnosing VL. The test was found to be highly sensitive, specific, simple, and reproducible, and results can be obtained within 10 to 15 min (7). To the best of our knowledge, the use of rK39 to detect VL infection in sputum samples has not been reported. This is perhaps the first early report of the use of rK39 strips (InBios, Seattle, WA) to detect active VL cases using sputum samples.

The assay was performed according to the manufacturer's protocol. Briefly, the bottom of the absorbent pad of an rK39 strip was dipped in a freshly collected sputum sample for 5 min. Two drops of the chase buffer provided with the kit was added to the pad and was allowed to migrate up to the strip by capillary action. The results were read after 10 min. The appearance of a red upper (control) band indicated the proper functioning of the test, and that of a red lower (test) band suggested the presence of anti-rK39 IgG in the sputum. The test was considered positive if two bands, viz., upper and lower, were present, whereas the test was considered negative if only the upper, control band appeared. A total of 505 sputum samples from confirmed VL patients and controls were screened with the rK39 strip test. Of the 126 microscopically confirmed VL cases, 125 (99.2%) were found positive by rK39 in sputum samples and all by rK39 in blood samples (100%) (Table 1). However, in controls from areas where the disease is endemic and where it is not endemic, the rK39 test was found to be more specific for sputum than for blood because sputum did not show any positive reaction, whereas 2.5% positivity in controls from areas of endemicity and no positivity in controls from areas of nonendemicity were observed using blood samples. This test was also found to be more specific in samples from the control group of patients with other diseases, because none of the sputum samples cross-reacted with the rK39 strip, whereas the blood samples of seven (5.73%) of these patients showed a positive reaction. The 2.5% and 5.73% positivities by rK39 strip test for blood samples from healthy controls from areas of endemicity and the control group with other diseases, respectively, were false positives because the positive samples in both groups did not show a *Leishmania donovani*-positive reaction by PCR (6). Moreover, these subjects did not exhibit clinical symptoms of VL, such as fever, pancytopenia, and hepatosplenomegaly. Even the follow-up over more than 6 months did not show conversion of any of these cases to symptomatic kala-azar cases. Furthermore, the PCR results (6) for sputum samples from both these groups were also negative for *L. donovani* infection. Lastly, it was not possible ethically to subject these cases to splenic or bone marrow aspiration.

TABLE 1. Comparison of sensitivities and specificities of rK39 strip test in diagnosis of kala-azar using sputum samples versus serum samples from *L. donovani* body-positive VL patients and controls

Group	No. in group	No. (%) of samples that were positive:[a]		
		For *L. donovani* bodies in SA or BMA	By rK39 strip test	
			Serum	Sputum
Confirmed VL patients	126	126 (100)	126 (100)	125 (99.2)
Healthy controls from areas of endemicity	155	NA	4 (2.5)	0 (0)
Healthy controls from areas of nonendemicity	102	NA	0 (0)	0 (0)
Control patients with other diseases[b]	122	NA	7 (5.73)	0 (0)
Total no. of samples	505			

[a] SA, splenic aspirate; BMA, bone marrow aspirate; NA, not applicable.
[b] Other diseases included tuberculosis ($n = 61$), leprosy ($n = 39$), and malaria ($n = 22$).

The present data clearly demonstrate that though the newly developed sputum-based rK39 test is only 0.80% less sensitive, it is much more specific than the rK39 test for diagnosis of VL using blood samples. This test can be easily adapted for routine diagnosis of VL using sputum samples. This will be highly beneficial for diagnosis of VL in difficult field conditions, as the test is purely noninvasive, uses easily collectable samples, and needs no special equipment or sophisticated technology, and the results can be read visually.

Finally, this diagnostic tool can be useful for the kala-azar

elimination program, which is scheduled to end by 2015 in the Indian subcontinent (8).

All authors declare no conflict of interest.

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