

Review Article

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Visceral leishmaniasis (kala-azar): Challenges ahead

R.K. Singh, H.P. Pandey* & S. Sundar

Infectious Diseases Research Laboratory, Department of Medicine, Institute of Medical Sciences &

**Department of Biochemistry, Faculty of Science, Banaras Hindu University, Varanasi, India*

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Indian visceral leishmaniasis (VL) is a parasitic disease caused by a haemoflagellate *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.

Key words Control measures - diagnosis - drug resistant - treatment - visceral leishmaniasis

Leishmaniasis, caused by 20 species of *Leishmania* and transmitted by 30 species of sand fly, is characterized by diversity and complexity¹⁻⁴. Most of *Leishmania* infections are zoonotic and rodents and canids are reservoir host. Only two *Leishmania* species can maintain anthroponotic, human-human cycle, these species are *L. donovani* responsible for visceral leishmaniasis (VL) in Indian subcontinent and east Africa and *L. tropica*, which is responsible for cutaneous leishmaniasis (CL) in the Old World⁵. Female sand fly of genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World are the only proven vector responsible for transmission of the disease⁶. In India,

Phlebotomus argentipes is the only proven vector for the disease.

Leishmaniasis has been considered a tropical affliction that constitutes one of the six entities on the World Health Organization tropical disease research (WHO TDR) list of most important diseases⁷. It occurs in 88 countries in tropical and temperate regions, 72 of them developing or least developed. An estimated 350 millions population is at risk and 10 million people are affected from this disease worldwide¹. Two million cases occur annually however, there is a gross underreporting of the cases from endemic regions, and there has been

a progressive increase in the cases of leishmaniasis being reported from the newer areas⁸.

Indian visceral leishmaniasis (kala-azar, Black fever), caused by *L. donovani*, is the systemic form of disease. In India, about 100,000 cases of VL are estimated to occur annually. Of these, the State of Bihar accounts for more than 90 per cent of the cases⁹. Epidemiology of VL is changing due to widespread migration of population and emerging HIV/VL co-infection. The risk of VL among AIDS patients increases by 100-1000 times in endemic areas, while VL accelerates the onset of AIDS in HIV infected people.

Clinical manifestations

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 hypoalbuminemia⁶. 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)^{10,11}.

Diagnosis

The diagnosis of VL is complex because other commonly occurring diseases such as malaria, typhoid, and tuberculosis share its clinical features; many of these diseases can be present along with VL (as co-infection). Further, sequestration of the parasite in the spleen, bone marrow or lymph node complicates this issue. Following methods can make laboratory diagnosis of kala-azar.

Demonstration (microscopy) and isolation of parasite (culture): This is the most commonly used method for diagnosing VL in patients. Samples of infected tissues are obtained and the organisms (amastigotes) are either seen in Giesma or Leishman

stained smears of the tissues or cultured from them. The presence of parasite in splenic, bone marrow, lymph nodes aspirates and liver biopsy and buffy coat of peripheral blood can be demonstrated by microscopy¹² (Fig.). After identification, the parasite density can be scored microscopically by means of logarithmic scale ranging from 0 (no parasites per 1000 oil immersion fields) to +6 (>100 parasites per field)¹³. The most commonly used tissue specimens are splenic and bone marrow. Though splenic smear is highly sensitive (95%) than bone marrow but it carries a small but definite risk of serious haemorrhage^{14,15}. However, due to high sensitivity and unavailability of better alternatives, demonstration of parasite in splenic smears is the only gold standard for the diagnosis of VL in patients.

Culture of parasite can improve the sensitivity of detection but often done only when other methods fail^{16,17}. Parasite culture is required for obtaining sufficient number of promastigotes to use as an antigen for immunological diagnosis and speciation,

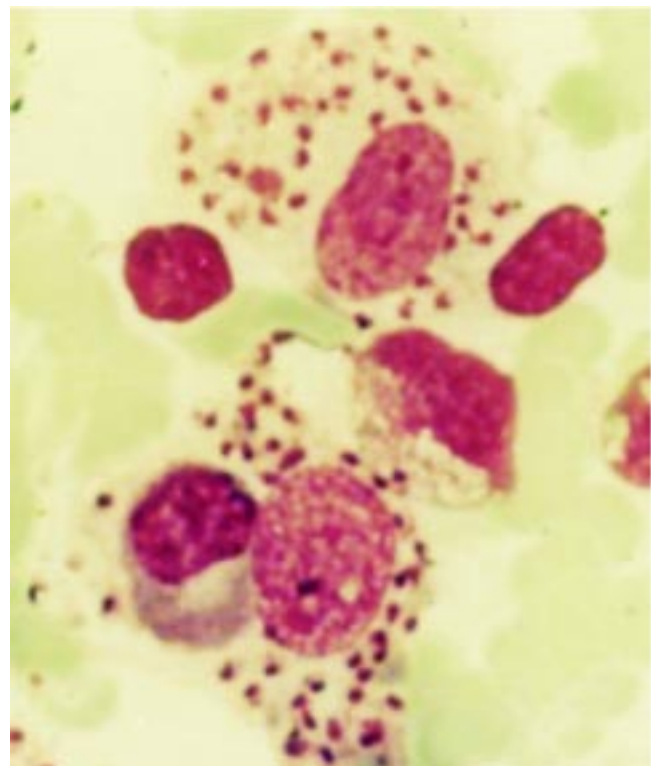


Fig. Microphotograph showing intracellular and extracellular *Leishmania donovani* bodies in splenic aspirate from a patient with visceral leishmaniasis.

inoculating susceptible experimental animals and *in vitro* screening of drugs, *etc.* However, in general, both smear staining and culture should be performed since smears negative lesion can be cultured positive and culture negative lesion can be smear positive^{17,18}.

Species identification is generally not required since none of species other than *L. donovani* is known to cause Indian VL¹⁹. However, identification of an organism to the species level is helpful epidemiologically, and is also important for the treatment and the prognosis determination for global travelers who are not immune to parasite and tend to develop unusual manifestation of the diseases^{20,21}. Species identification can be done by any of the methods such as species-specific isoenzymes pattern by cellulose acetate electrophoresis²², typing by species specific monoclonal antibodies²³, kDNA amplification by choosing primers from conserved regions of different leishmanial species kDNA minicircle^{24,25}, analysis of the *in vitro* promastigotes released antigenic factors, which are different for different leishmanial species²⁶.

Serological methods: Serological methods are highly sensitive and being non-invasive they are comparatively more suited for diagnosing VL in endemic regions. These methods are either based on detection of antibodies (produced against parasite by polyclonal activation of B cells) or antigens. Many conventional methods for antibodies detection for instance gel diffusion, complement fixation test, indirect haemagglutination test, indirect fluorescent antibody detection test (IFAT), and counter current electrophoresis have been evaluated with varying sensitivities and specificities²⁷⁻³¹. However, except IFAT, which is used on limited scale, these tests are rarely used for routine diagnosis of VL. The sensitivity of the IFAT varies from 80 to 100 per cent in various studies and specificity 96 to 100 per cent³²⁻³⁴.

Currently, the most used methods for diagnosis of VL are direct agglutination test (DAT) and enzyme linked immunosorbent assay (ELISA). DAT was introduced about two decades ago rapidly followed by its improved version for field use^{32,35}. DAT has been found to 91-100 per cent sensitive and 72-100 per cent specific in various studies elsewhere in the

world³⁶⁻³⁹. In India also it has been reported to be 95-100 per cent sensitive and 100 per cent specific^{34,40,41}. However, in spite of its excellent diagnostic accuracy, its use is limited in India mainly due to two reasons; non-availability of standardized antigen (freeze-dried and liquid antigen) and its adaptability at the rural centres. Further, despite its simplicity and economy, its evaluation has not been done extensively in Indian VL, and its commercialization failed to take off during earlier years in India. A fast agglutination-screening test (FAST) has been developed in the recent years, which can rapidly detect (<3 h) antileishmanial antibodies in serum samples and on blood collected on filter paper. This test uses the freeze-dried antigen and utilizes only one serum dilution, making the test less cumbersome and readily reproducible⁴².

ELISA is now being used as potential serodiagnostic tool for VL. Though this technique is highly sensitive, its specificity depends upon the antigen used. Two types of antigens, crude soluble antigen (CSA); obtained by freezing and thawing of live promastigotes, and a recombinant antigen (rk39) a conserved part in kinesin region can be used for diagnosis of VL⁴³⁻⁴⁵. The sensitivity of ELISA with CSA has been found in the range of 80-100 per cent, but the cross reaction with other infectious disease that mimics the symptoms also have been recorded^{27,46-49}. The rK39-based detection has been found to be 100 per cent sensitive and specific in diagnosis of VL by ELISA^{47,48,50}. The rK39 antigen has been found quite predictive of onset of disease manifestations in VL patients. The antigens are also being used in monitoring disease after chemotherapy and predicting clinical relapse since the antibodies titres against these specific antigens are closely associated⁴⁸. In addition, rK39 ELISA has high predictive value for detecting VL in immunocompromised patients, like those with AIDS. Several other recombinant antigens like recombinant gene B protein (rGBP) from *L. donovani*, rORFF from *L. infantum*, rgp63, rk90, rk26 from *L. chagasi* have been developed and tested⁵¹⁻⁵⁴. However, these tests require a well-equipped laboratory and cannot be employed on a wider scale and in field conditions. An immunochromatographic strip (InBios, USA) using

rK39 antigen and based on lateral flow is now commercially available in form of antigen impregnated nitrocellulose paper strips adapted for use under field conditions. The strip test is very easy to use in field and in initial studies; it was found to be 100 per cent sensitive and 91-98 per cent specific in Indian VL patients^{55,56}. Moreover, it has been used successfully as a diagnostic guide in suspected cases⁵⁷. In a recent evaluation, it has been found quite useful and a reliable indicator of VL in India⁵⁸.

Antigen-based immunodiagnostic tests are found to be more specific than antibodies based methods and are also useful in immunocompromised patients^{40,59}. Two urinary antigens of 72-75 and 123 kDa have been reported to be very useful in diagnosis and prognosis of kala-azar with sensitivity of 96 per cent and specificity of 100 per cent⁵⁹. A commercial KATEX; a new latex agglutination test for detecting leishmanial antigen in urine of patients with VL has shown sensitivities between 68-100 and 100 per cent specificity in preliminary tests⁶⁰. However, these tests are still under the preliminary phase evaluation and their commercialization is still far from the reality.

DNA detection methods: A test of cure in kala-azar is a debatable issue. In spite of significant development of diagnostic tools, none of the immunological methods have proved to be useful as a test of cure. The test based upon antibodies detection may remain positive for several years due to high and persistent antibodies titres in patients and hence fails to detect past and present infection and in immunocompromised patients⁴⁷. The detection of *Leishmania* DNA via polymerase chain reaction (PCR) is a definite breakthrough in the diagnosis and prognosis of VL as a non invasive method with excellent sensitivity and specificity. A variety of nucleic acid detection methods targeting both DNA and RNA have been developed. Different DNA sequences in the genome of *Leishmania* like ITS region, gp63 locus, telomeric sequence, sequence targeting rRNA genes and 18SrRNA, ssUrRNA and both conserved and variable regions of kDNA minicircles have been documented in diagnosis and prognosis of kala-azar⁶¹⁻⁶⁴.

The most suitable target for the DNA based diagnosis is kinetoplast DNA minicircle (kDNA)^{65,66}. The *Leishmania* PCR assays using peripheral blood as clinical specimen showed to be a highly efficient non invasive alternative with sensitivity varying from 80-100 per cent^{25,62,67-70}. PCR can be proved more useful in the prognosis of VL since none of the available methods can be used as test of cure. However, in prognosis PCR is still far from standardization and has been done only by few workers and mostly in HIV/VL co-infected patients^{62,67,69-71}. Few workers have also evaluated PCR as test of cure in immunocompetent patients suggesting the association between clinical improvement and clearance of *Leishmania* DNA from peripheral blood^{72,73}. We have also shown the usefulness of PCR as a test of cure in VL patients⁷⁴. Recently, a PCR-ELISA technique has been developed that is able to detect a minimum of 0.1 promastigotes or 1 fg of genomic material⁷⁵. PCR assay with buffy coat preparation to detect kDNA was 10 times more sensitive than that with whole blood preparations and particularly good results were obtained when proteinase K based method were used⁷⁶. Besides being a highly sensitive and specific tool for the diagnosis and prognosis of VL and useful method for species identification, it can also be used to distinguish between relapse and re-infection in treated VL patients^{77,78}. Additionally, a positive PCR result in healthy endemic controls may lead to the conclusion that they may suffer from VL⁷⁹. In these healthy endemic controls, a combination of DAT (which shows low titre in healthy controls) and PCR may be helpful in defining the status of these patients. Sensitivity and specificity of some of the commonly used diagnostic methods are summarized in the Table.

Treatment

The treatment options for VL are limited and far from satisfactory. All the drugs available need to be given parenterally except miltefosine, and are potentially toxic. Throughout the world pentavalent antimonials compounds (Sb^v) have been the mainstay of antileishmanial therapy for more than sixty years. The organic pentavalent antimonials were first used in 1912 soon after the recognition of *Leishmania*

Table. Sensitivities and specificities of various methods used for diagnosis of visceral leishmaniasis

Method and test or tissue used	Sensitivity (%)	Specificity (%)
<i>Immunodiagnosis by antibody detection:</i>		
IFA test ^{32,34}	55 - 70	70 - 89
DAT ^{34,49}	90 - 100	72 - 95
ELISA with CSA ⁴⁷	80 - 100	84 - 95
ELISA with rK39 ^{48,49}	100	100
Rapid strip test with rK39 ^{56,57}	100	93 - 97
<i>Antigen detection:</i>		
KATEX ⁶¹	68 - 100	100
DNA detection (PCR) ⁶⁶	90 - 100	100

IFA, indirect fluorescent antibody detection test; DAT, direct agglutination test; ELISA, enzyme linked immunosorbent assay; CSA, crude soluble antigen; PCR, polymerase chain reaction

species as cause of leishmaniasis in 1904⁷. Pentostam (sodium stibogluconate) and glucantime (meglumine antimoniate) have been the mainstay therapy for kala-azar. Due to high cost (approx 200 US \$ per patients) of branded sodium stibogluconate, a generic sodium antimony gluconate (SAG, Albert David Ltd, India, 13 US \$ per patient) is being used to treat patients satisfactorily without any significant difference in final cure^{80,81}.

These drugs have been used successfully for several years to treat patients suffering from VL in the endemic region of Bihar State. Unfortunately, the parasite has become resistant to this drug in these regions. Till late 1970s a small daily dose (10mg per kg, 600 mg maximum) for short duration (6-10 days) was considered adequate, when unconfirmed reports suggested 30 per cent treatment failure with this regimen from four most severely affected districts Muzaffarpur, Samastipur, Vaishali and Sitamadi in Bihar⁸². An expert group on kala-azar revised recommendations to use Sb^v in two 10 days (20 mg per kg, 600 mg total) courses with an interval of 10 days⁸³ and an improvement in cure rates (99%) were noted⁸⁴. Few years later, Thakur *et al* randomized patients to receive Sb^v 20 mg per kg (maximum 600 mg) either for 20 days or longer and found only 86 per cent cure rate⁸⁵. In the same year, WHO expert committee recommended that

Sb^v be used in doses of 20 mg per kg per day up to maximum of 850 mg for 20 days, and a similar regimen for 20 days in cases of treatment failure⁸⁶. However, following the above recommendations Thakur *et al* reported a decline in cure rate to 71 per cent after 20 days of treatment at the same dose⁸⁷. One year later Jha *et al* found only 64 per cent cure rate in hyperendemic regions of Bihar⁸⁷. We also observed that Sb^v (20 mg per kg without upper limit) cured only 60 per cent⁸⁸. Incidentally, only 2 per cent patients from neighbouring State of eastern UP failed treatment⁸⁹. Thus high level Sb^v unresponsiveness existed in Bihar State though the drug continued to be effective in other areas.

Reasons of drug resistance

The reason for the emergence of resistance is widespread misuse of the drug, as Sb^v is freely available in India, and is easily accessible over the counter. In human to human (anthroponotic) transmission once Sb^v resistance gets established, it spreads exponentially and organisms sensitive to the drug get eliminated quickly, and drug resistant parasites continue to circulate in the community. In the endemic regions, VL patients (73%) first consult unqualified quacks who might not use the drug in appropriate doses⁹⁰. It has been observed that only a minority (26%) of patients was treated according to prescribed guidelines, and irregular use and incomplete treatment were of common occurrence⁹⁰. Further, it is a common practice to start with small dose and gradually build up the dose over a week. Drug free intervals are given with the belief that it will prevent renal toxicity. Many times the daily dose of drug is split into two injections, to be given twice daily. These practices presumably expose the parasites to drug pressure, leading to progressive tolerance of parasite to Sb^v. These facts point towards the reasons contributing significantly to the development of drug resistance in Bihar⁹¹. Further, experiments with intramacrophageal Sb^v sensitivity assays suggest that the refractoriness to Sb^v in Bihar is due to emergence of drug resistant strains, as clinical isolates from unresponsive patients needed 3-5 times greater concentration of Sb^v to achieve similar leishmanicidal activity (ED₅₀ and ED₉₀, respectively) than those isolates from Sb^v responsive patients⁹².

Unfortunately, little is known about the mechanism of underlying drug resistance as seen in human visceral leishmaniasis. After administration, pentavalent antimonials are converted into trivalent compounds for its antileishmanial effect. The reduction of pentavalent to trivalent compound takes place either in macrophages⁹³ or in the parasite⁹⁴. In the later case, loss of reductase activity of parasite may lead to resistance. This is supported by the observation that Sb^v resistant *L. donovani* amastigotes lose their reductase activity⁹⁴. Molecular studies have identified an ATP binding cassette (ABC) transporter system, p-glycoprotein A (PGPA) involved in the metal resistance^{95,96}. PGPA is a member of multidrug resistance protein family, whose substrate includes organic anions and drugs conjugated to glutathione, glucuronate or sulphate. *Leishmania* contains glutathione as well as trypanothione (TSH) formed by conjugation of glutathione with spermidine. Transport experiments using radioactive conjugates clearly showed that PGPA recognized and actively transported the metal conjugates⁹⁷. Thus, PGPA might be conferring resistance either through efflux from *Leishmania*⁹⁶ or by sequestering metal thiol conjugates into a vacuole^{97,98}. In a laboratory generated multidrug resistant (MDR) *L. tropica* line overexpressing a P-glycoprotein-like transporter displayed significant cross-resistance to miltefosine⁹⁹. Defective uptake of miltefosine by resistant *L. donovani*¹⁰⁰ lines appeared to be through point mutations on a plasma membrane aminophospholipid translocase¹⁰¹.

Although an increase in the level of TSH has been reported from most of the resistant mutants amplification, expression of PGPA is not universal phenomenon^{102,103}. It has been shown that increased TSH may be mediated by *gsh-1* amplification (glutamylcysteine synthase) and/or ornithine decarboxylase (ODC) overexpressions^{104,105}. Moreover, butathione sulphoxemine (BSO), a specific inhibitor of *gsh* can reverse resistance to Sb^v in *Leishmania*¹⁰⁶. Most of these observations have come from resistant laboratory strains of *Leishmania*. The gene, recently identified in some of our antimony unresponsive isolates clearly suggests to be involved in conferring resistance to the parasites, however, the exact mechanism of resistance development remains

to be elucidated¹⁰⁷. Since the amplified sequence in clinical isolates did not hybridize with PGPA gene or multidrug resistance gene (*mdr*), suggesting involvement of some other mechanism in drug resistance in these strains. Another 7 Kb DNA fragment has also been identified, which was able to restore Sb^v reduction activity in mutants to near wild type levels¹⁰⁸.

Other therapeutic options

In India Sb^v has been the backbone of anti VL therapy because of its low cost 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:

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 patients refractory to Sb^v¹⁰⁹. 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 reported¹¹⁰ but its efficacy gradually declined over the years. It now cures only 70 per cent of patients^{87,111}. 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^v for kala-azar patients¹¹⁰⁻¹¹³. However, it has been used in treatment of both Old and New World cutaneous and mucocutaneous leishmaniasis¹¹⁴⁻¹¹⁶.

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* species¹¹⁷. 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*¹¹⁸. 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^{112,119}. 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 Sb^v 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.

Lipid formulation of amphotericin B : The need to develop less toxic, more effective formulation of amphotericin B has led to three new clinical formulations of amphotericin B in which deoxycholate has been replaced by other lipids. These formulations are liposomal amphotericin B (L-AmB: Ambisome), 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 toxicity¹²⁰. 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 ambisome for five consecutive days induces high cure rates¹²¹. 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 cure¹²². In another trial, a single total dose (5 mg of ambisome/kg) was compared with a similar dose administered over 5 days and final cure was achieved in 91 and 93 per cent patients respectively¹²³. Safety of liposomal amphotericin B permits administration of total dose requirement in a single infusion^{122,124}. However, prohibitively high cost makes these compounds unaffordable in VL endemic countries like India.

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 Sb^v 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*¹²⁵. Miltefosine has been approved in India for treatment of VL at a dose of 50-100 mg (~2.5 mg/kg) for four weeks^{126,127}. It has also been found safe and effective in paediatric patients^{128,129}. 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.

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 India¹³⁰⁻¹³². 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^{133,134}.

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, USA¹³⁵. 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^{135,136}.

Azoles and other steroid biosynthesis inhibitors: The azoles, like ketoconazole and triazoles, itraconazole and fluconazole produce an anti-leishmanial effect by blocking ergosterol synthesis¹¹⁸. 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*¹³⁷. Till date this drug has not been tried in India.

Cytokines : Leishmania infection progress to kala-azar in individuals who fail to initiate Th1 response, which is mediated by IL-2 and IFN- γ ^{138,139}. Interferon- γ is one of the principal activators of macrophages. Interferon- γ as adjuvants to Sb^v has been used successfully in VL with high cure rate in comparison to Sb^v alone⁸⁸. Later, it was observed that interferon- γ (daily dose 100 $\mu\text{g}/\text{m}^2$) though improved the response rate to antimony, but overall cure rate was less than 50 per cent¹⁴⁰. However, steep decline in the response rate to antimony rendered the addition of IFN- γ ineffective.

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.

Control methods and strategies

It is almost impossible to devise a single control strategy to for leishmaniasis control. A strategy combining the following three approaches can help in eradication of the disease; (i) a vector control strategy at national level is need of hour. The classical example of efficacy of this strategy is the almost disappearance of VL cases in India in 1960s when insecticides were used extensively as a part of the National Malaria Eradication Programme (now National Vector Borne Disease Control Programme). This approach is frequently hampered by the logistics of insecticides along with DDT use since production of DDT is now stopped and the other available insecticides are expensive; hence, this strategy is not in use either locally or by government of India in VL affected regions. Commercial production of pyrethroids impregnated fabrics (bed nets and curtains) or insecticides paints in a slow release emulsifiable solution should be encouraged to prevent the transmission of the disease; (ii) serological diagnosis at the infection stage, if followed by a prompt treatment, will prevent the evolution to overt disease and reduce morbidity and mortality. It will also reduce the parasite load and transmission rate, as in India and humans are known reservoir host for leishmania parasites; and (iii) health education of the population and physicians serving in the endemic area to improve the awareness regarding transmission, clinical features of the disease and importance of complete treatment, modes of prevention can form effective control strategy.

It is likely that in coming years a satisfactory diagnostic tool and therapeutic modalities will be developed and vaccine development is also not far from reality.

Conclusion

Despite significant developments in various fields, VL remains a serious public health problem

in many part of country. In most of the affected regions, only a basic level of control exists and funding, logistic and management problems are the cause of severe deficiencies, particularly in the reliability of the reporting system, the quality of diagnosis and the availability of the first line drugs. Control measures should be specifically adapted to the local epidemiology. Further, for better VL control tools for diagnosis that could be used for prognosis as well as treatment are still needed. Growing resistance of the parasite to the cheapest antileishmanial drug (*i.e.*, pentavalent antimony) and potential of the parasite to become resistant against other available first line drugs, the currently used treatment pattern needs to be revised. There is an urgent need for experimental and clinical studies on combination of drugs to prevent resistance to miltefosine and amphotericin B and to develop a policy to prevent drug resistance in anthroponotic areas. A strategy should also be developed for monitoring of the resistance. A new drug, which would allow a safer, shorter and cheaper treatment and would be easier to administer orally, a non invasive alternative to the invasive method of parasitological diagnosis, identification of the cost-effective surveillance system and control strategies to reduce the mortality rate, a most suitable vector control approach, identification and quantification of risk factors to better focus the control activities and prevent epidemics, are the most important aspects for the control and complete eradication of the disease from India as well as from other endemic countries.

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Reprint requests: Dr R.K. Singh, Infectious Diseases Research Laboratory, Department of Medicine
Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India
e-mail: rakeshbhu@yahoo.com