REVIEW ON DIAGNOSTIC METHOD AND TREATMENT OPTIONS FOR KALA AZAR IN INDIA

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Summary

Visceral leishmaniasis or Kala-azar is a intracellular protozoal infection caused by Leishmania donovani and transmitted by phlebotomine sandflies. Kala-azar is a major public health problem in the areas of its prevalence, principally India and its neighbors Bangladesh and Nepal, and Brazil and Sudan. In India the disease is found in Bihar, Jharkhand, West Bengal and pockets of eastern Uttar Pradesh. A national health programme to eliminate the disease by 2010 is in operation in India. The programme relies on case management, vector control, community involvement in control activities and capacity building as the principal components of the elimination strategy. The Kala-azar elimination programme is a centrally sponsored programme, under which the cost of all materials, i.e. insecticides, diagnostic kits, drugs and cost of operations is borne by the Central Government.

Key words: Amphotericin B, Visceral Leishmaniasis (kala-azar).

Introduction: Indian visceral leishmaniasis (VL) is a parasitic disease caused by a haemoflagellete *Leishmania donovani* and transmitted by the bite of sand fly Phlebotomus argentipes. It affects various age groups. In India about 1,00,000 cases of VL are estimated to occur annually; of these, the State of Bihar accounts for over than 90 per cent of the cases. Diagnosis of VL typically relies on microscopic examination of tissue smears but serology and molecular methods are better alternatives currently. Notwithstanding the growing incidence of resistance, pentavalent antimony complex has been the mainstay for the treatment of VL during the last several decades. The second line drugs such as amphotericin B, lipid formulations of amphotericin B, paromomycin and recently developed miltefosine are the other alternatives. In spite of significant development in various areas of Leishmania research, there is a pressing need for the technological advancement in the understanding of immune response, drug resistance and the pathogenesis of leishmaniasis that could be translated into field applicable and affordable methods for diagnosis, treatment, and control of the disease **[1].**

Clinical manifestations of kala azar: VL comprises a broad range of manifestations of infection. Infection remains asymptomatic or subclinical in many cases or can follow an acute or chronic course. The clinical symptoms are characterized by prolonged and irregular fever often associated with rigor and chills, splenomegaly, lymphadenopathy, hepatomegaly, pancytopenia, progressive anaemia, weight loss and hypergammaglobulinaemia (mainly IgG from polyclonal B cell activation) with hypoalbunemia. It is always fatal if left untreated. After recovery, some patients (50% in Sudan and 1-3 % in India) develop post kala-azar dermal leishmaniasis (PKDL) **[2-3].**

Life Cycle of Leishmaniasis: *Leishmania* are alternatively hosted by the insect (Flagellated promastigote) and by mammals (intracellular amasitgote stage). The bite of an infected sandfly results in the intradermal inoculation of metacyclic leishmaniae (promastigote). Their establishment in the mammalian host is facilitated by sand fly saliva delivered at the same time, which enhances the *leishmania* infectivity **[4].** Sandfly saliva contains various pharmacologically active substances which prevent haemostatic mechanisms of the host, and cause vasodilatation & local immune suppression **[5].** With in the dermis of the mammalian skin, the metacyclic promastigotes escape complement activation then phagocytosed by macrophages within which they transformed into amastigotes, and they have the capacity to resist intracellular digestion **[6].**

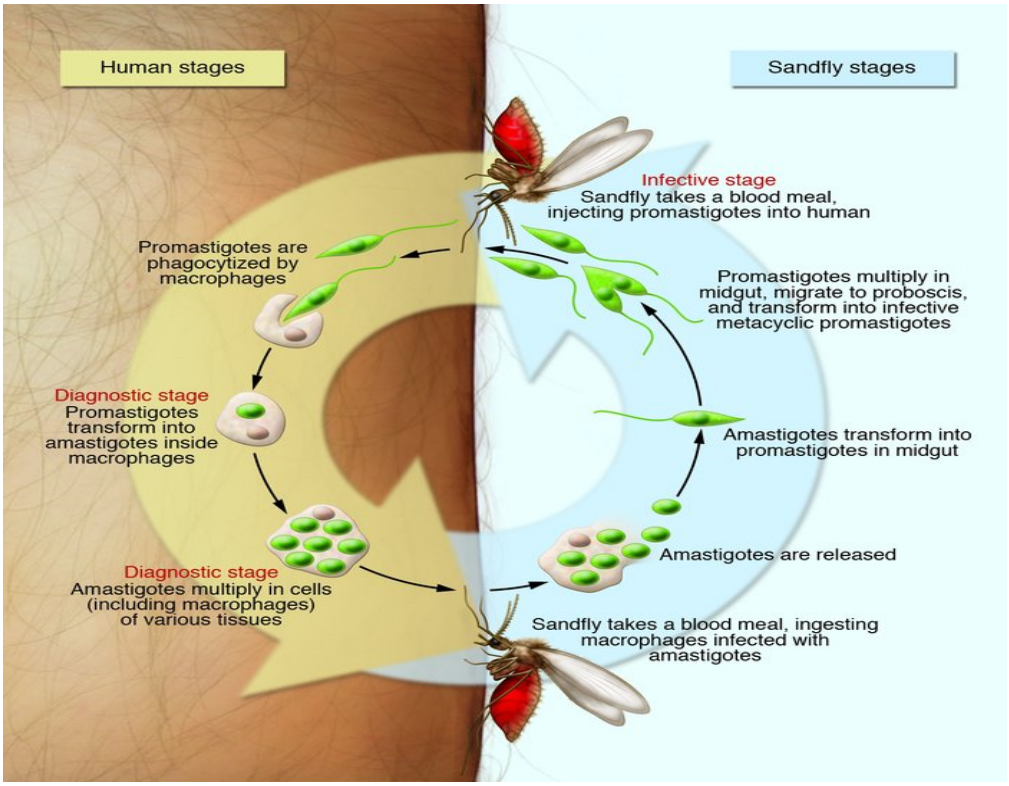

Figure 1: Life cycle of *Leishmania*: (http://www.jci.org/articles/view/33945/Figureure/3)

Diagnostic methods for kala-azar:

Non-leishmanial: A reduction in the number of red and white blood cells and platelets (pancytopenia) was found to be highly specific (98%) for VL in suspected clinical patients in Nepal but the sensitivity was low (16%) **[7].** Marked polyclonal hypergammaglobulinemia (the production of high titres of non-specific antibody), a common finding in VL, can be detected by a formol gel test (FGT; also called the aldehyde test), which is still used in East Africa and Asia because of its simplicity and low cost. However, as the sensitivity of this test is poor (as low as 34%59), some experts have recommended its use be discontinued **[8].**

Parasite detection: The visualization of the amastigote form of the parasite by microscopic examination of aspirates from lymph nodes, bone marrow or spleen is the classical confirmatory test for VL. Although the specificity is high, the sensitivity of microscopy varies, being higher for spleen (93–99%) than for bone marrow (53–86%) or lymph node (53–65%) aspirates **[9-13].** However, spleen aspiration can be complicated by lifethreatening haemorrhages in ~0.1% of individuals and therefore requires considerable technical expertise **[14],** as well as facilities for nursing surveillance, blood transfusion and surgery. Moreover, the accuracy of microscopic examination is influenced by the ability of the laboratory technician and the quality of the reagents used. The detection of parasites in the blood or organs by culture or by using molecular techniques such as PCR is more sensitive than microscopic examination but these techniques remain restricted to referral hospitals and research centres, despite efforts to simplify them **[15].**

Antibody-detection tests: Several tests that detect specific anti-leishmanial antibodies have been developed, but all have two major limitations. First, though serum antibody levels decrease after successful treatment, they remain detectable up to several years after cure. Therefore, VL relapse cannot be diagnosed by serology. Second, a significant proportion of healthy individuals living in endemic areas with no history of VL are positive for anti-leishmanial antibodies owing to asymptomaticinfections. The seroprevalence in healthy populations varies from <10% in low to moderate endemic areas, to >30% in high-transmission foci or in household contacts. Antibody-based tests must therefore always be used in combination with a standardized clinical case definition for VL diagnosis. 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. Two serological tests have been specifically developed for field use and have been sufficiently validated — the direct agglutination test (DAT) and the rK39-based immunochromatographic test (ICT). The DAT is a semi-quantitative test that uses microtitre plates in which increasing dilutions of patient's serum or blood are mixed with stained killed L. donovani promastigotes. If specific antibodies are present, agglutination is visible after 18 hours with the naked eye. This test has been extensively validated in most endemic areas. Thirty studies were included in a recent meta-analysis, which gave sensitivity and specificity estimates of 94.8% (95% confidence intervals (CI), and 97.1% (95% CI, 93.9–98.7), respectively. The performance of the DAT was influenced by neither the region nor by the Leishmania species. Freeze-dried antigen is more robust than liquid antigen. The DAT is simpler than many other tests but it requires equipment such as microtitre plates and micropipettes, well-trained laboratory technicians and regular quality control. The storage of the antigen at 2–8°C once it has been dissolved and the prolonged incubation time are other drawbacks. The fast agglutination screening test (FAST) is a simplified (single serum dilution at a cutoff of 1:800 or 1:1600) and more rapid (2–3 hours) version of the DAT, and its diagnostic accuracy seems comparable, but further validation is needed. rK39 is a 39-amino acid repeat that is part of a kinesin-related protein in Leishmania chagasi and which is conserved within the L. donovani complex90. An rK39- based ELISA showed excellent sensitivity (93–100%) and specificity (97–98%) in many VL-endemic countries. The test was then developed into an ICT, or dipstick, format that was more suitable for field use. A meta-analysis that included 13 validation studies of the rK39 ICT showed sensitivity and specificity estimates of 93.9% (95% CI, 87.7–97.1) and 95.3% (95% CI, 88.8–98.1), respectively83. Recently, the excellent diagnostic performance of rK39 ICT was confirmed in India and Nepal. However, this test has been shown to be less accurate in East Africa92. For reasons that remain unclear, Sudanese

patients seem to develop lower titres of antibodies against rK39 than do Indian patients, although the format of the test might be a factor, as other brands of ICT performed better in this region.

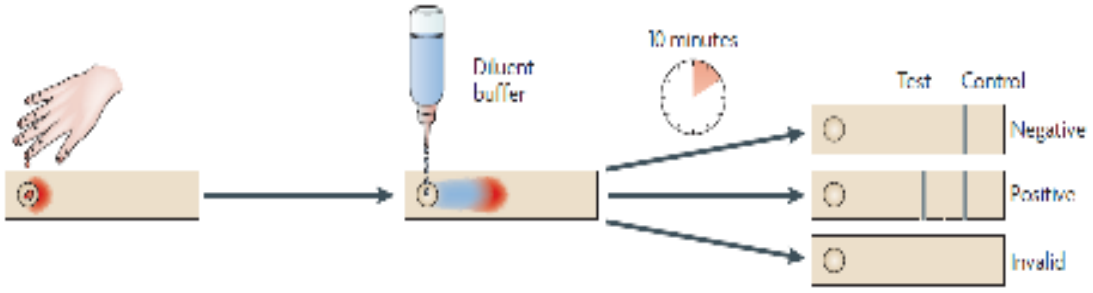

Figure 2: rK39 dip stick test

rK39 ICTs are easy to perform, rapid (10–20 minutes), cheap (around US$1 per test) and give reproducibleresults. They are currently the best available diagnostic tool for VL for use in remote areas, and their wide distribution and use within an appropriate VL diagnostic algorithm should be promoted. The case-management strategy of the VL elimination programme planned for the Indian subcontinent , which is based on the treatment of suspected clinically infected individuals who have positive rK39 ICT results, is supported by solid scientific evidence. Given that several counterfeit VL ICTs have already been found in the Indian subcontinent, the need for rigorous quality standards and regulation of diagnostics should be addressed at the same time **[16].**

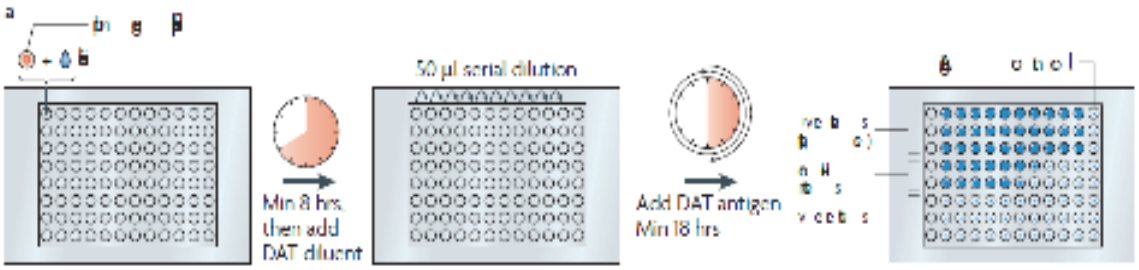

Figure 3: Direct agglutination test (DAT) based immunochromatographic test (ICT)

Antigen-detection tests: In theory, antigen-detection tests should be more specific than antibody-detection tests as they avoid cross-reactivity and can distinguish active from past infections. A latex agglutination test detecting a heat-stable, low-molecular-weight carbohydrate antigen in the urine of VL patients has shown promising initial results **[17-18].** Several studies conducted in East Africa and the Indian subcontinent showed good specificity but only low to moderate (48–87%) sensitivity **[19-22].**

Treatment Options: The objectives of treating kala-azar should be to cure the patient of intracellular parasites, prevent relapse and keep the costs to a minimum. Clinical relapse of kala-azar usually occurs Within 6 months after completion of therapy. If relapse occurs, patients should be treated again with an antimonial If the response is not satisfactory or in case of

primary unresponsiveness, other drugs should be used. These drugs are required in many patients due to increasing drug resistance and treatment failures reported from India **[23].**

In India Sb has been the backbone of anti VL therapy because of its low cast and excellent effectiveness. However, with its declining efficacy in VL hyperendemic regions of north Bihar, it should no longer be used as the first line drug in areas with high resistance. Fortunately we have the following other alternatives to treat VL **[24].**

Pentamidine: To circumvent the problem of clinical resistance to antimony in India, pentamidine has been tried for the treatment of VL and was the first drug to be used for patient refractory to Sb **[25].** The pentamidine regimen consisted of a dose 4 mg per kg given three times per week until initial parasitological cure was achieved. Initially high cure rate were reported **[26]** but its efficacy gradually declined over the years. It now cures only 70 per cent of patients. This drug is associated with serious adverse events like insulin dependent diabetes mellitus, shock, and hypoglycaemia and death in significant proportion of patients. The declining efficacy, resistance and serious toxicity associated with the drugs have made it unsuitable as a viable alternative to Sb for kala-azar patients. However, it has been used in treatment of both Old and New World cutaneous and mucocutaneous leishmaniasis **[27-29].**

Amphotericin B: Amphotericin B is the most effective antileishmanial drug, which induces high cure rates. Use of formulation of amphotericin B, a pollen antibiotic, for treatment of leishmaniasis is biochemically rational because the target of amphotericin B is ergosterol like sterols, which are the major membrane sterols of Leishmania species **[30].** Due to high affinity of amphotericin B for sterols, aqueous pores are formed in the membrane leading to increased membrane permeability and killing of Leishmania **[31].** Amphotericin B is now being more widely used for VL and constitutes the major advance in antileishmanial chemotherapy during the last 10 yr. At dose of 0.75-1.0 mg per kg for 15 infusions on alternate days, it cures more than 97 per cent of patients. Occasional relapse (1%) might occur with amphotericin B, which can be treated successfully with the same drug. It has been recommended as first line drug in India by the National Expert Committee for Sbv in refractory regions of VL. Primary resistance to this drug is unknown. However, the need for hospitalization for prolonged periods, high cost of the drug, equipment required for dose monitoring and high incidence of adverse events (occasionally serious) are the major drawbacks **[32].**

Lipid formulation of amphotericin B: The need to develop less toxic, more effective formulation of amphotericin B has led to three new clinical formulation of amphotericin B in which deoxycholate has been replaced by other lipids. These formulations are liposomal ampho B (L-AmB:Ambiosome), amphotericin B colloidal dispersion (ABCD:Amphocil) and amphotericin B lipid complex (ABL:Abelcit). These substitutes are well taken by reticuloendothelial system and poorly taken by kidney, the major target of organ toxicity **[33].** Adverse effects of the conventional amphotericin B can be circumvented without compromising with the efficacy of the drug. It is possible to deliver high doses of drugs over short periods. The dose requirement varies from region to region. In Indian subcontinent a small dose (3.75 mg/kg) of ambiosome for five consecutive days induces high cure rates **[34].** In another study, a single dose (15 mg/ kg) was compared with amphotericin B over 15 days (1 mg/kg) and all patients in both the groups had a final cure. In another trial, a single total dose (5 mg of ambiosome/kg) was compared with a similar dose administered over 5 days and final cure was achieved in 91 and 93

per cent patients respectively **[35].** Safety of liposomal amphotericin B permits administration of total dose requirement in a single infusion. However, prohibitively high cost makes these compounds unaffordable in VL endemic countries like India **[36].**

Miltefosine: Miltefosine, an alkyl phospholipids developed as an anti-tumour agent, has excellent antileishmanial activity. It is used orally and has undergone extensive trials against VL in Bihar. It has been found uniformly effective in naïve as well as Sbv refractory patients. In all clinical studies, a cure rate >94 per cent has been found consistently with this drug. This drug has mild gastrointestinal adverse events like vomiting and diarrhoea in 40 and 20 per cent patients, respectively. The exact mechanism of its action is not known but it probably interacts with the cell membrane of Leishmania **[37].** Miltefosine has been approved in India for treatment of VL at a dose of 50-100 mg (~2.5 mg/kg) for four weeks. It has also been found safe and effective in paediatric patients. This drug with mild side effects can become an important tool in containing the epidemics of VL. However, there are certain major limitations. Miltefosine has a median long terminal half-life of 154 h, which could encourage development of clinical resistance, and the best way to use this drug would be to use as a combination multi drug therapy. It is teratogenic and abortifacient, which means the drug cannot be used in pregnancy, and females with child bearing potential must observe contraception for the duration of treatment and an additional two months. Further, rapid therapeutic response coupled with unsupervised treatment can severely affect compliance, and bring a premature end to this very important arsenal against leishmania **[38].**

Paromomycin (aminosidine) : Paromomycin is an aminoglycoside antibiotic with unique antileishmanial activity. It acts synergistically with antimonials in in vitro and the combination has been used effectively in India. The drug is effective, well tolerated and as cheap as conventional amphotericin B. Its efficacy has been demonstrated in India and a dose of 16 mg per kg intramuscularly for 21 days has cured 93 per cent of patients **[39-40].**

Sitamaquine: Sitamaquine, a primaquine analogue (8-aminoquinolene), is another orally administrable compound. To date, little is known about its efficacy and toxicity. It has been in the process of development for over 8 yr by SmithKline Becham (now Glaxo SmithKline) and Walter Reed Army Institute of Research, USA. Till date there are no reports of its trial in VL patients in India. However, it has been tested in VL patients in Kenya and Brazil with limited success **[41].**

Azoles and other steroid biosynthesis inhibitors: The azoles, like ketoconazole and triazoles, itraconazole and fluconazole produce an anti-leishmanial effect by blocking ergosterol synthesis **[42].** Varying results have been reported from small-uncontrolled poorly designed clinical trials in both VL and cutaneous leishmaniasis (CL). In a study in Saudi Arabia, fluconazole showed a cure rate of 79 per cent in patients of CL caused by L. major **[43].** Till date this drug has not been tried in India.

Cytokines: Leishmania infection progress to kalaazar in individuals who fail to initiate Th1 response, which is mediated by IL-2 and IFN-r **[44].** Interferon-r is one of the principal activators of macrophages. Interferon-r as adjutants to Sb has been used successfully in VL with high cure rate in comparison to Sb alone. Later, it was observed that interferon-r (daily dose 100 μg/m2) though improved the response rate to antimony, but overall cure rate was less than 50 per cent.

However, steep decline in the response rate to antimony rendered the addition of IFN-r ineffective **[45].**

Liposomal Drug Delivery System: The most exciting development in the treatment of kala-azar has been the development of various drug delivery systems. Liposomes ar e phospholipid vesicles that are engulfed by the cells of the reticuloendothelial system, resulting in direct delivery of the bound drug to the target cells and hence reduced side effects. Three lipid formulations of amphotericin-B are presently being employed in clinical trials of patients with kala-azar. The commercially available liposomal preparation of amphotericin B AmBisome has been used in a dose of 50 mg/d in patients with refractory kala-azar **[46].** Presently, it is recommended in a dose of 3 mg/kg/d for 5 days followed by the same dose on days 14 and 21. In immunocompromised patients, it is administered in a dose of 3 mg/kg/d for 5 days and then 4 mg/kg/d on days 10, 17, 24, 31 and 38. Nephrotoxicity has been reported in about 19% cases compared with 34% of patients who receive the conventional drug. Infusion-related fever and chills are seen in 17% and 18% cases respectively, as compared to 44% and 54% with conventional drug. The cost of 50 mg of this preparation is nearly Rs. 11,000. The second compound, amphotericin B cholesterol dispersion (Amphocfl) consists of cholesterol sulphate and amphotericin B (1 : 1 molar ratio) in diskshaped particles and has been used_successfully in patients with kala-a~ar. No serious side effects except fever and chills have been recorded. The mean time for the decline in fever was only 4.2 days which was much shorter than the conventional drug. The third reparation is amphotericin B lipid complex of ABLC (AmBiosome$^{(R)}$) which is administered in a dose of 3 mg/kg/d for 5 days as infusion2L Recent trials conducted in India have shown a dose of 2 mg/d for 3-5 days to be effective in most patients with refractory kala-azar **[47].**

Combination therapy: In view of emergence of parasite resistant to the first line antileishmanial drug and potential emergence of resistance to limited alternative drugs, currently used monotherapy needs to be revised. Combination therapy with multiple drugs similar to that employed in tuberculosis, HIV and leprosy appears to be an important approach for treatment of leishmaniasis. A combination of potent drugs, one with short half life, which would rapidly bring down the parasite load below which new mutants are less likely to emerge and a second drug with long half life, which will kill the remainder parasites may be used to prevent this infection. This combination therapy will also help in shortening the duration of treatment. Unfortunately, there are only few drugs available for combination. A combination of miltefosine and paromomycin, due to less toxicity may fulfill this objective. Antimonials will be less suitable in combination with miltefosine because of toxicity and variation of parasite sensitivity **[48].**

Current issues in Leishmania Infection:

- Leishmania is given less importance than other more prevalent infectious diseases.
- Climate change may be responsible for the extension of endemicity of leishmaniasis to previously nonendemic countries.
- New diagnostic tests should improve diagnosis, especially on the field, in resource-poor areas.
- Leishmania infection may relapse in patients with HIV and is difficult to eradicate, if at all, in this setting. There have been calls for leishmania infection to be officially recognised as an AIDS-defining illness.
- Anthroponotic transmission may occur in injecting drug users who share needles.

- The leishmania genome has been unravelled, but an effective vaccine is not yet available **[49].**

Challenges in the chemotherapy of kala-azar:

- Widespread resistance to antimonials.
- Safe and cost effective medications with less toxicity.
- HIV leishmania co-infection and its rising incidence.
- The non availability of an effective vaccine **[50].**

Problems:

- The parasite is intracellular.
- The parasite induces immunosuppression.
- Increased unresponsiveness to antimonials, toxicity &hospital monitoring, long duration for
- amphotericin –B. High cost of amphotericin- B liposomal formulation.
- Resistance development in monotherapy.
- Immunodepressed patients are difficult to treat **[51].**

New therapeutic agents in pre-clinical phases:

- Lichochalcone A – derived from Chinese liquorice plant Glycyrrhiza, has shown reasonable oral efficacy in experimental models of VL as well as Cutaneous leishmaniasis (CL).
- Another compound from a Vietnamese plant Maesa balanasae showed significant activity in VL models.
- Isopropylquinolines, isolated from Galipea longiflora inboligia, has also shown activity in VL models.
- Biphosphonates, used for osteoporosis, have been found to be effective against both VL.
- Two of these drugs, risedronate and pamidronate, have also been found to be effective **[52].**

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