Visceral leishmaniasis (VL) is a systemic disease that is fatal if left untreated and is caused by an obligate intracellular protozoan parasite of genus leishmania. VL is typically caused by the Leishmania donovani complex which includes three species: L. donovani, the causative organism for VL in the Indian subcontinent and East Africa; L. infantum, causes VL in the Mediterranean basin; and L. chagasi, which causes the disease in Central and South Africa. Sandfly is the only vector responsible for transmission of leishmaniasis. It inoculates the flagellar promastigotes into the skin of the host, which are taken up by the macrophages and dendritic cells, and then transformed into aflagellar amastigotes, and divide by binary fission, leading to ultimate rupture of the cells and dissemination to the reticulo-endothelial system of the body and produce 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. In India, PKDL occurs 6 months to 3 years after treatment, whereas in Sudan it occurs either concomitantly with VL or within a few months [1].

**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. [2] Transmission in Indian subcontinent is anthropoantonic (man to man), whereas in Mediterranean regions and Americas, it is zoonotic, and dog is the main reservoir.

Immunosuppression due to HIV increases the risk of VL by 100- to 1000-fold in endemic areas. Leishmania-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:**
The clinical manifestation of VL lacks specificity as its clinical manifestations are shared by a number of commonly occurring diseases like malaria, typhoid, tuberculosis, etc.. Therefore, confirmatory tests are required for diagnosis. Moreover, the sequestration of the parasite in the spleen and bone marrow necessitates embarking upon invasive procedures for demonstration of parasites.

Laboratory diagnosis of leishmaniasis can be made by the following:

i. Demonstration of parasite in the tissue of relevance.

ii. Immunodiagnosis by detection of parasite antigen or anti-leishmanial antibodies,

iii. Detection of parasitic nucleic acid in tissue samples.

**Demonstration of parasite**
Definitive diagnosis is made by demonstration of the parasite in splenic or bone marrow aspirate or buffy coat of blood stained with Giemsa or Leishman staining. Splenic aspiration is the gold standard for diagnosis of kala-azar with sensitivity exceeding 95%. However, it is associated with risk of fatal haemorrhage in inexperienced hands as the procedure requires training and expertise. The sensitivity of bone marrow smear is only about 60 to 85%, it is a painful and cumbersome procedure.

**Immunodiagnosis**
Antibody detection: There is polyclonal activation of B cells in VL, resulting in marked elevation of levels of IgG and IgM against various proteins and haptns. Several serological techniques are based on detection of these antibodies.

The specific serodiagnostic tests utilise leishmanial antigen to detect antibodies like direct agglutination test (DAT), IFAT (indirect fluorescent antibody test), ELISA (enzyme linked immunosorbent assay), and immunochromatographic strip test.
Tests like microELISA, IFAT, CIEP are highly sensitive and specific, however, these can be performed in sophisticated laboratories with modern equipment and skilled personnel. Unfortunately because of these reasons, these test could not become popular in endemic regions.

**DAT:** It is a highly sensitive and specific tool for the diagnosis of VL. It uses trypsinised and stained whole promastigotes as antigen. Agglutination is observed after overnight incubation of the patient’s serum. Unfortunately only two European laboratories produce standardized lyophilized antigen, and these are quite expensive. Thus high cost, prolonged incubation period, cumbersome procedure, and need for refrigeration limited its use in dedicated field laboratories like that of MSF in Sudan.

**rK39 immunochromatographic strip test:** It is a simple, rapid, and cheap test with excellent sensitivity and specificity; it does not require any specific expertise, and can be easily used in difficult field conditions. Though there are several rapid tests marketed, but only two (InBios, USA and DiaMed, Switzerland) have been validated and FDA has approved only the test of InBios. In this test, the antigen is immobilised in a nitrocellulose membrane. Goat antiprotein A is attached to the membrane above this band to detect IgG. A protein A-gold conjugate is used as the immunochromatographic detection reagent. After a drop of blood serum is placed on the absorbent pad followed by 4 drops of buffer, in the presence of anti-rk 39 IgG antibodies, two pink lines appear; the upper control line indicates the presence of IgG and proper test functioning, whereas the lower line indicates positive test result. Several studies from the Indian subcontinent have shown it to be 100% sensitive and 93-98% specific. Limitations include positivity of the test in healthy endemic controls (~20%), and in successfully treated patients for several months to years, making it useless in diagnosis of relapses or reinfections. Notwithstanding these limitations, this test has proven to be one of the best tools for the diagnosis of VL in the Indian subcontinent.

**Antigen detection:** A latex agglutination test (KATEX) for detecting leishmanial antigen in urine of patients with VL has shown sensitivity between 68 and 100% and specificity of 100% in preliminary trials, and is undergoing further development.

**Molecular diagnostic methods**

PCR has been used extensively for the diagnosis of VL. By using this technique, the conserved DNA sequence of leishmania is amplified using a broad range of clinical samples like peripheral blood, skin smears, bone marrow, splenic aspirates. PCR in various clinical samples has shown excellent sensitivity and specificity. PCR can be used to assess the success of treatment, as a negative PCR is a strong indicator of cure. However, PCR results from healthy endemic controls may be positive, as it might amplify dead parasite DNA leading to misdiagnosis. Nevertheless, PCR is a highly sensitive and specific test for the diagnosis of VL and PKDL. Cheaper and simpler versions of PCR need to be developed for field applicability of this excellent diagnostic tool.

**TREATMENT OF VL**

In general, treatment of leishmaniasis is far from satisfactory. Most antileishmanial drugs are toxic and have to be given parenterally for prolonged periods. Once successful therapy is instituted there is a prompt return of temperature to normal, regression of spleen and recovery of blood counts towards normal. An initial cure can be declared if there is clinical improvement and no parasites are seen at the end of treatment. Complete regression of splenomegaly may take several months, best indicator of final cure is freedom from a clinical relapse at six months follow up.

**Drugs:**

**Pentavalent antimonials**

Treatment of VL centered on Pentavalent antimonials like sodium stibogluconate (Sb”) and meglumine antimoniate for several decades. Recommended dose is 20 mg/kg/day iv/im for 28-30 days. Adverse effects like gastrointestinal symptoms, myalgia, arthralgia, and metallic taste are common. Pancreatitis, especially in HIV co-infected patients, cardiotoxicity and sudden deaths are serious side-effects. Monitoring of patients with serum chemistry, complete blood count, and electro-cardiography should be done. Corrected QT interval in ECG helps to detect cardiotoxicity and its prolongation (> 0.5 secs) signals the likely onset of serious and fatal cardiac arrhythmias, culminating into death.

In regions endemic for VL, response to these drugs is excellent, except in Bihar. However, for the treatment of PKDL, it has to be used for 120 days in India and for at least 2 months in Sudan. It is still the least expensive and the most cost-effective treatment option and remains the first line of therapy, except in regions with high levels of resistance (north Bihar and adjoining areas of Nepal). Though, it remains a toxic drug with mortality being 2-5% reported in different studies from all parts of the world, so it should soon be replaced with safer drugs.

**Pentamidine isethionate**

A diamidine compound was used for the treatment of antimony resistant VL. However, it is a toxic drug with severe toxicity like insulin dependent diabetes, hypotension, and death. Further, over the years its efficacy also decreased and now use of this drug is abandoned.

**Amphotericin B (Ampho B)**

Ampho B is a polyene antibiotic, used extensively as an antifungal drug, has excellent antileishmanial activity. It is being used as a first line drug in regions with high levels of Sb” resistance. The drug is administered at a dose of 0.75 - 1 mg/kg for 15 IV infusions either daily or on alternate days, induces high cure rates of nearly 100%. Most common adverse effect includes infusion reactions like high fever with chills, and thrombophlebitis. Other adverse reactions are nephrotoxicity, hypokalaemia, thrombocytopenia, myocarditis and occasional death. These reactions necessitate close monitoring and hospitalization up to 4-5 weeks, leading to increase in the cost of treatment. In north
Bihar, where Sb\(^\text{r}\) resistance is high, National Expert Committee recommends amphotericin B deoxycholate to be used as the first line drug.

**Lipid formulations of amphotericin B**

Toxic effects of amphotericin B deoxycholate have been largely ameliorated with the advent of lipid formulations of amphotericin B. In these formulations, deoxycholate has been replaced by other lipids which facilitate its preferential uptake by reticuloendothelial cells of liver, spleen and bone marrow, thus achieving targeted drug delivery to the parasite resulting in increasing efficacy and reduced toxicity. Due to targeted delivery the tolerance is improved and adverse effects including hypokalemia and nephrotoxicity are generally reduced. Moreover, it is possible to deliver large doses of the drug over short duration.

Three such lipid associated formulations of amphotericin B have been tested extensively in the treatment of visceral leishmaniasis: (i) liposomal amphotericin B (AmBisome; Gilead Sciences, Foster City, CA, USA); (ii) amphotericin B lipid complex [Abelcet (ABLC); The Liposome Co, Princeton, NJ, USA]; and (iii) amphotericin B colloidal dispersion [Amphocil (ABCD); Sequus Pharmaceutical; Menlo Park, USA]. AmBisome is licensed in several European countries and USA for primary treatment of Visceral leishmaniasis (VL).

There is a regional variation in the response to liposomal Ampho B. In immunocompetent patients in Europe and South America, total doses of 18-24 mg/kg, and in Kenya 14-18 mg/kg given over 10 days cured 90-100 per cent patients. In Indian VL, a dose of 6 mg/kg (2 mg/kg x 3) cured 100 per cent and 3.75 mg/kg cured 89 per cent patients. In a subsequent study employing a single dose of 7.5 mg/kg of AmBisome, 90 per cent patients were cured with minimal adverse events.

Results from a recent three armed study in Bihar where a direct comparison was made between conventional amphotericin B (1 mg/kg/day on alternate days for 30 days) and AmBisome and Abelcet (both at a dose of 2 mg/kg/day for 5 days) showed that though the overall cure rates of amphotericin B were comparable with AmBisome or Abelcet being 96 vs. 96.2 per cent, respectively, the lipid formulations had an upper edge as they produced distinctly lower toxicities, notably the absence of nephrotoxicity and significantly lower infusion reactions.

Alternatively, single dose regimens for AmBisome (5 mg) was comparable with a similar dose administered for 5 days with similar cure rates of 91 and 93 per cent respectively; this single dosage showed excellent tolerance and safety coupled with a tremendous economic impact as hospital stay would be was considerably reduced. Through the WHO initiative, AmBisome is now available at 10% (18-20 $) of its original cost for developing countries including India.

**Paromomycin**

This aminoglycoside is known to have antileishmanial activity. A randomised, unblinded, controlled Phase II trial in Bihar was designed to assess the efficacy and tolerability of paromomycin alone compared with Sb\(^\text{r}\). Cure at the end of a six-month follow-up was achieved in 77.93 and 97% patients treated with paromomycin 12, 16 and 20 mg/kg/day for 21 days, respectively, and only 63% in patients receiving Sb. It was thus established that paromomycin alone was effective for the treatment of VL in the Sb\(^\text{r}\)-resistant areas of Bihar.

These encouraging results led to the Phase III study in Bihar in 2003–2004, where paromomycin 11 mg/kg body weight (15 mg/kg as the sulfate) i.m. for 21 days (n = 502) was shown to be non-inferior to amphotericin B (1 mg/kg i.v. alternate day for 30 days; n = 165) with final cure rates of 94.6 versus 98.8%, respectively. No nephrotoxicity was seen, and reversible ototoxicity as observed in 1.6% patients. Paromomycin is manufactured in India and the treatment cost of an adult is between 10-15 US$. Its affordable cost coupled with a 3 weeks treatment schedule and excellent safety and efficacy puts it in line to be used as a first line drug.

**Miltefosine**

It is an alkylphospholipid analogue initially developed as an oral anticancer drug, and is the first highly efficient oral drug to be registered in India for the treatment of VL in a large randomised phase III trial on VL patients aged > 12 years, the long-term cure rate was 94% after 28 days of miltefosine. Miltefosine commonly induces mild gastrointestinal side effects such as anorexia, nausea, vomiting (38%) and diarrhea (20%); however, these are usually brief and resolve even as the treatment is continued. Transient asymptomatic elevation of hepatic transaminases and rarely renal insufficiency may be observed with miltefosine. The recommended daily dose of miltefosine is 50-100 mg (for body weight of < 25 or > 25 kg respectively) for 4 weeks, and 2.5 mg/kg for children. Miltefosine is teratogenic in rats and should not be used in pregnant women or those with child bearing potential in whom adequate contraception cannot be assured for the duration of treatment and for a further 3 months after treatment. Being first oral drug and its ease of administration, miltefosine has been chosen as the drug for Elimination Programme for VL from the Indian Subcontinent in which India, Nepal and Bangladesh are participating.

**Sitamaquine (WR-6026)**

This orally administrable primaquine analogue was developed by the Walter Reed Army Institute of Research. In recently concluded phase II studies in India, 89 and 100% were cured with sitamaquine 1.75 and 2.0 mg/kg/day for 28 days. Sitamaquine alone was effective for the treatment of VL in the Sb\(^\text{v}\)-resistant areas.

**Cytokine therapy**

IFN-\(\gamma\) is one of the principle activators of macrophages and has shown its ability to control leishmanial infection in animals. Results of studies in India have been disappointing with IFN-\(\gamma\) with cure rate less than 50%. Decline in the response rate of antimony rendered the addition of IFN-\(\gamma\) ineffective. In a developing country...
like India field applicability of these products remains a distant possibility.

**Combination therapy:**

Efforts to improve the treatment of VL and develop new therapeutic approaches have moved steadily forward in the past decade. This progress has been driven, in part, by the emergence of large-scale resistance to conventional pentavalent antimony (Sb) treatment, and in parallel with the development of new drugs for VL. There is also growing interest in combination therapy to treat visceral leishmaniasis, as practiced, for example, in the treatment of tuberculosis, HIV infection, and malaria. Potential advantages of 2-drug chemotherapy in the treatment of kala-azar include:

1. **Less toxicity** (as a result of lower drug doses and/or shorter treatment courses);
2. **Convenience**, better compliance, and lower costs, resulting from less lengthy treatment; and
3. **Possibly reduced likelihood** of developing resistance to either agent.

The combination of sodium stibogluconate and paromomycin was found to be safe and effective in early trials conducted in India and East Africa and has been successfully used in Sudanese patients. In a comparative trial of treatment in southern Sudan, patients (200) were randomised to receive Sb\(^{-}\) 20 mg/kg/day for 30 days (Group S, \(n = 99\)) or Sb\(^{-}\) at 20 mg/kg/day plus paromomycin 15 mg/kg/day for 17 days (Group AS, \(n = 101\)). At days 15–17, microscopy of aspirates showed that 95% in the combination group were negative for parasites compared with 81% in those receiving Sb\(^{-}\) alone. [17] In a pilot study in Bihar, India, paromomycin 12 mg/kg/day in combination with sodium stibogluconate 20 mg/kg/day for 20 days proved efficacious and well tolerated in patients with VL. [18] However, in view of the resistance of Sb\(^{-}\) in Bihar, there is also understandable concern about how to best preserve the efficacy of new single-agent treatments that are now being deployed, especially in regions of endemicity, such as India.

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 liposomal ampho B with either miltefosine or paromomycin, may fulfill this objective. The results of a phase II study conducted in India suggest that treatment with single-dose L-AmB followed by 7–14 days of miltefosine is active against Indian kala-azar and results in high cure rates. [19]

**HIV and VL**

Leishmania co-infection in HIV patients is regarded as an emerging disease. Though initially Southern Europe was the biggest focus of HIV/VL co-infection, however, introduction of HAART led to a considerable decline in the incidence, on the other hand incidence is significantly high in Africa, especially Ethiopia, and steadily rising in the Indian subcontinent. In HIV co-infection, the leishmania parasite may be present outside the reticulo-endothelial system, e.g., in the peripheral blood in HIV infected patients making these patients a reservoir of infection. Parasite load in the peripheral blood is so high that transmission among intravenous drug users by use of shared syringes has been reported.

VL-HIV co-infected patients pose a considerable diagnostic challenge as they present with atypical presentations like gastrointestinal involvement (stomach, duodenum, or colon); ascites; pleural or pericardial effusion; involvement of lungs, tonsils and skin. [20] Amastigotes may be demonstrated in buffy coat preparations and may be demonstrated in unusual sites. For HIV co-infected patients, the sensitivity of antibody-based immunologic tests is low. Conventional PCR as well as PCR-ELISA and real time PCR has shown high sensitivity in bone marrow and peripheral blood samples in VL-HIV co-infection, and has also been used to monitor treatment in these patients. [20] The treatment of leishmaniasis in HIV infected patients is made difficult by increased drug toxicity, frequent relapses, and high parasitaemia. Experience from Southern Europe indicates that the initial responses to Sb\(^{-}\), conventional amphoterics, B, or ABLC are significantly less (~40 - 65%) and serious reactions are frequent. In terms of both safety and efficacy, the best results are observed with AmBisome; an extended course of AmBisome, to a total dose of 40 mg/kg, is the drug of choice in these patients. HAART is the cornerstone of management of HIV/VL co-infection in addition to antileishmanial therapy.

**CONCLUSION**

Although much can be achieved with the existing tools, there is a definite need for continued investment in diagnostics, treatment and prevention of VL. rK39 ICTs perform well for the primary diagnosis of VL in the Indian subcontinent, but further test development and evaluation is required to find biomarkers of the active disease in the backdrop of several pitfalls in antibody-based diagnostic system. Development of a sensitive and easy to use non-invasive antigen-detection test for the diagnosis of VL, and to assess treatment efficacy also remains an important challenge.

Further investment in drug development is badly needed to fill the pipeline with novel compounds, as all of the current drugs have one or more drawbacks. For treatment of VL, both Sb\(^{-}\) and Ampho B are potentially toxic, and have to be administered parentally for prolonged periods (>30 days). A steady decline in the efficacy of Sb\(^{-}\) in endemic areas of Bihar has rendered this drug useless in this region. Lipid formulations of Ampho B are a major advance in the treatment of VL and now well within reach of the National Control Programme.

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. Recent breakthrough is the development of
paromomycin, which with its excellent efficacy, low cost, shorter duration of administration and good safety profile has the potential to be used as first line.

In the meantime, the evaluation of combination therapies with existing drugs remains a priority. Immunochemotherapy is another approach that should be considered. The development of improved therapies should go hand-in-hand with the development of practical tools to monitor drug resistance.

No vaccine is available, however, an effective vaccine would significantly improve VL control. Vector control, which is the most efficient tool for control of vector born disease like VL, has taken a back seat. This should form the back bone of any control programme.

REFERENCES