Visceral Leishmaniasis: Current Concepts in Management

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INTRODUCTION

Visceral leishamaniasis (VL, Kala - azar) is a disseminated intracellular protozoal infection caused by *leishmania donovani* complex which includes three species, *L donovani, L infantum* and *L chagasi*. As many as 500,000 new cases of VL occur world wide every year, 90% of which occur in five countries; Bangladesh, Brazil, India, Nepal and Sudan[1]. In India, Bihar, adjoining areas of West Bengal, Jharkhand and Uttar Pradesh account for about half the world's burden of VL[2].

VL is characterized by prolonged fever, splenomegaly, progressive anemia, pancytopenia, hypergammaglobulinemia and increased susceptibility to serious infections. VL is uninformly fatal if left untreated. After recovery, some patients (50% in Sudan and 1-3% in India) develop post kala azar dermal leishmaniasis (PKDL)[3]. PKDL patients serve as human reservoir for the protozoan parasite and play an important role in VL transmission.

Leishmaniasis results in a life long latent infection and if immunoparesis supervenes *leishmaniae* can become opportunistic pathogens through reactivation or new infection. Leishmania-HIV coinfection is regarded as an emerging disease in Southern Europe, Brazil and Africa[4]. Leishmania/HIV co-infection impose specific difficulties in terms of diagnosis and treatment. The usual clinical features (fever, weight loss, hepatosplenomegaly, inflammation of the lymph nodes) are not always present. In these patients leishmaniasis can present with gastrointestinal involvement, ascitis, pleural or pericardial effusion, involvement of lungs, tonsil, skin and even as a widely disseminated disease. The presence of leishmania parasite in the peripheral blood makes these patients a reservoir and source of infection for the vectors, and transmission among intravenous drug users by the use of shared syringes have also been demonstrated. In these patients treatment failure, relapses due to drug resistance and drug toxicity are very common.

Diagnosis

The diagnosis of VL is complicated by the fact that its clinical features are shared by a number of commonly occurring diseases like malaria, typhoid, tuberculosis etc., the parasite is sequestered in the tissues (spleen, bone marrow, lymph nodes) and poor economic conditions prevail in the endemic region. All these factors lead to difficulty in the diagnosis.

The diagnosis of leishmaniasis can be made by

  i. Demonstration of parasite

 ii. Immunodiagnosis

    a. Detection of antileishmanial antibodies

    b. Detection of parasite antigen

iii. Molecular diagnostic methods

Demonstration of Parasites

The definite diagnosis of VL is accomplished by demonstration of amastigotes in the tissue of relevance like spleen, bone marrow, lymph node or in the buffy coat of peripheral blood. The sensitivity of splenic smear is excellent (>95%), but the procedure may be associated with fatal intraabdominal hemorrhage in untrained hands. It should be avoided in patients with a platelet count of <40,000/l and a prothrombin time of >5 seconds over control. The diagnostic yield of bone marrow smear is low (60-85%) and the procedure is painful and cumbersome[4]. Although demonstration of parasite

remains the gold standard its use in endemic areas is limited, by factors like low yield of tissue aspirates (except splenic aspirate), traumatic procedure and requirement of trained personnel for the identification of amastigotes. Cultures are now rarely used for the routine diagnosis of VL.

Immunodiagnosis

Patients with VL develop high titre of antibodies against parasite antigens which are useful in the serodiagnosis of VL. There are many serological tests for the diagnosis VL, among which indirect fluorescent antibody test (IFAT), direct agglutination test (DAT), ELISA and rk39 based immunochromatographic stip test are commonly used.

IFAT has been used in a limited scale is the diagnosis of VL as it cross reacts with patients of tuberculosis, leprosy and trypanosomiasis[5]. Moreover, the procedure is cumbersome and not suitable for field conditions.

DAT measures antileishmania antibody titres using a freeze dried antigen[6]. A recent metaanalysis showed that DAT has sensitivity of 94.8% and specificity of 85.9%[7]. However, multiple steps, prolonged incubation time, non-standardization of test readings and handling of multiple samples are some of the handicaps of this test. Nevertheless, it is used as a tool for the diagnosis of VL in the field laboratories of Sudan, where patients with a titre of 1:6400 are treated and those between 1:400 and 1:6400 need a splenic aspirate to confirm the diagnosis.

ELISA is a valuable test in the diagnosis of VL. Previously crude soluble antigen, was used as antigen but now many recombinant antigens are available. The most promising recombinant antigen is rk39 which is a member of kinesin family containing 39 amino acid repeat derived from *L. chagasi*. Studies with this antigen have shown very high sensitivity and specificity in the diagnosis of VL by ELISA[8,9]. In the prevailing field conditions, sophisticated methods like ELISA cannot be employed and there is a need for a simple, rapid, cheap test with a good sensitivity and specificity.

The rk39 based immunochromatographic strip test is one such test which is cheap, rapid, simple and has high sensitivity (93.9%) and specificity (90.6%) as shown in a metaanalysis.[7] However, there is a regional difference in the test; with the sensitivity being higher is South Asia (~99%) as compared to Sudan[10]. This difference could be due to differences in the antibody responses observed in different ethnic groups. These serological test though very convenient have certain drawbacks. They remain positive for several years after

cure and thus cannot be used to detect relapse or re infection. Another limitation of this test is seroreactivity in healthy endemic controls which could be due to subclinical infection in these countries[11]. Notwithstanding these drawbacks the rk39 based immunochromatographic strip test is an excellent tool for the diagnosis of VL in the Indian subcontinent. Serological test has another limitation as they may be negative in upto 50% of HIV positive patients co-infected with visceral leishmanias[12]. Tests are therefore needed that can identify active disease and are useful in immunodeficient patients.

Antigen Detection

Antigen detection is more specific than antibody based test. They are also useful in patients with immunosuppression. A latex agglutination test (KATEX) has been developed to detect leishmanial antigen in the urine of patients of VL which disappears after cure. It has been tested in Asia and Africa and specificity has ranged from 47-95%[13,14]. Further studies are needed to validate this test.

Molecular Diagnostic Methods

PCR assays are highly sensitive and specific tests for the diagnosis of VL which can be performed with a broad range of clinical samples[15,16]. PCR analysis of whole blood and bone marrow samples have shown excellent sensitivity in patients with VL and HIV co-infection. Simple field adaptable versions of PCR needs to developed before it can be used in the prevailing conditions.

TREATMENT

Sodium Stibogluconate

Sodium stibogluconate a pentavalent antimony compound (Sb^v) is the drug of choice for the treatment of VL globally. It is given parenterally in a dose of 20 mg/kg daily for 30 days. However, in the last few years over 60% of patient in North Bihar are unresponsive to Sb^v[17]. The exact mechanism of action is not known. It has been demonstrated that antimony alters the thiol redox potential which makes the parasite susceptible to oxidative stress. The adverse effects of the drug are nausea, vomiting, metallic taste, pancreatitis, myelosuppression, liver and kidney damage. ECG (QTc) renal and liver functions should be monitored regularly. Mortality from Sb^v treatment range from 3-12%, and it is time to consider other drugs as first line treatment for VL as safer drugs become available.

Caution must be exercised before using Sb^v from new manufactures as bad batches causes fatal cardio-toxicity[18]. Sb^v is also used in PKDL which requires prolonged treatment >120 days in India and 60 days in Sudan.

Pertamidine Isethionate

Pertamidine isethionate is a diamidine compound previously used as a second line treatment for VL. Its use has been abandoned because it causes irreversible insulin dependent diabetes mellitus.

Amphotericin B and its Lipid Formulation

Amphotericin B is an antifungal macrolide antibiotic derived from *Streptomyces nodosus*. It has high affinity for ergosterol which is the main component of leishmania and causes cationic and anionic influx via formation of aqueous pores resulting in cell lysis. It is given in a dose of 0.75-1 mg/kg for 15-20 infusions either daily or on alternate days and has consistently produced cure rates of about 97%[19]. It has now become the drug of choice in North Bihar. The adverse effects are infusion based reaction like high fever, chills, rigor, thrombo-phlebitis and occasionally serious toxicities like myocarditis, severe hypokalemia, renal dysfunction and even death. To ameliorate these toxic effects, lipid formulations of amphotericin B have been devised in which deoxycholate is replaced by other lipids. This facilitates its preferential uptake by reticuloendothelial cells i.e. achieving targeted drug delivery and resulting in increasing efficacy and reduced toxicity. The commercially available lipid formulations are:

  i. Liposomal amphotericin B (Ambisome)
 ii. Amphotericin B lipid complex (Abelect)
iii. Amphotericin B colloidal dispersion (Amphocil)

In Indian VL, ambisome in a dose of 6 mg/kg (2 mg/kg×3) cured 100% patients and 3.75 mg/kg cure 89% patients[20]. Ambisome is also recommended in immuno-compromised patients in a total dose of 40 mg/kg spread over 38 days. However, this has not been compared with shorter regimens and relapse is very common. These lipid formulation do not have nephrotoxicity and have lower infusion reactions, they require shorter hospital stay and have cure rates comparable to amphotericin B, however, their cost is prohibitive.

Miltefosine

It is an alkyl-phospholipid derivative originally registered as an anti-neoplastic agent. It is the first orally active antileishmanial agent. Studies have shown cure rates of 94% and efficacy has also been reported in Sb^v resistant cases[21]. Doses recommendation are 50 mg single dose in patients weighing <25 kg and 50 mg twice daily for those >25 kg for 28 days. In children daily dose of 2.5 mg/kg for 28 days in recommended. Adverse effects includes gastrointestinal disturbances and renal toxicity. Miltefosine is teratogenic, it is contraindicated in pregnancy and women of child bearing age group.

Paramomycin

Paramomycin (Aminosidine) is obtained from cultures of *Streptomyces rimosus* and belongs to the class of aminoglycosides. Studies in India have shown that the cure rates with paramomycin are 93-97%[22]. A pivotal phase III trial has just been completed in Bihar, and preliminary analysis suggested that its efficacy is comparable to other licensed drug and tolerability is excellent. Dose is 15 mg/kg/day of paromomycin sulfate (11 mg/kg of base) for 21 days. A major advantage of this drug is its low cost. The was approved in India for indication of VL, based on the pivotal phase III trial on 31 August 2006, and it is the first time that this drug has been permitted anywhere in the world.

Sitamaquine

It is another orally active agent which has shown antileishmanial activity. In a multicentric phase II trial in India, sitamaquine demonstrated excellent anti-leishmanial activity at a daily dose of 1.75-2 mg/kg for 28 days[23]. More studies are needed to evaluate clinical efficacy and safety issues with this drug.

Immunomodulations

Leishmania infection is associated with a depression of T helper type 1 cell and preferential expansion of T helper type 2 cells, and therefore modulating T helper cells towards Th1 response is considered as a promising strategy. However, a large randomized study in India comparing Sb^v alone with Sb^v IFN gamma showed disappointingly low cure rates[24].

CONCLUSION

The emergence of leishmaniasis in new geographic areas (Southern Europe), new host population (e.g. HIV infected persons) and changing clinical profile of infected persons has increased the need for rapid field applicable diagnostic test, safe inexpensive and effective treatment regimens. With the availability of drugs like miltefosine, paramomycin, lipid formulation of amphotericin B, short course combination chemotherapy may become the mode of therapy of VL in the future.

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