K39 Strip Test - Easy, Reliable and Cost-Effective Field Diagnosis for Visceral Leishmaniasis in India

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Abstract

Objective: A firm diagnosis of visceral leishmaniasis (VL) requires demonstration of the parasite in splenic or bone marrow aspirate. The aim of this prospective study was to assess the usefulness of K39 strip test as a noninvasive method of diagnosing visceral leishmaniasis under field conditions by testing serum antibody to the leishmanial antigen K39.

Material and Methods: One drop of serum/blood was applied to the sample application pad on the test strip, which was diluted with 2 drops of chase buffer solution. The development of two visible red lines indicates the presence of IgG anti-K39.

In the first phase of the study (2001), a total of 200 patients (Active VL-70, ex-VL-30, healthy endemic control-20 and patients with other tropical diseases-80) were tested with the K39 strip test at the School of Tropical Medicine, Kolkata.

In the second phase of the study (2002), the test was applied in a remote tribal area of West Bengal where an epidemic of VL had occurred. Thirty-two patients were identified in 207 villagers of the affected area; all of them were tested with the K39 strip test.

Results: In the first phase, all VL and ex-VL cases gave positive results (100%). Ten percent of the healthy endemic controls were positive. The test results were negative in all other prevalent tropical diseases (100%). The estimated sensitivity of the test was 100% and the specificity was 98.18%.

In the second phase of the study, all 32 patients of the epidemic were shown to be positive. All patients were treated with sodium stibogluconate injections and they recovered uneventfully.

Conclusions: K39 strip test is ideal for rapid reliable field diagnosis of visceral leishmaniasis. The test has high sensitivity and specificity but it remains positive long after treatment (up to 3 years).

INTRODUCTION

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. Thus aldehyde test remains the test of choice in majority of rural centers. Other serological tests use crude antigen preparations and one lacking in specificity. Thus cases are undiagnosed or misdiagnosed and the epidemic that began in 1977 continues unabated. But a good news in this bleak picture is the recent development of a recombinant product of 39 amino acid repeats (K39) encoded by a kinesin related gene of Leishmania chagasi. Enzyme linked immunosorbent assay (ELISA) using the K39 antigen has high sensitivity and specificity in the diagnosis of Indian VL and post kala-azar dermal leishmaniasis (PKDL). A promising ready to use strip test has also been developed as a rapid test for use in difficult field conditions including primary health care centers. We examined the sensitivity and specificity of one of the manufacturers’ immunochromatographic strip assay in a tertiary care hospital in Kolkata over a period of two years in the first phase and applied this test in a recent outbreak of VL in a remote village of Bardhaman district of West Bengal state in the second phase, to find its (K39) utility in Indian setting.
MATERIAL And METHODS

InBios International, Inc. Seattle, USA supplied the immunochromatographic strips for qualitative detection of antibodies to VL in serum. The test is a membrane-based immunoassay; the membrane is precoated with K39 antigen on the test line region and chicken anti-protein A on the control line region. During testing the serum sample reacts with the dye conjugate (Protein A-colloidal gold conjugate), which has been precoated in the test device. The mixture then migrates upwards on the membrane by the capillary action to react with the K39 antigen on the membrane and generates a red line. Regardless of the presence of antibodies to VL, as the mixture continues to migrate across the membrane to the immobilized chicken anti-protein A region a red-line at the control line region will also appear. The test is positive when two red lines appear in the middle of the nitrocellulose membrane, negative when only one redline appears and invalid when no line appears. One drop of serum is added to the sample application pad on the test strip, which is to be diluted with two drops of chase buffer solution (provided with the test kit). The result is to be read in 10 minutes. According to the manufacturer’s guide, 20 to 30 µl of serum is required. We modified the test by using only one drop of blood directly instead of serum in the second phase of the study in the field. Outcome of result was same.

In the first phase of our study (2001) at the School of Tropical Medicine, Kolkata, a total of 200 subjects including healthy endemic controls, cases with active VL as well as past history of VL and patients with other tropical diseases were tested with K39 strip test.

RESULTS

The Table 1 gives details of the test results. All 70 VL cases (parasitologically proved) gave positive results. Different investigators did parasitological diagnosis and K39 strip test; each one was unaware of other’s result. In all prevalent tropical diseases (malaria, filaria, tuberculosis, leprosy, AIDS, toxoplasmosis, hepatic amoebiosis, chronic hepatitis B and C) the test result was negative and no cross-reaction was noted. In 10 cases of autoimmune disorder (rheumatoid arthritis and systemic lupus erythematosus) test results were also negative. Out of 20 healthy controls of endemic areas only two were weakly positive. Thirty subjects with past history of VL showed positive results at time points 1,3,6,12,24,36 months after complete successful therapy. There were five patients for each time point. On the basis of these results the test is found to have 100% sensitivity and 98.18% specificity for diagnosis of VL including both past and present cases.

In the second phase of our study (2002) the test was applied in a remote tribal area of a West Bengal state, where an epidemic outbreak of fever with hepatosplenomegaly occurred. Thirty-two patients were identified in a total population of 207 villagers. Fifteen patients were under 14 years of age (47%). Number of male patients was 21 (39%).

Table 1: Results of K39 strip test at the School of Tropical Medicine, Kolkata (in the first phase)

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No</th>
<th>Positive No (%)</th>
<th>Negative No (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parasitologically proved VL cases</td>
<td>70</td>
<td>70 (100%)</td>
<td></td>
</tr>
<tr>
<td>Person with past history of VL</td>
<td>30</td>
<td>30 (100%)</td>
<td></td>
</tr>
<tr>
<td>Healthy endemic control</td>
<td>20</td>
<td>2 (10%)</td>
<td>18 (90%)</td>
</tr>
<tr>
<td>Patients with prolonged fever</td>
<td>20</td>
<td>2 (10%)</td>
<td>20 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasmodium falciparum malaria</td>
<td>10</td>
<td>10 (100%)</td>
<td></td>
</tr>
<tr>
<td>Bacteriologically positive TB cases</td>
<td>10</td>
<td>10 (100%)</td>
<td></td>
</tr>
<tr>
<td>Filaria</td>
<td>5</td>
<td>5 (100%)</td>
<td></td>
</tr>
<tr>
<td>Hepatic amoebiosis</td>
<td>5</td>
<td>5 (100%)</td>
<td></td>
</tr>
<tr>
<td>AIDS with associated infections</td>
<td>10</td>
<td>10 (100%)</td>
<td></td>
</tr>
<tr>
<td>Systemic lupus erythematous</td>
<td>5</td>
<td>5 (100%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>102</td>
<td>98</td>
</tr>
</tbody>
</table>

**Cases were ultimately diagnosed to be chronic viral hepatitis (B,C), cirrhosis of liver (alcoholic, idiopathic), chronic myeloid leukaemia and lymphoma.**

Duration of illness was one month to one year with average duration of 5.3 months (SD ± 3.03 months). Four patients were afebrile (12.5%). All patients had anaemia, weight loss, splenomegaly (from just palpable to 15 cm below left costal margin) and hepatomegaly (from just palpable to 9 cm below right costal margin). Two young male persons of the village had past history of VL and they were treated with sodium stibogluconate injections about 4 months back by a private practitioner of nearby subdivisional town. Sandflies were collected from the dwelling houses - all of which were Phlebotomous argentipes and 50% were fully blood-fed. Clinico-epidemiological findings suggested VL as causative factor for the outbreak. Nine patients were randomly selected irrespective of age and sex for bone marrow or splenic aspirations according to the platelet count. Leishman Donovan (LD) bodies were identified in all cases. All 32 patients were examined with K39 strip test. The results were universally positive. Different investigators did parasitological examination and strip test. All patients were treated with sodium stibogluconate injections (20 mg/kg body weight per day for 20 days intramuscularly - the maximum dose was 8.5 ml). All patients recovered uneventfully.

DISCUSSION

Very few studies have been done with K39 strip test for diagnosis of visceral leishmaniasis. Whereas in Sudan K39 strip test was positive in 67% of parasitologically confirmed
VL cases and 3% of endemic controls, in Nepal both sensitivity and specificity was 100%, and in a previous study from eastern India sensitivity and specificity of the strip test were estimated to be 100% and 98% respectively. The result of our study is similar as that of Sundar S et al who studied the patients from Bihar and Uttar Pradesh. We performed the test on VL patients of West Bengal province mainly. As VL occurs in these three provinces of India only, our study along with the previous one conclusively proves the strength of the test in patients representative of the population in whom this test would be applied in normal clinical practice as well as in epidemic set up.

Sundar S et al excluded patients who gave a history of previous VL. But we included 30 such patients who gave past history of VL successfully treated with sodium stibogluconate or amphotericin B injection 1 month to 3 years before. Astonishingly all 30 patients along with two healthy endemic controls gave positive results, though previous workers reported rapid decline of K39 titers in individuals with subclinical infection or in patients with overt symptoms who were treated and cured (by ELISA data). This seems to be the only drawback of the test; the test remains positive long after cure and though rarely, may become positive in healthy endemic subjects.

Otherwise 100% sensitivity and 98.18% specificity found in the present study proving that K39 epitope is conserved in Indian strains of Leishmania donovani and the test can be utilized in our settings. The test becomes positive early in the course of the disease (within one month) but it remains positive even after 3 years of complete treatment. So it is difficult to identify the relapsed or reinfeected VL cases, but fresh cases of clinically manifest VL can be reliably diagnosed with this test. The test is very easy to perform and no special equipment is needed; even the paramedical staff can be trained to do this test. The test is reasonably cheap (around 1$), does not require refrigeration and has a long shelf-life. The most important aspect of the test is that it gives result within a few minutes. In all of our parasitologically proved cases of VL the test gave positive result in less than a minute. In only two cases of endemic healthy controls positive result was obtained in 10 minutes when a very faint pink line appeared in test line region in comparison to a bright red line in top control line region. Though any line that appeared was regarded as a positive test result but degree of brightness and visibility can be regarded as a measure to distinguish between positive result in active VL/ex-VL cases and that in healthy endemic control. The barely visible faint positive reaction in the later group probably reflects low antibody titer due to subclinical infection in endemic area.

We think the rest is suitable for difficult field conditions in peripheral health centers in eastern India for easy diagnosis and management of VL cases. The test will be very helpful in epidemic outbreaks of VL. The wider use of K39 strip test would improve the specificity of field diagnosis of VL. Extensive field trials are needed to establish our opinion that the test can be adopted as the method of choice for diagnosis of VL at primary healthcare level. The test has the potentiality to replace all other diagnostic methods for VL, including invasive procedures (splenic or bone marrow aspiration) in future. Aspiration may however be necessary for identification of specific infecting species and research purposes. Aspiration of bone marrow or spleen would also clearly be needed for relapsed or reinfected cases of VL for which minority of cases may have to be referred to district or tertiary care hospital. Additional field-testing of the K39 strip test in other parts the world where VL is endemic is needed to confirm the applicability of our findings.

Acknowledgement

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REFERENCES