Laboratory Diagnosis of Visceral Leishmaniasis

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The diagnosis of visceral leishnianiasis (VL) is based upon the demonstration of "Leishman Donovan bodies" in smears obtained routinely from bone marrow, spleen, liver and lymphnodes of patients. Inoculation of infected tissues into specific culture media and susceptible animals like BALB/c mice and hamsters facilitates the diagnosis. Microscopic examination of splenic aspirates from patients generally yield higher positivity rates when compared with aspirates from bone marrow, liver or lymph nodes[1-4]. Splenic aspiration however, is best avoided in children under 5 and in patients with platelet counts of below 5000 per cubic mm. Leishmania parasites am not usually found in the peripheral blood smears of patients, unless they are severely immunocomprornised[5]. Occasionally, the parasites have been demonstrated in the skin snip smears or biopsies[6], nasal and oral secretions, tonsillopharyngeal mucosa and urine centrifugates of VL patients[7,8].

The use of blood serology for the detection and confirmation of disease has been practised since the early twenties when Napier[9], developed the aldehyde test. This test is based on the principle of precipitating increased amounts of globulins in the sera of patients by fonualdehyde. Ancient as it may be, the aldehyde test is still very helpful in facilitating diagnosis in areas where VL is endemic and little other facilities for diagnosis are available[10]. Other serological techniques, such as, the complement-fixation test[11], the indirect haemagglutination test[14,15] and the counter immunoelectrophoresis[15-17] have also been successfully employed for the serological diagnosis of VL.

The more sensitive techniques like the Indirect Fluorescent Antibody Test[16,17], and the Enzyme-Linked Immunoassay[20] (ELISA), demonstrate varying degree of cross reactivity with sera of patients with malaria. American trypanosomiasis and Hansen's disease[18-22]. Most serum cross reactions, though can be eliminated if the assays are carried out at higher semm dilutions[23,24]. The competitive ELISA employing monoclonal antibodies has been evaluated to be a highly sensitive and specific technique[25]. The Direct Agglutination Test[26] (DAT) is also a simple and economic diagnostic test and has a high sensitivity and specificity[4,27-31]. Antibodies to leishmartia have also been detected in the saliva of patients using the DAT[32].

Major surface antigens of the parasite are expressed both by the promastigotes and amastigotes of the Leishmania species[33,34]. These surface antigens react with sera from Kala-azar patients[35]. Small amounts of circulating antigens have been detected in patients suffering from L. donovani infections using competitive ELISA[36]. Similarly, circulating L. donovani glycoproteins have also been identified in VL patients by monoclonal antibodies[37]. Parasite antigens have also been detected within the circulating high molecular weight immune complexes in the sera of patients with L. chagasi infections. Immunizations of BALB/c mice with these precipitates of these immune complexes elicit specific anti-leishmania antibodies[38].

During the nineteen eighties, the new era of molecular biology techniques dawned upon the horizon of diagnosis in VL, promising more reliable and direct methods of diagnosis and eliminating possibly the need for culturing the organisms. Radioactive kinetoplast DNA (kDNA) probes were developed first and these distinguished between New World species[39,40]. A potentially sensitive e DNA probe (Lmet 2), specific for L. donovani complex recognized both promastigotes and amastigotes of the L. donovani complex. It can identify as few as 100 parasites on dot blots and parasites in field prepared squashed blots of sandflies[41]. It has also been shown to react with dot or touch blot preparations obtained from

splenic aspirates, liver and skin biopsies of patients[42]. The sensitivity of this probe was further enhanced when adapted to a non-radioactive chemiluminescent detection system[43]. The sequence analysis of L. donovani kDNA has led to the development of synthetic oligonucleotides, which have been used as primers in the polymerase chain reaction (PCR) to amplify L. donovani kDNA from very small amounts of splenic aspirates and blood samples of VL patients[44].

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