Management of visceral leishmaniasis: Indian perspective

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ABSTRACT

Diagnosis and treatment of Indian visceral leishmaniasis (VL) is extremely unsatisfactory. For diagnosis, demonstration of parasites in splenic/marrow smears remains the gold standard, though k39 rapid strip test is a useful method in regions where access to parasite demonstration is difficult. pentavalent antimony remains the mainstay for the treatment of all forms of leishmaniasis globally; however, development of large-scale antimony resistance in Bihar has necessitated search for alternative drugs. Amphotericin B is the most effective, though toxic, drug for patients with refractory VL. Lipid formulations of amphotericin B, though safe and effective, are too expensive to be useful for poor patients of this region. These hold advantage as large quantity of the drug can safely be given over a short period of time, thus leading to a decrease in the hospital stay to a few days instead of several weeks. Oral miltefosine, an alkyl phospholipid, has recently been approved and marketed in India for the treatment of VL. Miltefosine cures 94% patients with VL if given in a daily dose of 50–100 mg for 28 days. Most common adverse events are mild vomiting and diarrhea. Paromomycin, an amino glycoside, is undergoing a pivotal phase-III clinical trial, and is likely to be approved and available to patients with VL at an affordable cost. To protect the already scarce inventory of antileishmanial drugs, it is time that combination chemotherapy is introduced for the treatment of VL in India.

KEY WORDS: Amphotericin B, kala-azar, miltefosine, paromomycin, pentavalent antimony, visceral leishmaniasis

Leishmaniasis encompasses a group of diseases, caused by the obligate intracellular parasite of the genus *Leishmania*. The disease is transmitted by sandfly, which inoculates the flagellated promastigotes into the skin of the host.[1] In humans, these promastigotes are taken up by macrophages or dendrite cells and transformed into flagellar amastigotes. The future course of infection depends upon the strain of *Leishmania* and the immune response mounted by the host. Visceral leishmaniasis (VL, kala-azar) is the systemic and disseminated form of the disease, where the primary target of infection is the bone marrow, the spleen and the liver. It is typically caused by *L. donovani* complex, which includes three species, *L. donovani* (Indian subcontinent and East Africa), *L. infantum* (Mediterranean basin) and *L. chagasi* (Latin America). VL, the most severe form of Leishmaniasis, is characterized by prolonged irregular fever, splenomegaly, hepatomegaly, progressive anemia and pancytopenia along with hypergamm-aglobulinemia. It is uniformly fatal unless treated. Although VL is endemic in 62 countries, 90% of the estimated 500,000 new cases, which occur annually, are confined to the rural areas of India, Nepal, Bangladesh, Sudan and Brazil; as many as one-half of these cases occur in India.[2] There are 30–100 subclinical infections for every overt case of VL.[3] Malnutrition, immunosuppressive drugs or immunocompromised state (HIV infection) can convert these subclinical cases into clinical disease.[4],[5] HIV–*Leishmania* co-infection is being regarded as an emerging disease, especially in Southern Europe where 25–70% of adults with VL have AIDS as well. VL is now being considered as an important opportunistic infection, among AIDS cases in the Mediterranean basin.[5]–[7] With the spreading global pandemic of HIV infection, the HIV–VL co-infection continues to rise, notably in India and Brazil. In these countries, it threatens to urbanize the VL infection, which is essentially a disease of rural areas.

VL in different parts of the world shows considerable epidemiological variation and the clinical presentation also varies considerably; consequently the control measures, case finding and treatment modalities differ in different geographical areas. The scope of the present article is essentially a review of the management of Indian VL, which will be discussed under two heads: (i) diagnosis of VL and (ii) treatment of VL.

Diagnosis of VL

Clinical features of VL are shared by other commonly occurring diseases, such as malaria, typhoid and tuberculosis. In the early infection before the classical triad of fever, splenomegaly and pancytopenia has set in, the diagnosis remains elusive, leading to considerable delay in diagnosis.[8] Moreover, since the parasite is largely sequestered in the spleen, liver and bone marrow, their demonstration entails embarking upon traumatic interventions, which further adds complexity in making the diagnosis.

The definitive diagnosis of VL requires demonstration of the parasite. This is done by (i) demonstration of the parasite by light microscopic examination of stained splenic or bone marrow aspirate, in vitro culture or animal inoculation (ii) detection of parasite DNA in tissue specimen or in peripheral blood.[9] Although, the parasite can be demonstrated in buffy coat of peripheral blood and in bone marrow aspirate, the diagnostic sensitivity of splenic aspirate is highest (~98%). In preparations stained with Giemsa or Leishman stain, the amastigote appears as round or oval bodies measuring 2–3 mm and found intracellularly in macrophages or monocytes. The presence of dark red or violet nucleus and kinetoplast in the pale blue cytoplasm of amastigotes helps in identifying them.[10] Although when performed by experienced hand splenic aspiration is safe and relatively painless as compared to bone marrow aspiration, it has the potential of causing fatal bleeding from the enlarged and soft spleen. This risk can be obviated to a certain extent by deferring splenic aspiration in patients with a platelet count of less than 40,000/ml and prothrombin time not more than 5 s over control.[9] Moreover, the sensitivity of the test is subject to the observer's expertise and requires searching several fields to demonstrate scanty parasites. Culture of the parasite in monophasic (Schnieder's insect medium or Graces' medium) or diphasic (Novy–McNeal Nicolle Medium) medium or animal inoculation in Golden Hamster improves the sensitivity of detection, but these methods are seldom used outside of research laboratories.

Detection of parasite DNA in the peripheral blood is emerging as a non-traumatic and sensitive tool for the diagnosis of VL; primers are designed to amplify the conserved sequences found in minicircles of kinetoplast DNA (kDNA) which is an eminently suitable target for PCR, as kinetoplasts possess thousands of copies of minicircle DNA.[11]–[13] In a study reported from India, in which a species-specific primer for *L. donovani* (LD1primer) was used, the sensitivity of PCR with whole blood from VL patients was 96%.[14] We also evaluated this primer in parasitologically proved VL patients. PCR could detect Leishmanial DNA from peripheral blood of 100 out of 101 patients (sensitivity >99%) and it was 100% specific (S. Sundar, unpublished). However, until this process is standardized and widely available, the diagnosis of VL will essentially rest upon the demonstration of parasite from tissue specimen. Technical skill, expertise and a well equipped laboratory, which are essential for all of the above-mentioned tests, are seldom available in the areas where VL is prevalent.

There has been a need for rapid, accurate, field-suitable and non-invasive methods for diagnosis of VL. Traditionally, increase in immunoglobulins in VL patients has been exploited to suspect the diagnosis of VL. Aldehyde test, Chopra Antimony test and Napier formal gel, have been used for this purpose. However, these tests lack specificity and are highly unreliable. Subsequently, a range of assays were developed to detect specific antileishmanial antibody [ELISA and Direct Agglutination Test (DAT)].[15]–[19] These methods have been widely used the world over. However, the usefulness of these assays is limited by their variable sensitivity and specificity besides the requirement for electricity, refrigeration and a well equipped laboratory. A recombinant product of 39 amino acid (k39) repeats found in kinesin gene of *L. chagasi* has been highly conserved on amastigotes of *Leishmania* species that cause visceral infection.[20],[21] When used in ELISA testing, circulating anti k39 IgG was detectable in 98–100% of Asian patients with VL.[19],[22],[23] A promising ready to use immunochromatographic strip test impregnated with k39 has been developed for field conditions, obviating the need for costly infrastructure. When tested with blood and serum samples from Indian patients, the strip tested positive, with overall sensitivity of 100% and specificity of 97%.[24],[25] It has emerged as an important tool in evaluating VL patients in field conditions. However, due to the inherent limitation of antibody-based tests and considering the arduous therapy of VL, it is not considered optimal to initiate therapy based on serological tests alone. Antibody-based tests fail to differentiate between current and past infection as well those with subclinical infection. The persistence of antibodies post treatment, limits its usefulness in relapsing patients.[19] They also tend to lose sensitivity in immunocompromised hosts.[5],[26] Notwithstanding these limitations, encouraged by the high titer of anti-k39 antibody produced in Indian VL patients, we evaluated the utility of this test in making non-invasive clinical management of suspected VL patients. In this study, 143 Indian patients with suspected kala-azar were included. Out of the 120 strip test positive symptomatic subjects, 119 responded to amphotericin B (Amp B), while in 23 strip test negative subjects, only two subsequently proved to be suffering from VL. The result suggested that k39 strip test when used judiciously, taking into consideration the clinical presentation, can obviate the need for organ aspiration.[27]

Treatment of VL

The treatment options for VL are limited and far from satisfactory. Pentavalent antimonials (sodium stibogluconate; Sb^V) and Amp B have been the two drugs with proven efficacy for treatment of VL. Both the drugs are given parenteraly and are potentially toxic. Paromomycin, an aminoglycoside antibiotic, is currently undergoing Phase-III clinical trail. Recently, for the first time an oral antileishmanial drug "Miltefosine" has proved effective in treating Indian VL and has been registered for treatment of VL.[28],[29]

For more than six decades, Sb^V has been the cornerstone of the treatment of leishmaniasis including VL, in most parts of the world, and 98–99% of patients of VL respond well to the drug.[30] Generic sodium stibogluconate from Indian manufacturers (Albert David Ltd., Kolkata, India) is cheaper than the branded product (Pentostam; GSK), and has been reported to be equally effective and safe in controlled studies from different parts of the world.[31]–[34] For treatment of Indian VL, it is used as daily intravenous (IV) or intramuscular (IM) injections in a dose of 20 mg/kg body weight for 30 days.[35] However, the hyperendemic areas of VL in North Bihar have the unique distinction of being the only region in the world from where widespread primary failure of Sb^V has been reported.[36],[37] Like elsewhere in the world, in India as well, Sb^V has been used to treat VL for several decades. Till the late

1970s, a small daily dose (10 mg/kg; 600 mg maximum) for 6–10 days was considered adequate for treatment.[38] Increasing refractoriness to Sb^V led to periodic escalation of the dose of 20 mg/kg/day for 30–40 days, which was reached in the late 1980s.[39],[40] Any increase in the dose beyond this would seriously compromise the safety of the patient. However, with this dosage schedule also, primary unresponsiveness was 52%, whereas 8% patients relapsed.[41] Incidentally, the drug is still responsive in the neighboring state of Uttar Pradesh, where only 2% patients failed treatment.[36] Thus, Sb^V still remains the treatment of choice for VL in regions like West Bengal or Eastern Uttar Pradesh, and Jharkhand. The resistance is on account of relapse after suboptimal treatment with Sb^V, which leads to selection of resistant mutants, and these are recycled in anthroponotic foci with a high rate of transmission. Due to this fact, other antileishmanial drugs are likely to meet a similar fate and there is urgent need to protect these drugs before we lose them. The problem is likely to worsen with the increasing incidence of HIV/VL co-infection, as the response to treatment is not as good in these patients and relapse is a rule. These relapsing patients may provide a human reservoir for resistant *Leishmania*.[5],[42]

Pentamidine isothionate was the first drug to be used in Sb^V refractory patients.[43] However, the declining efficacy of the drug, coupled with serious adverse effects like hypoglycemia, shock, occasional death and above all insulin-dependent diabetes mellitus in a significant proportion of patients, lead to almost abandonment of the drug.[44]–[46]

For Sb^V refractory patients, Amp B is currently the alternative treatment of choice. It is a polyene antibiotic used as an antifungal drug, but it also has excellent antileishmanial activity. In Sb^V refractory regions in India, it has been used extensively with excellent results. Amp B is used in doses of 0.75–1 mg/kg in 5% dextrose infusion up to a total of 15–20 infusions either daily or on alternate days with efficacy of nearly 100%, and resistance has not been reported.[46],[47] It has been recommended as a first line drug by the Indian National Expert Committee for Sb^V refractory regions.[32] In a recent multicenter study, when Amph B was used as a comparative drug on 99 patients in the dose of 1 mg/kg IV every other day for a total of 15 injections, a final cure rate of 97% was achieved at 6 months follow up.[48] Its drawbacks include high cost, requirement for hospitalization for 5–6 weeks, limited and erratic availability and the need for close monitoring. It is also a toxic drug and its adverse reactions include – universal occurrence of infusion-related fever, chills, vomiting and diarrhea, high incidence of thrombophlebitis and serious and occasionally fatal hypokalemia, renal impairment and cardiac toxicity.

To improve the safety of conventional Amp B, lipid formulations have been developed in which deoxycholate has been replaced with other lipids. Clinical application of lipid formulations of Amp B has been one of the most remarkable developments for chemotherapy of VL. The lipid Amp B is taken up by the macrophages and very little free drug is available, minimizing the organ toxicity. These formulations have the advantages that a large daily dose can be given permitting the total dose to be delivered over a short period, minimizing the hospital stay and cost and several fold increase in the capacity of the hospitals to treat larger number of patients. AmBisome (Liposomal Amp B; Gilead Sciences, Foster City, CA, USA) is the only liposomal drug approved by the US FDA for treatment of VL. Several studies are available using this compound for the treatment of Indian VL. In a multicenter trial, a total dose of 3.75 mg, delivered over 5 days in equally divided doses, cured 89% patients with Sb^V refractory VL.[49] In the same study, a total dose of 15 mg/kg cured 97% patients. However, in a dose of 5 mg/kg administered as single total dose or over 5 days cured 91 and 93% patients, respectively.[50] In another large multicenter study with liposomal amp B in which 203 patients were enrolled at four treatment centers, a total dose of 7.5 mg/kg given as a single dose cured 90% patients.[51] Adverse events were minor and were seen in a small proportion of patients. This kind of treatment is a real breakthrough in the treatment of VL as patients can be treated in a day care setting with a minimum of monitoring. Most importantly, single-dose treatment increases the scarce hospital bed capacity to more than 30 folds. Unfortunately, due to the extremely prohibitive cost of the drug, it remains beyond the reach of most patients. Another Lipid-associated Amp B, Amp B lipid complex (Abelcet; ABLC, Enzon Inc., Piscataway, NJ, USA), has excellent efficacy in Indian VL.[52] In a series of studies, it was established that ABLC in a dose of 10–15 mg/kg delivered over 5–10 days cures 90–100% patients including those with refractory VL While experience with amphocil (Amphotec) (Amp B cholesteryl sulfate complex; InterMune Inc., Brisbane, CA, USA) has been limited, in a study we used it in doses of 7.5, 10 and 15 mg delivered over 6 days, and a cure rate of $\geq$96% was achieved in all the three groups (S. Sundar, unpublished), infusion-related adverse reactions were common.

In a recently conducted head to head comparison, we looked at the efficacy and tolerability of conventional Amp B (1 mg/kg on alternate days for 30 days), liposomal Amp B (2 mg/kg/day for 5 days) and ABLC (2 mg/kg/day for 5 days), in treatment of VL patients. The final cure was similar in all the groups, but infusion reactions were universal in Amp B group; lesser with ABLC and least with liposomal Amp B.[53]

A liposomal Amp B preparation has been developed in India and is safe and effective in VL. The regimen of 2 mg/kg daily for 10 days cured 100% patients; 3 mg/kg daily for 5 days was efficacious in 90.9%, and 3 mg/kg daily for 7 days in 100% of the unresponsive cases of visceral leishmaniasis.[54] This drug is now commercially available as Fungisome, and needs to be sonicated before administration. Its major advantages are reduced toxicity, shorter duration of treatment, efficacy in resistant cases and reduced cost.

Paromomycin (aminosidine), an aminoglycoside, has excellent antileishmanial activity.[55] It has been used either alone or in combination with Sb^V. In several studies in Africa and India, its efficacy in the treatment of VL has been demonstrated.[56]–[59] In a phase-II multicenter randomized controlled trial of aminosidine (paromomycin) for treating VL in north Bihar, Paromomycin was used in daily doses of 12, 16 or 20 mg/kg

intramuscularly for 21 days. A cure rate of more than 90% was achieved at 180 days follow-up.[57],[60] A pivotal phase-III multicenter trial on the safety and efficacy of Paromomycin in four different centers of Bihar is nearing completion after which this drug is likely to be registered in several countries including India. The drug is now being manufactured in India and the treatment cost is likely to be between US$10–20 for the whole treatment course for an adult patient.

Miltefosine (Impavido®) is an alkyl phospholipid developed as an anti-tumor agent, and has excellent antileishmanial activity. It is the first orally effective antileishmanial drug, which is uniformly effective in both naïve as well as Sb^V refractory patients.[28],[29],[61] In all clinical studies, it cured ³94% patients. It is safe and effective in daily doses of 100–150 mg administered for a duration of 28 days.[28],[48],[61],[62] Its adverse events include mild-moderate vomiting in ~40% patients, and diarrhea in ~20%. In a significant proportion of patients, there is asymptomatic transient elevation of hepatic transaminases. Rarely, it can cause skin allergy and renal insufficiency. In several pediatric studies, it cured 94% of children if a daily dose of 2.5 mg was used for 28 days. Tolerance was good with mild gastrointestinal adverse events like vomiting and diarrhea occurring in 26% children.[63],[64] It was approved in India in 2002 for the treatment of VL at a daily dose of 50 mg in patients weighing <25 kg, and 100 mg in those ≥25 kg for 4 weeks. The drug has a long terminal half-life (~7 years). The fact that Miltefosine is administered orally and produces early symptomatic improvement predisposes it for incomplete treatment unless the treatment is strictly supervised. All these factors could encourage development of resistance to the drug and premature end of this very important arsenal against Leishmania unless it is handled and used judiciously.

Sitamaquine is an orally administrable primaquine derivative, and though on the horizon for several decades, is still in the early stages of development. Several studies in Kenya, Brazil and India have been done, but in a few patients nephrotoxicity has been observed.[65],[66] Thus, more studies are needed to clearly delineate its safety profile before this drug can reach clinics.

HIV/VL co-infection

In patients with HIV infection, VL is considered to be an AIDS-defining illness, and though predominantly a disease of Mediterranean region and African countries like Ethiopia, its incidence in India is increasing. The treatment of choice is Amp B or its liposomal formulation. Unfortunately, relapse is a rule in co-infection. Reconstitution of immunity by highly active antiretroviral therapy (HAART) has led to a decline in the incidence of VL co-infection in Spain significantly.

Conclusion

VL in India affects more than 100,000 persons every year. The problem is likely to get aggravated by the emerging epidemic of HIV infection. Diagnosis and treatment of this disease is far from satisfactory. Splenic aspirate, although risky, remains the gold standard for the diagnoses of VL. Polymerase chain reaction, using primers to amplify Leishmania kDNA, may emerge as an important tool for the diagnosis of this condition. Out of the battery of serological tests available, k39-based immunochromatographic strip test has become an important and cost effective tool for diagnosis of VL in field conditions, notwithstanding the inherent limitation of antibody-based tests.

For treatment of VL, both Sb^V and Amp B are potentially toxic, and have to be administered parentally for prolonged periods (≥30 days). A steady decline in the efficacy of Sb^V in endemic areas of Bihar has rendered this drug useless in this region. Lipid formulations of Amp B are a major advance in the treatment of VL; however, their prohibitive cost puts them beyond the reach of most patients. There is an urgent need to develop this technology indigenously, so that the drug can be reasonably priced to be within reach of poor patients. Miltefosine is the first oral antileishmanial drug, which has proved highly effective in Indian VL. However, there is a potential for development of resistance to this drug by parasite, unless it is judiciously used. Paromomycin, another parental antileishmanial drug, is undergoing phase-III multicenter trials in India.

In view of the emergence of parasite resistant to antileishmanial drugs, and limited number of effective antileishmanial drugs, there is an urgent need to revise the strategy of treatment. Combination therapy with multiple drugs is a viable option, which needs to be given serious consideration.

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