LEISHMANIASIS-CURRENT BURDEN AND TRENDS

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OVERVIEW OF THE DISEASE

Visceral Leishmaniasis (VL) is typically caused by obligate intercellular parasite, *Leishmania* donovani complex which includes three species: *L. donovani*, the causative organism for VL in the Indian subcontinent and East Africa*; L. infantum* and *L. chagasi*, causes VL in the Mediterranean basin, in Central and South Africa.

Natural transmission of leishmaniasis is carried out by female sand flies. VL is a systemic disease characterised by prolonged fever, splenomegaly, hepatomegaly, weight loss, progressive anaemia, pancytopenia, and is complicated by serious infections. After recovery, some patients (50% in Sudan and 1 - 3% in India) develop post kala-azar dermal leishmaniasis (PKDL) characterised by depigmented macules, indurated plaques, and nodules.

EPIDEMIOLOGY

An estimated 500,000 cases of VL occur every year worldwide. India, Nepal, Bangladesh, Sudan, and Brazil account for 90% of cases. The annual incidence of VL in India is approximately 100,000 cases, and the state of Bihar accounts for more than 90% of these[1]. Environmental changes like deforestation, urbanisation, migration of non-immune people to endemic areas and new settlements in zoonotic foci have led to the increase in the incidence of leishmaniasis.

Immunosuppression due to AIDS increases the risk of VL by 100- to 1000-fold in endemic areas. VL-HIV co-infection is a growing problem in Southern Europe, Brazil and Africa. Cases of co-infection have been reported from 35 countries around the world. These patients have low cure rates, frequent relapses and high parasite load, making them a reservoir for the spread of the infection.

DIAGNOSIS OF VL

Confirmatory test are required to make the diagnosis of VL as the clinical manifestation of VL is similar to commonly occurring diseases like malaria, typhoid, tuberculosis, etc. Laboratory diagnosis of leishmaniasis can be made by the following:

1. Demonstration of parasite

   Demonstration of the parasite in splenic or bone marrow aspirate is required for definitive diagnosis. Splenic aspiration is regarded as the gold standard for diagnosis of kala-azar with sensitivity exceeding 95%.[2] The sensitivity of bone marrow smear is only about 60 to 85%, besides being traumatic. However, it is associated with risk of fatal haemorrhage in inexperienced hands as the procedure requires training and expertise.

   Recognition of the parasite demands experience and thorough search. Amastigotes appear as round or oval bodies inside monocytes and macrophages with pale blue cytoplasm, large red nucleus, and deep red or violet rod-like body called kinetoplast at a right angle to the nucleus.

2. Serological tests:

   Though widely used Antibody based tests have two major drawbacks. First, up to 32% healthy

individuals living in endemic areas with no history of VL are positive for anti-leishmanial antibodies owing to asymptomatic infections. Secondly, serum antibody levels remain detectable after cure for several years therefore; VL relapse cannot be diagnosed by antibody detection. The direct agglutination test (DAT) and the rK39-based immunochromatographic test (ICT) are two serological tests have been specifically developed for field use. Other serological tests based on indirect fluorescence antibody (IFA), enzyme-linked immunosorbent assay (ELISA) or western blot have shown high diagnostic accuracy in most studies but are poorly adapted to field settings

DAT - In this test, coomassie brilliant blue stained whole promastigotes are incubated with sera of the patients and agglutination observed after an overnight incubation. Initially, aqueous antigen was used but it had the drawback of cold chain requirement, and short life. Now, freeze dried antigen has been developed which can be transported at ambient temperature. In a meta-analysis of studies using DAT, it had sensitivity and specificity estimates of 94.8% and 85.9%, respectively.[3] However, the major disadvantage of DAT is the need of multiple pipetting, long incubation time, high cost of antigen and limited production facility of quality controlled antigen.[4,5]

rK39 is a 39-amino acid repeat that is part of a kinesin-related protein in *Leishmania chagasi* and is conserved within the *L. donovani* complex.

Immunochromatographic strip tests (ICTs) based on rK39 are easy to perform, rapid, cheap and yields reproducible results. A meta-analysis that included 13 validation studies of the rK39 ICT showed sensitivity and specificity estimates of 93.9 % and 95.3 % respectively.[3]

rK28 antigen has recently been introduced as a candidate for serological diagnosis of VL. When compared to the rk39 ELISA in a micro-ELISA format the specificity of the rK28 antigen in non-endemic, endemic healthy and different disease controls was 100%, 94.17% and 95.45% respectively, and 99.6% sensitivity, which was similar to that of rK39 ELISA.[6] Rapid format of rK28, though available, is yet to be commercialized and tested in the Indian subcontinent.[7]

Recently a novel *Leishmania donovani* antigen (BHUP2) was identified which had a sensitivity of 94% whereas specificity in endemic healthy control, non endemic healthy control, and disease control were 98%, 100%, and 97%, respectively. Although preliminary, this 37kD protein has a strong potential for further development as a diagnostic tool.[8]

3. Antigen Detection:

   Antigen detection is more specific than antibody-based immunodiagnostic tests and is expected to broadly correlate with the parasite load. A latex agglutination test detecting a heat-stable, low-molecular-weight carbohydrate antigen in the urine of VL patients has shown promising initial results. Several studies conducted in East Africa and the Indian subcontinent showed good specificity but only low to moderate (48–87%) sensitivity.[9] The latex agglutination test correlated well with cure in a high proportion (97–100%) of patients during anti-leishmanial treatment.[9] Efforts are being made to improve the performance of this technique, as it holds promise as a test of cure, for which none of the current serological tests can be employed.

4. Molecular diagnosis:

   PCR for detection of parasite DNA in blood or bone marrow aspirates is more sensitive than microscopic examination which helps in detecting more asymptomatic infection. However these methods are limited to tertiary care centers.

   Thus to conclude, currently rK39 based rapid tests are widely used for the diagnosis of VL, however, their continued positivity long after cure and in a high proportion of individuals living in the endemic area are its major drawback.

TREATMENT OF VL

In general, treatment of leishmaniasis is far from satisfactory. Except for oral miltefosine, all antileishmanial drugs are given parenterally. Also, most of the drugs are toxic and require prolong hospitalization.

Efficacy of Miltefosine, Paromomycin, Single dose liposomal Amphoterecin B and combination therapy has revolutionized the conventional treatment of VL.

PENTAVALENT ANTIMONIALS

Sodium stibogluconate in doses of 20 mg/kg body weight for 28–30 days has been standard first-line medicines in India. Large scale misuse of this drug in Bihar, which included improper doses, splitting of daily dose, substandard batches of drugs were the main reasons behind the emergence of widespread antimony resistance in this region.[10] The level of unresponsiveness reached as high as 60% in India, and adjoining Nepal. Due to the emergence of high level of resistance sodium stibogluconate lost its utility in this region.

AMPHOTERICIN B DEOXYCHOLATE

The most commonly used drug for treatment of refractory VL in India is Amphotericin B deoxycholate. Amphotericin B has high cure rate (~100%) at a dose of 0.75 – 1 mg/kg

for 15 – 20 intravenous infusions. However, this drug has many adverse effects, which necessitates close monitoring and hospitalization for 4-5 weeks which ultimately increases the cost of therapy.

Lipid formulations of amphotericin B have lower toxicity and shorter duration of therapy. The total dose requirement for treatment of visceral leishmaniasis varies by geographical region. In India (*L. donovani*), a total dose of ≥10 mg/kg results in a cure rate of > 95% while a total dose of 18–21 mg/kg, has 90–100% efficacy in southern Europe. Thus far prohibitively high cost of these lipid formulations has been a limiting factor in their use in endemic countries including India. Of all the lipid formulations, liposomal amphotericin B (Gilead Sciences, USA) has been tested most widely in all the leishmaniasis affected regions including India, and is the only antileishmanial drug approved by the Food and Drug Administration, USA. After a series of studies with multiple and single doses, it was established that L-AmB is highly effective in Indian VL.[11,12]

In a breakthrough study from India, 412 patients were randomly assigned in a 3:1 ratio to receive either liposomal amphotericin B (at a dose of 10 mg per kilogram of body weight) as a single dose or the conventional amphotericin B deoxycholate administered in 15 infusions of 1 mg per kilogram, given every other day during a 29-day hospitalization. Cure rates at 6 months were similar in the two groups: 95.7% (95% confidence interval [CI], 93.4 to 97.9) in the liposomal-therapy group and 96.3% (95% CI, 92.6 to 99.9) in the conventional-therapy group. The single dose treatment along with a preferential pricing makes single infusion of the liposomal preparation an excellent option for the Indian subcontinent[13]. WHO has recommended as a safe and effective alternative requiring a single day of admission

MILTEFOSINE

The only oral antileishmanial agent miltefosine was registered for use in India in March 2002. Its limitations includes monitoring of gastrointestinal side effects, occasional hepatic- and nephrotoxicity and high cost. As miltefosine is teratogenic, women of child-bearing potential have to observe contraception for the duration of treatment and for an additional three months, due to its long half-life of approximately one week, which also makes it vulnerable to the rapid development of drug resistance. In a recent Phase IV trial of the drug in India, the final cure rate was only 82% by intention to treat and 95% by per protocol analysis.[14] These findings suggest that monitoring miltefosine therapy is imperative to prevent emergence of resistance. Miltefosine is currently being used in the elimination initiative in the three countries.

PAROMOMYCIN (AMINOSIDINE)

In the phase III trial of Paromomycin in the Indian subcontinent it has been shown to be noninferior to Amphoterecin B and was approved by the Indian government in August 2006 for the treatment of patients with visceral leishmaniasis.[15] The advantages of this agent is its cost, approximately US $10 per patient. The disadvantages are need for administering intramuscular injection, monitoring of serum transaminases and inadequate data regarding its use in pregnancy

COMBINATION THERAPY

The idea behind combination therapy is increased activity through use of compounds with synergistic or additive activity, preventing the emergence of drug resistance, lower dose requirement thereby reducing chances of toxic side effects and cost, and increased spectrum of activity. Multi drug therapy is expected to reduce the probability of development of resistant parasites, thereby prolonging the useful therapeutic lifespan of the existing drugs.

Single infusion of L-AmB (in most instances, administered in an outpatient setting) followed by a brief self-administered course of miltefosine has shown to be an excellent option against Indian kala-azar.[16]

In a phase 3 trial in India, three drug combinations (single injection of 5 mg/kg L AmB and 7-day 50 mg oral miltefosine or single 10-day 11 mg/kg intramuscular paromomycin; or 10 days each of miltefosine and paromomycin). All the combinations showed an excellent cure rates (>97% in all arms) and were non inferior to the standard treatment.[17]

As the efficacy and required dosage of the antileishmanial agents vary in different areas the World Health Organization recently published the treatment recommendation for VL based on these regional differences. Recent WHO treatment recommendations (2010) for VL and PKDL caused by *L. Donovani* are given in Table 1 and 2. According to WHO, ideal treatment for VL should cure the patient, reduce the risk for relapse and for PKDL and reduce transmission of resistant parasites. To ensure compliance and completion of the course of therapy, direct observation should be implemented, mainly for oral miltefosine.[18]

VACCINE

Leishmaniasis is unique among parasitic diseases because a single vaccine has the potential to protect against more than one species and be successful at both treating and preventing disease. Unfortunately there is no vaccine approved for VL, though several vaccine development programmes are underway.

TREATMENT OF HIV/VL CO-INFECTION

These patients have high parasite burden, a weak immune response, respond poorly to treatment and have a high relapse rate. Pentavalent antimonials are more toxic to HIV patients, who require close monitoring for pancreatitis and

Table 1 : Recommended treatment regimens for visceral leishmaniasis, ranked by preference (Adopted from the Control of the Leishmaniases, WHO Technical Report Series 949, 2010)

Anthroponotic visceral leishmaniasis caused by* L. donovani *in Bangladesh, Bhutan, India and Nepal

1. Liposomal amphotericin B: 3–5 mg/kg per daily dose by infusion given over 3–5 days period up to a total dose of 15 mg/kg (A) by infusion or 10 mg/kg as a single dose by infusion (A)
2. Combinations (co-administered) (A)
   - liposomal amphotericin B (5 mg/kg by infusion, single dose) plus miltefosine (daily for 7 days, as below)
   - liposomal amphotericin B (5 mg/kg by infusion, single dose) plus paromomycin (daily for 10 days, as below)
   - miltefosine plus paromomycin, both daily for 10 days, as below
3. Amphotericin B deoxycholate: 0.75–1.0 mg/kg per day by infusion, daily or on alternate days for 15–20 doses (A)
4. Miltefosine: for children aged 2–11 years, 2.5 mg/kg per day; for people aged 12 years and < 25 kg body weight, 50 mg/day; 25–50 kg body weight, 100 mg/day; > 50 kg body weight, 150 mg/day; orally for 28 days (A)

   or

   Paromomycin: 15 mg (11 mg base) per kg body weight per day intramuscularly for 21 days (A)
5. Pentavalent antimonials: 20 mg Sb5+/kg per day intramuscularly or intravenously for 30 days in areas where they remain effective: Bangladesh, Nepal and the Indian states of Jharkhand, West Bengal and Uttar Pradesh (A)

Rescue treatment in case of non-response: conventional amphotericin B deoxycholate infusions or liposomal amphotericin B at higher doses

cardiotoxicity[19]. The best option for these patients are L AmB at 4 mg/kg given on day 1-5, 10, 17, 24, 31 and 38.[20] Secondary prophylaxis to prevent relapses has been reported in several publications, but more evidence from clinical trials is needed to establish a beneficial effect.[19] Initiation of HAART dramatically decreases the incidence of VL coinfection and also increases the interval between relapses. Therefore; HAART in combination with antileishmanials should be advocated strictly in these patients.

VL ELIMINATION PROGRAMME

The governments of India, Nepal and Bangladesh have launched a regional VL elimination programme as these countries harbour 67% of the global VL disease burden. The target of this programme is to eliminate VL as a public health problem in these countries by 2015, by using a local approach to reduce the annual incidence of VL to less than 1 case per 10,000 individuals.

The rationale for VL elimination in the Indian subcontinent are as follows: the disease in this area is anthroponotic, with humans being the only reservoir and *Phlebotomous argentipes* sandflies the only known vector; availability of new and more effective drugs and a rapid diagnostic test, the rk39 immunochromatographic test, are available that can be used in the field; there is strong political commitment and inter-country collaboration; and the disease is endemic in only a limited number of districts.

Table 2 : Post-kala-azar dermal leishmaniasis (Adopted from the Control of the Leishmaniases, WHO Technical Report Series 949, 2010)

East Africa

1. Pentavalent antimonials: 20 mg Sb5+/kg per day intramuscularly or intravenously for 30–60 days, when indicated (C)
2. Liposomal amphotericin B: 2.5 mg/kg per day by infusion for 20 days, when indicated (C)

Bangladesh, India, Nepal

1. Amphotericin B deoxycholate: 1 mg/kg per day by infusion, up to 60–80 doses over 4 months (C)
2. Miltefosine orally for 12 weeks at dosage as above (A)f

CONCLUSION

Though rK39 based immunochromatographic strip test remains one of the most sensitive, simple, rapid and affordable test for the diagnosis of VL, the biomarkers of active disease and parasite resistance and test of cure, are urgently needed.

Many effective advances have been made in the treatment of VL like the single dose L-AmB, combination therapy and newer drugs like paromomycin. However, the emergence of drug resistance is further complicating the control of leishmaniasis. As we do not have newer antileishmanial drugs, combination therapy by reducing the duration of therapy and decreasing the chances of developing resistance should be encouraged. The use of vaccines and immunomodulators for prophylactic as well as therapeutics would go a long way in the control of the disease.

Therefore, for effective VL elimination programme implementation an early diagnosis and complete treatment of cases, integrated vector management, effective disease surveillance through passive and active case detection, social mobilization and partnership building at all levels and clinical and operational research should be the five pronged strategy to combat this disease.

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