Leishmania infections are worldwide in distribution. The disease is endemic in the tropical and subtropical regions of 88 countries. There are an estimated 12 million cases worldwide; 1.5 to 2 million new cases occur every year. Cutaneous forms are most common (1 to 1.5 million cases per year), and 500,000 cases of visceral leishmaniasis (kala-azar, VL) occur every year. The resurgence of leishmaniasis, and its emergence in newer geographical areas, besides changing the clinical profile of infected patients, has put forward newer challenges in the areas of diagnosis, treatment, and disease control. VL is characterized by prolonged fever, splenomegaly, hepatomegaly, substantial weight loss, progressive anemia, pancytopenia, and hypergammaglobulinemia, and can be complicated by serious infections. It is the most severe form of leishmaniasis and, if left untreated, is usually fatal. Leishmania-HIV coinfection is regarded as an emerging disease especially in southern Europe, where 25 to 70% of adults with VL have AIDS as well. In the Indian subcontinent, the disease is almost exclusively caused by *L. donovani*.

Diagnosis of VL is complex because its clinical features are shared by a host of other commonly occurring diseases, such as malaria, typhoid and tuberculosis. Diagnosis of kala-azar is accomplished either by demonstration of amastigotes (LD bodies; aflagellar forms) in splenic or bone marrow smears or by culture of leishmania promastigotes (flagellar forms) from clinical specimen (most commonly in NNN media). While the sensitivity of splenic smears could be as high as > 95%, it carries the risk of severe/fatal haemorrhage, on the other hand bone marrow aspiration is painful, cumbersome and has a low sensitivity (60-85%). Culture can not be used for routine clinical diagnosis as it requires expensive equipment and expertise. In the endemic regions, splenic smear examination remains the most popular method for diagnosis, however, in patients with low parasite density, long searches are required to demonstrate the parasite. In addition to the difficulties associated with splenic aspiration, technical expertise remains a necessity for proper staining and microscopic demonstration of parasites.

Because of difficulties associated with parasite detection, several methods, based on non-specific rise in immunoglobulin levels, have been used for diagnosis of VL. Such methods (Napier’s formol gel or aldehyde test and the Chopra antimony test) are absolutely unreliable because of their non-specific nature with unacceptably low sensitivity and specificity, and these tests should have no place in the diagnosis of kala-azar, and best be abandoned. As in various other diseases, specific serodiagnostic tests for antibody detection have been employed in the diagnosis of kala-azar. Conventional methods for antibody detection included gel diffusion, complement fixation test, indirect hemagglutination test, indirect immunofluorescent antibody test (IFAT), and countercurrent immunoelectrophoresis. However, aside from practical difficulties at peripheral laboratories, the sensitivities and specificities of most of the above tests have been the limiting factors. Except for the IFAT, which is used on a limited scale, others are rarely used for routine diagnosis of VL. Direct agglutination test (DAT), based on agglutination of the trypsinized whole promastigotes, has been found to be useful in several endemic countries. DAT, in various studies including from India, has been shown to be 91 to 100% sensitive and 72 to 100% specific. Difficult field conditions, the fragility of aqueous antigen, the lack of cold chain, variations in the antigen batches, and lack of objectivity in test readings, have severely limited its applicability in India.

Attempts to commercialise it in India by Central Drug Research Institute, Lucknow failed to take off. Freeze-dried version of the antigen may overcome some of these handicaps, however, it has a long way to go for field application in India. Freeze-dried antigen need to be produced indigenously to make it affordable, and steps of the test simplified with one-step dilution and reduced incubation time for its meaningful application.

ELISA has been used as a serodiagnostic tool in leishmaniasis. The technique is highly sensitive, but its specificity depends upon the antigen used. Several antigens have been tried. Most commonly used is a crude soluble antigen (CSA) derived by lysing the leishmania promastigotes. Its sensitivity ranges from 80 to 100%, but cross-reactions with sera from patients with malaria, tuberculosis, and toxoplasmosis have been recorded. An amastigote-specific recombinant 39 amino acid antigen (rK39) derived from *L. chagasi* (etiological organism for kala-azar in Brazil), has been shown to be specific for antibodies in patients with VL caused by members of the *L. donovani* complex. This antigen, encoded by 117 base pair gene conserved in the kinesin region of the parasite, is highly sensitive and predictive of the disease. High antibody titers in immunocompetent patients with VL in India have been demonstrated. It has been found to be 100% sensitive and 100% specific in the diagnosis of VL by...
ELISA, and its titer correlates directly with the disease activity, and can be used in predicting response to chemotherapy. In Indian kala-azar, anti-rK39 antibody titers were 59-fold higher than those of antibody against CSA at the time of diagnosis, falling sharply with successful therapy during post-treatment and follow-up periods. Need of sophisticated equipment, skilled manpower, electricity and high cost have prevented widespread application of these techniques (some of which are excellent).

Because of the difficult conditions prevailing in areas of endemicity, there has been an urgent need for a simple, cheap, rapid and accurate test with high sensitivity and specificity. A promising ready-to-use immunochromatographic strip test based on rk39 antigen has been developed as a rapid test for use in difficult field conditions. In this issue of the Journal, Goswami et al report successful use of this rapid test in diagnosis of kala-azar in West Bengal. After our first report of its clinical usefulness five years ago, its utility has been demonstrated from all parts of the world. In Indian subcontinent, this rapid strip test is very successful, though not so much in Europe or Africa. In HIV coinfected patients with VL, its sensitivity is low (71%) with lower anti-rk39 titers, similarly in Sudan, in immunocompetent individuals, its sensitivity was 67%.

Limitations of rapid rK39 immunochromatographic test: A very important limitation of this test is the presence of antibodies in healthy controls hailing from the endemic region. While such reactions might be considered to be false positive, these probably represent subclinical infections, 12-39% healthy individuals might demonstrate positive strip test (unpublished observations), and a positive test in a patient suffering from an illness mimicking signs and symptoms of VL might lead to an erroneous diagnosis of VL. Though in individuals presenting with typical signs and symptoms of VL with suggestive laboratory findings (pancytopenia), a positive strip test might be a strong indicator of the disease, however, one needs to be careful in ones enthusiasm, not to treat those with false positive results. Persistence of antibodies well beyond cure, or in healthy individuals and inability of some patients to produce enough antibodies are inherent limitations with antibody-based diagnostics. These limit utility of this rapid test in predicting cure or diagnosing relapses as anti-rK39 antibody/strip test remain positive for years after successful cure.

Notwithstanding these limitations, the rK39 immunochromatographic strip test has proven to be versatile in predicting acute infection, and it is the only available format for diagnosis of VL with acceptable sensitivity and specificity levels which is also simple and inexpensive (1 to 1.5 US dollars for each strip), and it can be performed even by paramedics in prevailing difficult field conditions. It turns positive very early in the course of the disease. Recently US FDA has approved rK39 Strip test from InBios International, USA for diagnosis of kala-azar. Another format of this test by DiaMed, Switzerland is under evaluation.

Antigen detection: Antigen detection is more specific than antibody-based techniques. This method is also useful in the diagnosis of disease in cases where there is deficient antibody production (as in AIDS patients). A new latex agglutination test (KATEX) using monoclonal antibodies for detecting leishmanial antigen in urine of patients with VL has shown sensitivities between 68 and 100% and a specificity of 100% in preliminary trials. The antigen is detected quite early during the infection, and the results of animal experiments suggest that the amount of detectable antigen tends to decline rapidly following chemotherapy. These antigens are also detectable in blood and efforts are underway towards its further refinement.

DNA detection: Detection of parasitic DNA in blood after polymerase chain reaction (PCR) amplification has been applied in the clinical diagnosis of VL, and several Indian laboratories have developed primers which are species-specific and are able to detect very low levels of parasitemia. However, the technique is yet to be standardized for clinical application in India. PCR has the potential to predict cure or detect early relapse. But being highly sophisticated, it needs expensive equipment, skilled personnel, and running cost is likely to be very high. In a study reported from India the sensitivity of PCR with whole blood is 96% and 93.8% for VL and post kala-azar dermal leishmaniasis, respectively. PCR can also be used to distinguish between relapse and reinfection in treated VL patients using restriction fragment length polymorphism (RFLP) analysis. Important drawbacks of PCR include high possibility of cross-contamination, and positive results in healthy individuals from endemic regions, and these may lead to the erroneous conclusion that they suffer from VL.

Though many noninvasive tests are available for the diagnosis of leishmaniasis, none have become popular in areas of endemicity. They are expensive, require skilled personnel, expensive equipment, and electricity, and are technically demanding. Parasite diagnosis by splenic and marrow smear examination remain the “gold standard”, with their usual limitations. rK39 strip test in an important step forward in the diagnosis of VL, and it has the potential to be used under field conditions. It has already become popular in endemic regions of the country. Other tests, which are likely candidates for diagnosis and prognosis of leishmaniasis in the future, are KATEX and a field-adaptable version of PCR, which has to be simple, inexpensive, and easily available.

References


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**Announcement**

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