

Review

Changing trends in the epidemiology, clinical presentation, and diagnosis of Leishmania–HIV co-infection in India



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SUMMARY

Following the HIV epidemic, several countries have reported co-infections of Leishmania with HIV. Co-infection with these two pathogens results in rapid disease progression, more severe disease, and a poor response to treatment. A systematic review of the literature from India is presented herein. Since the first case of visceral leishmaniasis (VL) and HIV was published from India in 1999, a number of cases of HIV–Leishmania co-infection have been reported, but the proportion has been low (0.029–0.4%), as also reported in other countries where these two diseases are co-endemic. More than 89 cases of VL–HIV and 10 cases of cutaneous leishmaniasis (CL)–HIV have been published since 1999. Of these latter 10 cases, five had simple CL and five cases manifested with diffuse cutaneous leishmaniasis (DCL). In addition, one case of post-kala-azar mucocutaneous leishmaniasis in a patient with full-blown AIDS has also been reported. In two cases, it could not be ascertained whether they were cases of DCL or post-kala-azar dermal leishmaniasis from the description. Although the first case of VL–HIV co-infection was reported from the sub-Himalayan state of Uttarakhand, most cases have been reported from the VL endemic state of Bihar. HIV–Leishmania is not alarmingly high in India. Most cases were found to have occurred during 1997–2007. After that, the number of new cases decreased. This is most probably due to the low prevalence of HIV in VL and CL endemic regions and to the free supply of highly active antiretroviral therapy for HIV-infected patients.

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1. Introduction

Leishmaniasis is a parasitic disease caused by the haemoflagellate parasite, *Leishmania* spp.^{1,2} *Leishmania* is one of several genera within the family Trypanosomatidae, characterized by the possession of kinetoplast DNA (kDNA).^{3,4} There are more than 20 species of *Leishmania* that can cause human infection. Female sandflies belonging to the genus *Phlebotomus* and its six subgenera are the main vectors in the Old World³ and the genus *Lutzomyia* in the New World.⁴ On rare occasions, transmission may occur congenitally or as a result of blood transfusion.⁵

Leishmaniasis manifests mainly in three forms: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (MCL). VL is the most severe form of the disease and is often fatal if not treated.⁶ Globally the disease is endemic in 98 countries on five continents, with a total of 310 million people at risk. According to a World Health

Organization (WHO) estimate, 1.3 million new cases of leishmaniasis occur worldwide annually. This review focuses on the epidemiology of HIV and leishmaniasis in India, the immunological events that take place during co-infection with these two pathogens, and the challenges we face in diagnosing AIDS-associated leishmaniasis.

2. Epidemiology of visceral leishmaniasis in India

Of all cases of VL reported in the world, 90% occur in six countries: India, Bangladesh, Brazil, Ethiopia, South Sudan, and Sudan.^{1,2} The word kala-azar was first used in India, and before this term was coined, the manifestations of this disease were known by various other names.⁷ The causative agent of kala-azar remained unknown for several decades. In 1903, Major W.B. Leishman, who was posted in the Dum-Dum area of Calcutta (eastern India), published his microscopic observations in an issue of the *British Medical Journal*. He thought that he had observed trypanosomes. Two months later, Prof. Charles Donovan, Professor at the Madras Medical College, Madras (southern India), suggested that the

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causative agent was a new parasite. In the November 1903 issue of the same journal, Sir Ronald Ross, recognizing the contribution of both of these doctors, named the parasite *Leishmania donovani*.⁸

During the first 50 years of the twentieth century, kala-azar moved from the southern parts of India and remained endemic only in the eastern states of Assam, Bengal, and Bihar until the late 1990s. More recently, the epidemiology of Indian VL showed a shift east to west along the Ganges river, and new foci have been reported in eastern Uttar Pradesh,⁹ Uttarakhand,¹⁰ and Himachal Pradesh.¹¹ Interestingly, these foci have now become endemic for VL.¹² Sporadic cases have also been reported recently from Madhya Pradesh (central India),¹³ Gujarat (western India),¹⁴ Haryana (north India),⁵ and also from southern states of India¹⁵ (Figure 1).

Many of these cases could be associated with migration and blood transfusion, but in several of these cases, no obvious non-vector route could be established. Only two possible explanations could be proposed for this spread to sub-Himalayan and other parts of India. Either the infection has come from Nepal via some snow animal reservoirs, or through sandfly vectors, possibly transported to this region. These new foci also lead to questions¹⁶ on the dictum that post-kala-azar dermal leishmaniasis (PKDL) patients are the only reservoirs for new outbreaks and that Indian VL has an only anthroponotic cycle.¹⁷

In southern Europe and Mediterranean countries, VL is caused by *Leishmania infantum*, a zoonotic parasite, and is predominantly an urban and peri-urban disease.¹ In India, kala-azar is caused by *L. donovani* and is a rural poor man's disease.¹⁸ It more commonly occurs in socially less privileged communities residing on the outskirts of villages, providing more accessibility to the sandfly. The two parasite species are significantly different in several respects. While *L. infantum* is transmitted by *Phlebotomus perniciosus*, *Phlebotomus ariasi*, *Phlebotomus neglectus*, etc., *L. donovani* is mainly transmitted by *Phlebotomus argentipes*. *L. infantum* has a zoonotic cycle, while for *L. donovani*, no animal cycle has been proved and as yet it is considered to be an anthroponotic cycle only, although

recently some goats have been incriminated as possible reservoirs of *L. donovani* in India.¹⁹

Young children are more vulnerable and show severe disease manifestations due to malnutrition and a weak immune defence system.²⁰ The disease is characterized by irregular bouts of fever, substantial weight loss, swelling of the spleen and liver, and gross anaemia. In some areas of India, lymphadenopathy is reported in as many as 50% of cases.¹² Although people are often bitten by sandflies infected with *Leishmania* protozoa, most do not develop the disease and remain asymptomatic yet latently infected.²¹ However, disease presentations are severe among persons who are immunosuppressed, e.g. after organ transplantation,²² haematological malignancy,²³ auto-immune diseases,²⁴ and advanced HIV disease.²⁵

3. Epidemiology of HIV infection in India

HIV, the causative agent of AIDS, reached India within 3 years of its discovery in 1983. The first HIV-1-seropositive case was diagnosed in April 1986 in Tamil Nadu State of south India^{26,27} and the first clinical case of AIDS was diagnosed in Bombay in the same year.²⁸ However, the virus spread very fast to other neighbouring areas and soon a sharp increase in HIV incidence was noted (Figure 1). Within the next 3 years, the prevalence of HIV had increased from 1.8% to 28.6% in commercial sex workers,²⁹ and to 16.3% in professional blood donors in Tamil Nadu.³⁰ Seeing this rapid spread, the Government of India set up a national HIV surveillance program.^{31,32} The surveys carried out by the Indian Council of Medical Research (ICMR) showed that almost all cases were HIV-1 and only 1.3% were HIV-2 monoinfections. In 1.5% of cases, HIV-1 and HIV-2 dual infections were detected.³³ The HIV infection started spreading to the rest of India mostly through the migratory population.^{29,34} However, rural India by and large remained free of HIV until 1996.³⁵ Nevertheless, its spread was very slow to north India, where leishmaniasis has been endemic. The number of HIV cases continued to increase in India until 2006, at which point India had its maximum number of persons living with HIV (2.52 million). From 2006 onwards, the Government of India instituted several preventative measures, including highly effective information, education, and communication (IEC), safe blood transfusion, opt-out diagnostic services, and programmatic administration of highly active antiretroviral therapy (HAART). All of these measures resulted in a steady decline in new HIV infections. The 2013 data show that the number of persons living with HIV in India has come down to 2.1 million.³⁶

4. Immunology of Leishmania–HIV co-infection

Leishmania co-infection has emerged as a major complication in patients infected with HIV. HIV and VL are locked in a vicious circle of mutual reinforcement. In HIV-infected persons, VL quickly accelerates the onset of AIDS and shortens their life-expectancy.^{37,38} Also, HIV increases the risk of clinical VL more than 100 times.^{18,39} This duo produces a cumulative deficiency in the CD4+ T cell-dependent immune response, exponentially increasing disease severity and their consequences. A paradigm shift in cytokine networking also takes place (Figure 2). In the Mediterranean region and Europe, VL is the third most frequent opportunistic infection among HIV-positive patients.³⁹ For these reasons it was suggested that *Leishmania* parasites could be seen as a potential co-factor in HIV-1 pathogenesis.⁴⁰ Further, it is reported that lipophosphoglycan (LPG) on the surface of *L. donovani* amastigotes mediates CD4+ T cell activation, and *Leishmania* antigen-induced tumour necrosis factor alpha (TNF- α) production helps HIV-1 replication.⁴¹ This is further evident from the observation that successful anti-leishmanial treatment decreases the HIV viral load.⁴² Co-infection in THP-1

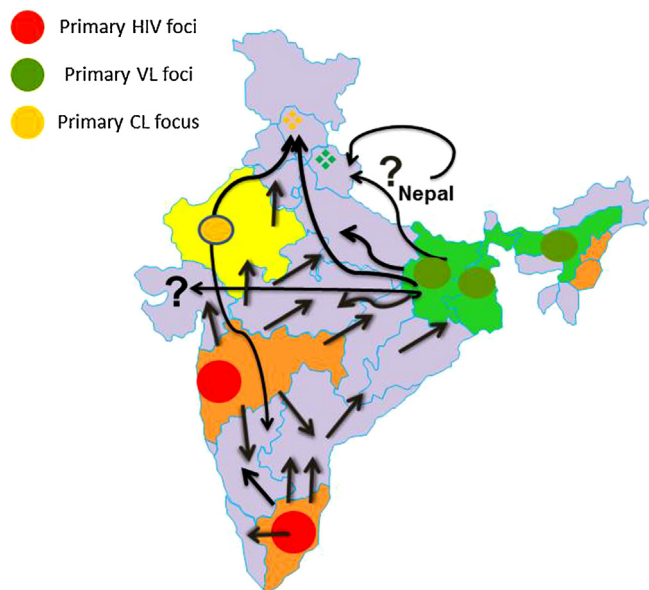


Figure 1. Diagrammatic representation of the spread of HIV-1 from primary foci (dark red spots) to the respective states (red) in southern and western India and eventually to the whole country. Primary foci of *Leishmania donovani* (dark green spots) in eastern states (green) of India spread upward to other parts of the country and became established as a new focus in the sub-Himalayan state (green dots) of Uttarakhand. Cutaneous leishmaniasis caused by *Leishmania tropica* remained endemic to Rajasthan State (yellow), but recently the disease has spread to the sub-Himalayan state of Himachal Pradesh and far south.

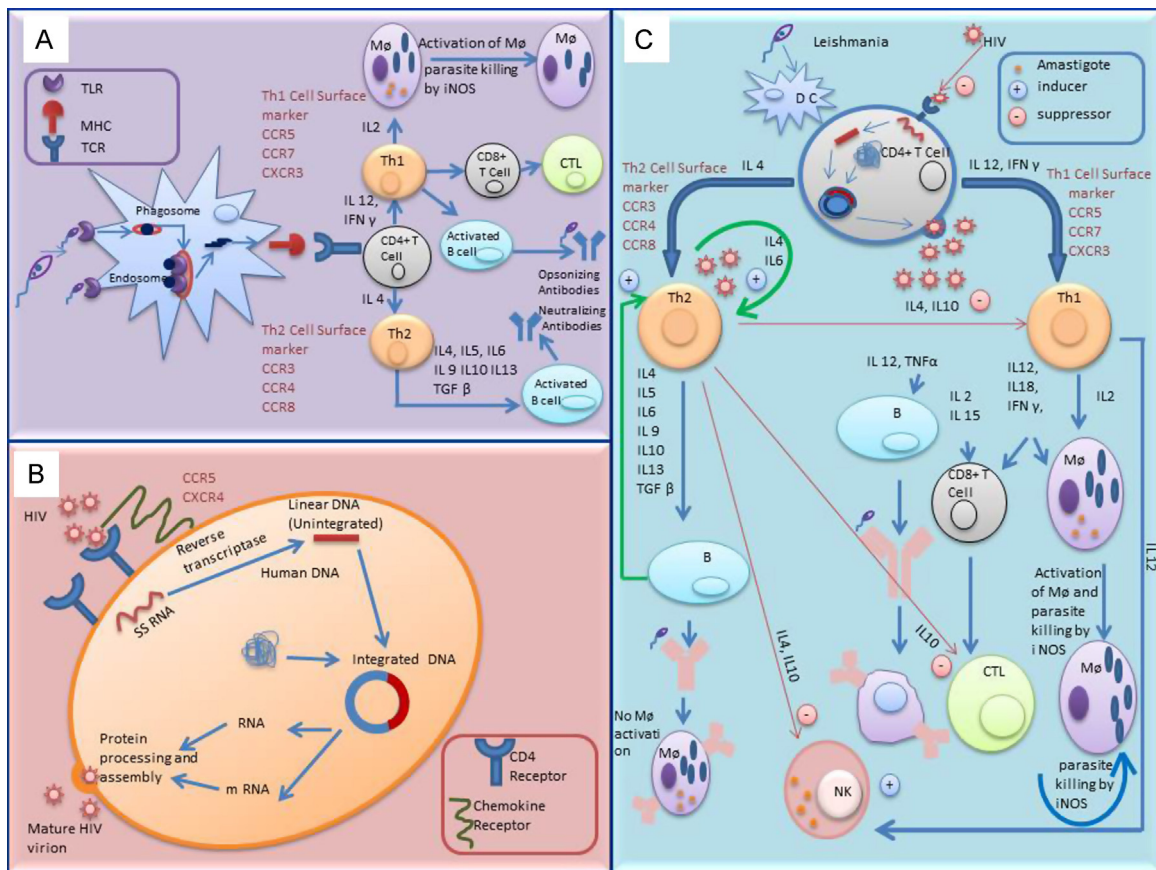


Figure 2. Schematic diagram depicting the immunological events and cytokine networking that take place during *Leishmania donovani* infection (panel A), HIV infection (panel B), and during co-infection of HIV and *Leishmania donovani* (panel C) in the human host.

macrophage cell lines increases the multiplication of *L. donovani* amastigotes in the macrophages. It was shown that a killed HIV preparation abrogates the proliferative response as well as interferon gamma (IFN-γ) production from the *L. donovani* antigen-induced PBMCs of healthy individuals.^{43,44}

5. Epidemiology of Leishmania–HIV co-infection

In the early 1990s a rapidly increasing trend of HIV–*Leishmania* was noticed, mainly in Spain and other neighbouring countries, when the HIV epidemic was at its peak. By 1997, the number of co-infected patients had peaked. Of the first 1700 cases of *Leishmania*–HIV co-infection reported to the WHO from 33 countries, 1440 were from the Mediterranean region,³⁹ mainly from Spain. During 1998–2001, the incidence of HIV–VL co-infection plateaued.⁴⁵ In Spain, most of the infections occurred through shared injection equipment.⁴⁶ After 2001, awareness about HIV–*Leishmania* co-infection increased, safe injection practices were promoted, and HAART was administered to most of these patients, which led to the decline in the number of new cases.⁴⁵ However, in Africa, particularly Ethiopia and Sudan, HIV–*Leishmania* co-infection is still regarded as an emerging disease.¹⁸ In 2013, the WHO stated that more than 35 countries had reported VL–HIV co-infection by the year 2012.¹

6. Epidemiology of Leishmania–HIV co-infection in India

Curiously no case of *Leishmania*–HIV co-infection was reported from India until 1999.¹⁰ This is most likely because the HIV epidemic remained localized to the western and southern parts of the country, where leishmaniasis is non-existent (Figure 1). Most

cases with a low CD4+ cell count died of tuberculosis or a tuberculosis-like illness, often due to misdiagnosis. The first case of VL–HIV co-infection was diagnosed at the All India Institute of Medical Sciences (AIIMS) in 1997 and published in 1999. This case came from a newly identified focus of VL in the sub-Himalayan (Kumaon) region of Uttar Pradesh, now a separate state known as Uttarakhand. The patient was a 27-year-old truck driver who came from Almora District. He was misdiagnosed with tuberculosis because kala-azar was never suspected in this leishmaniasis non-endemic area.¹⁰

Bihar State of India has remained VL endemic for the last several decades, but interestingly this state was untouched by the HIV epidemic until the year 1999. In 1999–2000 we carried out a community-based serosurvey¹⁹ to determine the prevalence of hepatitis B virus (HBV), hepatitis C virus (HCV), and HIV infection in patients with VL. The study found that of the 4050 inhabitants in the endemic area, 77 had confirmed leishmaniasis. Although the prevalence of HBV and HCV co-infections in the VL patients was extremely high, only one patient (1.3%) was found to be co-infected with HIV. This patient acquired HIV-1 from a commercial sex worker in Bombay (or Mumbai), where he used to work as labourer, and he was most likely the first documented case of VL–HIV co-infection from Bihar. In all probability, several such migratory labourers brought HIV from Bombay to Bihar.³⁵

During these early years, several subclinical cases of leishmaniasis²¹ started manifesting and various workers started publishing VL–HIV co-infection cases from Bihar and other parts of India.^{47–60} Thakur et al.⁴⁷ reported the first case of HIV–VL co-infection from Bihar who was treated successfully with miltefosine. In 2002, Lanjewar and colleagues⁴⁸ reported a series of autopsy findings from 195 AIDS cases in Bombay. They found that two cases had

histopathologically confirmed VL. Both were natives of the VL endemic area of Bihar.

Redhu et al.⁵⁰ found that out of 25 VL/PKDL cases seen at a Delhi hospital (AIIMS), six were co-infected with HIV-1. Of these, four were from the VL endemic state of Bihar, while two were from the newly identified focus of VL in Uttarakhand State. Later on, from the same hospital and in the same year, Mathur et al.⁵¹ reported that out of 104 VL cases included in their study, six (5.7%) were HIV-1 co-infected and one of the six patients was from Uttarakhand. Of note, there is a strong possibility of an overlap in cases reported by Redhu et al.⁵⁰ and Mathur et al.,⁵¹ especially the case from Uttarakhand.

In later years, most cases were reported by Sinha and colleagues from Patna, Bihar.^{52–54} A major study finding was that out of 55 VL–HIV cases treated with amphotericin B, 74.5% were cured, 14.5% relapsed, and 10.9% died within 2 years. Others are mostly case reports^{55–60} (Table 1). Thus, a total of 89 published records of VL–HIV cases from India were identified to the date at which this manuscript was submitted. There are also a few case reports of VL–HIV co-infection, but these are not so convincing.⁵⁵

As well as kala-azar or VL in AIDS patients, at least five cases of CL^{61,62} and five cases of diffuse CL (DCL)^{63–67} in HIV-infected patients have also been reported. Of these 10 cases of tegumentary leishmaniasis, all CL cases were from the endemic area of Rajasthan. Of the five DCL cases, three were from a CL endemic area, while one was from Kerala and one from Karnataka (Table 2). Both of the latter states are in southern India and are non-endemic for VL and CL.

As expected, HIV co-infected Indian patients presented with atypical manifestations. In 2004 we reported the first case of post-kala-azar mucocutaneous leishmaniasis (PKML). The patient was treated successfully with antiretrovirals and amphotericin B.⁶⁸ Later on Kumar et al.⁵⁴ also reported a case of VL–HIV co-infection presenting with oral ulcers and Nandi et al.⁵⁹ reported cases presenting with cervical lymphadenopathy. This patient was from VL non-endemic Etah District of Uttar Pradesh. There is confusion in the classification of two cases reported by Shah et al.⁶⁹. The authors do not give any reason why they identified these cases as PKDL without any suggestive history of VL, rather than DCL.

Out of the total 89 cases of VL–HIV co-infection reported so far from India, 28 were reported in the first 10 years (1997–2006) following the first case diagnosed in 1997. From 2007 to date, 61 more cases were added. In the second 9 years of reports of this co-infection (2007–2013), 197 332 VL cases⁷⁰ and 2.1 million HIV cases were reported to the Government of India. Thus, the rate of co-infection in these years is estimated to be only 0.029%. The rate did increase in later years, but it remains very low as compared to other VL and HIV co-endemic regions, such as southern Europe (20–40%) and some African countries (up to 70%). Even if we calculate the rate of HIV and VL cases only for the VL endemic states of Bihar and Jharkhand (separated from Bihar in 2000), it comes to only 6.86% per year. The average number of HIV cases in these two states has been 150 000 for the last 9 years. There could be several reasons for this low rate in India. The most important is the low rate of intravenous drug use⁴⁶ in VL endemic areas and the predominance of orthodox populations in northern India, which has witnessed a slow spread of HIV as compared to western and southern India. In VL endemic states, the incidence rate of HIV has remained <0.3%, while in south Indian states and Maharashtra, it has remained between 0.5% and 1.2%.⁷¹

7. Clinical manifestations and prognosis of Leishmania–HIV co-infection

In India, the two clinical forms are endemic, VL and CL (Figure 1). As discussed above, the visceral form is caused by *L. donovani*, while the cutaneous form is caused by *L. tropica*.² It is

well established that HIV-1 modifies the clinical presentation of leishmaniasis in co-infected patients, especially those with VL.^{37–39} Several atypical species and sub-species of *Leishmania* have been incriminated in the infection of HIV-infected subjects and can be called purely opportunistic species.^{2,39,50,68} In HIV-associated leishmaniasis, dermatropic species may disseminate to the reticuloendothelial system and various other organs including the viscera, and conversely the visceralizing species can manifest in an atypical manner. Dissemination is common and a fulminant presentation of VL is possible. Relapses are normal in these patients. Several cases of VL–HIV co-infection will not have splenomegaly.^{45,72}

In India several patients with HIV co-infection are reported to have shown disease that manifests in an atypical manner, delaying the diagnosis and management. Tuberculosis is the most common co-infection in Indian HIV-positive patients and several cases have been diagnosed with a triple infection of HIV, *Leishmania* sp, and *Mycobacterium* sp;^{10,49,51,53} many more might have died undiagnosed.

Vyas and Shah⁵⁸ reported probably the first case of visceral and concomitant PKDL in a patient from Rajasthan, an area endemic for CL only. The patient presented with non-tender papulo-nodular lesions all over the body and had hepatosplenomegaly and inguinal lymphadenopathy. Leishman–Donovan (LD) bodies could be demonstrated in aspirates from lymph node, nodular lesion, blood buffy coat, and bone marrow. Although the species was not identified, since the patient came from a *L. tropica* endemic region, *L. donovani* was less likely in this case. Nandi et al.⁵⁹ and Mishra et al.⁶⁰ also reported lymphadenopathy in their patients as the presenting clinical feature. Purohit et al.⁶⁵ reported a case of CL in HIV-infected patients in whom LD bodies could be demonstrated in skin as well as in bone marrow aspirate smears. Two cases of DCL have been reported from non-endemic areas of south India; both presented with multiple non-tender skin lesions.^{64,67} *L. donovani* and *L. tropica* have a different pathogenesis. While *L. tropica* usually acts purely as an opportunistic infection in HIV-infected patients, *L. donovani* is pathogenic in itself. Alvar et al.⁴⁵ described disseminated CL to be the major characteristic feature of leishmaniasis in HIV-positive patients.

Beside the visceral and tegumentary forms, *Leishmania* species can manifest in various other atypical forms. Hence, dermatologists, internists, and clinical microbiologists must be aware of such atypical manifestations. In 2004, we reported the case of a patient with Leishmania–HIV co-infection, who presented with multiple erythematous lesions on the tongue, mucosa of the nasal alae, conjunctivae, and soft palate, as well as on the skin of the face, after cured kala-azar.⁶⁸ The causative agent was *L. donovani*. No such manifestations of PKDL have been reported from India. Hence, we named this condition PKML. The treatment of these co-infected patients must be planned carefully. HIV-positive patients in India have a poor treatment success rate (74.5%) with a high relapse rate (14.5%) and high death rate (10.9%) even with best treatment regimen.⁵⁷

8. Leishmania and immune reconstitution syndrome in AIDS patients

Immune reconstitution syndrome (IRS), or immune restoration disease (IRD), is defined as an acute symptomatic or paradoxical deterioration of a pre-existing infection that is temporally related to the recovery of the immune system. Immune restoration may occur due to immunopathological damage associated with the reversal of immunosuppressive processes, such as with HAART for AIDS.⁷³ The pre-existing microbial infection could be either asymptomatic or mildly symptomatic. The resulting clinical manifestations of this phenomenon are diverse and depend on

Table 1

Clinical and demographic characteristics of VL–HIV co-infection in India (1997–2013)

SN	Disease form	No. of VL/VL–HIV co-infected Patients	Age, years	Gender and M/F ratio	Geographical origin	Visit to VL/CL endemic area	Clinical presentation and diagnostic criteria of Leishmania–HIV co-infection	CD4/CD8 count, cells/ μ l	Treatment	Outcome of VL	Reference
1	VL	5 cases 1 HIV-pos 4 HIV-neg	16–45	4 M, 1 F	Kumaon (UK) region of India	Never	All had been misdiagnosed as TB locally; diagnosis of VL confirmed in Delhi by demonstrating LD bodies and anti-rK39 ELISA	ND	SAG	Cured	Singh et al., 1999 [10]
2	VL	77 VL cases 1 HIV-pos 76 HIV-neg	11–40	80% M	Bihar	NA	Serosurvey for Leishmania–HIV co-infection carried out in VL endemic Samastipur District; confirmed by anti-rK39 ELISA	ND	SAG	Cured	Singh et al., 2000 [18]
3	VL	1 case	Not given	M	Bihar	NA	rK39 and splenic aspirate	ND	Miltefosine	Cured ^a	Thakur et al., 2000 [47]
4	VL	195 HIV cases, 2 had VL	21, 40	76% M	Reported from Mumbai	Both VL cases from Bihar	Post-mortem histopathological examination showed LD bodies in tissues	NA	NA	NA	Lanjewar et al., 2002 [48]
5	VL	1 case	37	M	RMRIMS Patna, Bihar	NA	Triple infection of HIV, VL, and disseminated TB; demonstration of LD bodies in splenic aspirate	310/550	L-Ampho B + HAART+ ATT (RHEZ)	Cured ^a of VL and TB	Pandey et al., 2005 [49]
6	VL/ PKDL cases ^b	25 cases 6 HIV-pos 19 HIV-neg	2.5–61	76% M	AIIMS, New Delhi	4 from Bihar, 2 from Uttarakhand	Fever and hepatosplenomegaly; one patient had triple infection of HIV, Leishmania, and <i>Mycobacterium tuberculosis</i>	ND	SAG	5 Cured with SAG, 1 died	Redhu et al., 2006 [50]
7	VL/ PKDL cases ^b	104 cases 6 HIV-pos 98 HIV-neg	21 (median)	M	AIIMS, New Delhi	5 from Bihar, 1 from Uttarakhand	Bone marrow aspirate showed the LD bodies for all patients	23–320	Ampho B/SAG + miltefosine	No details	Mathur et al., 2006 [51]
8	VL	280 HIV cases; 8 had VL	1– 45	Both	RMRIMS Patna, Bihar	NA	Direct agglutination test and demonstration of LD bodies in splenic aspirate; sensitivity of DAT was 80% and antibody titres were lower in co-infected patients	162/840 (mean)	Ampho B/ miltefosine + HAART	Improved	Sinha et al., 2006 [52]
9	VL	2 cases	40, 40	F, M	RMRIMS Patna, Bihar	NA	Splenic aspirate showed LD bodies for both the patients; one patient had triple infection of HIV, Leishmania, and <i>M. tuberculosis</i>	71/179 8/143	ART + ATT, ART + miltefosine +ATT	One patient cured ^a	Das et al., 2006 [53]
10	VL	1 case	35	F	Etah District western Uttar Pradesh	Never	Submandibular lymph node aspirate showed LD bodies; serological test (ELISA) for leishmaniasis was positive; patient had blood transfusion 10 years ago	12/18	No treatment could be initiated	Died on third day	Kumar et al., 2007 [54]
11	VL	1 case	55	M	RMRIMS Patna, Bihar	NA	Splenic aspirate showed the presence of LD bodies	180/643	Miltefosine + HAART	VL cured ^a but died after 6 months	Pandey et al., 2008 [55]
12	VL	1 case	32	M	Pune	No details	rK39 RDT done three times remained negative; bone marrow aspirate reported to have LD bodies?	57	L-Ampho B	Cured ^a	Yanamandra et al., 2011 [56]

Table 1 (Continued)

SN	Disease form	No. of VL/VL-HIV co-infected Patients	Age, years	Gender and M/F ratio	Geographical origin	Visit to VL/CL endemic area	Clinical presentation and diagnostic criteria of Leishmania-HIV co-infection	CD4/CD8 count, cells/ μ l	Treatment	Outcome of VL	Reference
13	VL	55 VL-HIV cases	30–40	83.6% M	RMRIMS Patna, Bihar		Demonstration of LD bodies and rK39 RDT; cases enrolled between 2007 and 2010	66 (median)	L-Ampho B + HAART	41 Cured, 8 relapsed, 6 died	Sinha et al., 2011 [57]
14	VL/ PKDL	1 case	34	M	Rajasthan	Never	Fever and tender papulo-nodular lesions on skin with umbilication; peripheral blood smear; aspirates from bone marrow and skin nodules showed LD bodies	187	Ampho B + HAART	Clinically cured	Vyas and Shah 2011 [58]
15	VL	1 case	6	M	Endemic area of Calcutta, India	NA	Child had cervical lymphadenopathy; both parents HIV-positive; lymph node biopsy showed LD bodies	Not done	Parents did not give consent for treatment	NA	Nandi et al., 2012 [59]
16	VL	1 case	18	M	Eastern Uttar Pradesh	NA	Cervical lymphadenopathy with hepatosplenomegaly; bone marrow aspirate showed the presence of LD bodies	Not done	L-Ampho B	Cured ^a	Mishra et al., 2013 [60]

AIIMS, All India Institute of Medical Sciences; ART, antiretroviral therapy; ATT, anti-tuberculosis therapy; CL, cutaneous leishmaniasis; DAT, direct agglutination test; F, female; HAART, highly active antiretroviral therapy; L-Ampho B, liposomal amphotericin B; LD, Leishman-Donovan; M, male; NA, not applicable as the case(s) were from an endemic area; ND, not done; PKDL, post-kala-azar dermal leishmaniasis; RDT, rapid diagnostic test; RHEZ, rifampin, isoniazid, ethambutol, pyrazinamide; RMRIMS, Rajendra Memorial Research Institute for Medical Sciences; SAG, sodium antimony gluconate; TB, tuberculosis; VL, visceral leishmaniasis.

^a Word 'cured' used by the original authors, without any details on whether they followed the patients for more than 6 months or not. Hence the author of this review does not make any claim as to whether these were clinically cured cases or parasitologically cured cases.

^b Some of these cases might have been common in both studies.

the associated pathogens viz. mycobacteria, parasites, viruses, or fungi.⁷⁴ The data regarding leishmaniasis-associated IRS from India and globally are very limited. There are only a few reports, and these are mostly from non-endemic countries.^{75,76} From India there is only a report by Patel et al.⁷⁷ who described a case of VL presenting with haemophagocytosis, fever, severe anaemia, and mild splenomegaly. The patient was put on HAART. His CD4+ cell count was 234 cells/ μ l. After 3 months of HAART, the patient returned with a high-grade fever and mild hepatomegaly but massive splenomegaly. This time his CD4+ count was only 57 cell/ μ l. A bone marrow aspirate revealed severely hypocellular marrow, myeloid hypoplasia, and megaloblastoid erythroid maturation with delayed haemoglobinization, but adequate megakaryocytes. Numerous LD bodies could also be demonstrated in the aspirate smear. The patient was treated successfully with amphotericin B.

9. Diagnosis of Leishmania-HIV co-infection

The laboratory diagnosis of HIV infection is well established, but the diagnosis of leishmaniasis per se, and especially in HIV-infected persons, may very often, if not always, be difficult. The clinical signs and symptoms of VL or CL in these patients pose several problems. Cases of kala-azar in non-endemic areas may be confused with and misdiagnosed as other similar conditions of infectious or non-infectious origin.² Similarly, numerous primary and secondary skin conditions are frequently over-diagnosed as early lesions of CL in endemic areas, and in non-endemic countries CL is frequently misdiagnosed as other diseases. Whenever, there is a clinical suspicion of leishmaniasis, the diagnosis should be confirmed by laboratory tests. A standard algorithm has been suggested for this.⁷⁸ Tests for both non-specific surrogate markers and specific tests should be taken into account. Amongst, various surrogate markers, interleukins and TNF- α can be used as prognostic markers. TNF- α increases significantly in acute persistent cases of kala-azar, but the levels come down sharply after parasitological cure.⁷⁹ The specific tests are based on the principle of detecting the causative agent as a whole, in parts, fragments, coatings, cellular or molecular products, or its footprints.

The confirmatory diagnosis of leishmaniasis, irrespective of the patient's HIV status, relies on the demonstration of parasite in the tissues of relevance, such as bone marrow, spleen, lymph nodes, or liver, the peripheral blood buffy coat, slit skin smears, or biopsies, as published elsewhere.^{2,78,80} Hence, only special considerations in the diagnosis of HIV-associated leishmaniasis will be discussed here.⁸¹

The hallmark of VL is hyperimmunoglobulinemia; this is in contrast to cutaneous and mucocutaneous leishmaniasis in which the humoral immune response is very poor. However, hyperimmunoglobulinemia may not be significant if the Leishmania infection has occurred after advanced HIV disease. A number of antibody detection methods have been developed to measure these immunoglobulins.^{2,78,80} Currently tests such as the indirect immunofluorescence assay (IFA), latex agglutination test (LAT), direct agglutination test (DAT), various enzyme immunoassays (EIA), etc., can be used for antibody detection. Antigens of the parasite can also be detected using these test formats, except DAT.

The sensitivity of the IFA test varies from 70% to 86.6% but it has high (>90%) specificity. The direct fluorescence antibody (DFA) test is more useful in the diagnosis of CL, MCL, and PKDL. In full-blown AIDS cases, where antibody titres are low and the parasite load is high, the DFA test may be more useful.^{2,78} The DAT is a highly specific and sensitive test and is endorsed by the WHO for the diagnosis of VL. The DAT has been found to be 91–100% sensitive and 72–100% specific in HIV-negative patients in various studies.^{81,82} However, in HIV co-infected patients its sensitivity

Table 2

HIV co-infected patients with non-visceral forms of leishmaniasis reported from India

SN	Disease form	Number of cases	Age, years	Gender and M/F ratio	Geographical origin	Visit to VL/CL endemic area	Clinical presentation and diagnostic criteria of Leishmania–HIV co-infection	CD4/CD8 count, cells/ μ l	Treatment	Outcome	Reference
1	CL	2 cases	35, 42	M	Ahmedabad, Gujarat	Resident of Rajasthan and Bihar	LD bodies demonstrated in skin scraping smears	224	Ketoconazole +ART	Only 1 patient improved	Mittal et al., 2005 [61]
2	CL	3 cases	40, 34, 28	All M	Bikaner, Rajasthan	NA	Skin smear showed LD bodies	290–380	(In 2) SAG + HAART + rifampin	1 Died, 1 cured, 1 refused Rx	Soni et al., 2011 [62]
3	DCL	1 case	38	M	Alwar, Rajasthan	South India	Demonstration of LD bodies in skin scrapes	95	Ketoconazole + ART	Lost to follow-up	Chaudhary et al., 2008 [63]
4	DCL	1 case	30	M	Kerala		Slit smear showed LD bodies	684	ART + SSG	Lost to follow-up	Mehta et al., 2009 [64]
5	DCL	1 case	33	M	Jodhpur, Rajasthan	NA	Microscopic examinations of skin smear and bone marrow examination revealed LD bodies	95	Rifampin + ketoconazole + ART	Died	Purohit et al., 2012 [65]
6	DCL	1 case	32	F	Bikaner, Rajasthan	NA	LD bodies on histopathology, identified as <i>Leishmania tropica</i> by PCR-RFLP	44	SAG +HAART	Did not improve clinically	Khandelwal et al., 2011 [66]
7	DCL	1 case	45	M	Karnataka	Never	Skin nodules showed LD bodies	250	No details	No details	Maresh et al., 2012 [67]
8	PKML	1 case	27	M	Bihar	One patient gave history of visiting Bihar	PKML, anti-RK39, anti-rKE16 positive	210, 170	Ampho B + ART	Cured	Singh, 2004 [68]
9	? DCL/ PKDL	2 cases	45, 52	M	Bikaner, Rajasthan		Demonstration of LD bodies in tissues		Rifampin + ketoconazole + HAART	No details	Shah et al., 2010 [69]

Ampho B, amphotericin B; ART, antiretroviral therapy; CL, cutaneous leishmaniasis; DCL, diffuse cutaneous leishmaniasis; F, female; HAART, highly active antiretroviral therapy; LD, Leishman–Donovan; M, male; NA, not applicable as the case(s) were from an endemic area; PKDL, post-kala-azar dermal leishmaniasis; PKML, post-kala-azar-mucosal leishmaniasis; RFLP, restriction fragment length polymorphism; SAG, sodium antimony gluconate; SSG, sodium stibogluconate; VL, visceral leishmaniasis.

Table 3Sensitivity and specificity of various recombinant and purified LPG *Leishmania* proteins in the diagnosis of visceral leishmaniasis using ELISA (95% CI)^a

Determinants	rKE16 ^b	rK39 ^b	rKLO8	rH2A	rH2B	rGBP	rLACK	rgp63	rP20	rPSA-2- GST	rPSA-2- TRI-GST	rPSA-2- MBP	Purified LPG
Sensitivity	100%	100%	98.1%	100%	100%	97%	97%	86%	68%	47%	36%	57%	92%
Specificity	100%	97%	96.1%	91%	92%	92%	84%	90%	95%	96%	85%	97%	92%
PPV	100%	92%	97.2%	80%	82%	82%	68%	76%	83%	81%	46%	88%	81%
NPV	100%	100%	97.4%	100%	100%	99%	88%	96%	89%	83%	79%	86%	97%

LPG, lipophosphoglycan; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

^a Source and cross references: references 2, 21, 45, 50, 78, 80, 82, 84.^b Only these two antigens are commercialized in the form of lateral flow rapid test dipsticks.

was reported to be significantly lower in Ethiopian patients (89%)⁸³ and very low in Indian patients (80%).^{52,57} EIA tests can be performed easily and are adaptable for use with various antigens.⁸⁴ The sensitivity and specificity of EIAs are greatly influenced by the antigen and the test format used.⁸⁰

A major breakthrough in the diagnosis of VL was reported in 1993 by Burns et al.⁸⁵ These authors characterized a novel kinesin-related 39 AA protein (Lc-rK39) from *Leishmania chagasi*.⁸⁶ Later our laboratory prepared another novel antigen (Ld-rKE16) from Indian strains of *L. donovani* with very high sensitivity and specificity.^{87,88} Other workers have also attempted to prepare good recombinant antigens,^{89,90} but with the exception of rK39 and rKE16, no other antigen has reached a commercial level.^{90,91} The sensitivity and specificity of various test formats using these two antigens have been evaluated extensively in both immunocompetent and in VL–HIV co-infected patients (Table 3).^{50,51,57,68,78} Although there is no significant difference in the performance of EIA-based tests between HIV-positive and HIV-negative VL patients, in quantitative terms the antibody titres remain several logs lower in VL–HIV co-infected patients.⁵⁰

Antigen detection tests would, in principle, provide a better means of diagnosis of active leishmaniasis, especially in VL–HIV co-infected patients, as the antigen levels are expected to theoretically correlate with the parasite load.^{2,45,50} Attar et al.⁹² developed a latex agglutination test kit (Katex) to detect antigen in urine samples, with satisfactory sensitivity and specificity in immunocompetent VL patients. This was found to carry a good prognostic value,⁵⁰ but its sensitivity in HIV co-infected patients has been relatively poorer elsewhere. The kit has not yet been evaluated on Indian VL–HIV co-infected patients. In HIV-negative Indian VL patients its sensitivity and specificity rates are reported to be between 47% and 67%, and between 98% and 99%, respectively.⁹³

Delayed hypersensitivity is an important feature of tegumentary forms of human leishmaniasis and can be measured by the leishmanin skin test (LST). The LST is mainly used as an indicator of the prevalence of CL and MCL in human and animal populations and for determining the cure of VL.^{94,95} The positivity rate of the LST was found to be 14% in active Indian kala-azar cases and 40.3% in cured VL patients. Even in healthy controls from a VL endemic area it was (false) positive in 21.6% of cases.⁹⁶

Molecular biology is increasingly becoming relevant to the diagnosis and control of infectious diseases. With increased information on DNA sequences and the advent of 'nucleic acid engineering', a number of strategies have been developed for diagnostic purposes. A variety of nucleic acid detection methods targeting species-specific DNA and/or RNA genes have been developed.⁹⁷ Of these gene amplification techniques, PCR has been the most rewarding because of its extremely high sensitivity, rapidity, and the ability to work with a broad range of clinical specimens.^{97,98} Various *Leishmania* gene target sequences can be used. These include, 18S-rRNA, small subunit rRNA (SSU rRNA), cysteine proteinase b, a repetitive genomic sequence of DNA, the mini-exon (spliced ladder) gene repeat, the

β-tubulin gene region, gp63 gene locus, internal transcribed spacer (ITS) regions, and micro-satellite DNAs such as maxi- and mini-circles of kDNA.^{2,97–102} Several studies have reported that the PCR assay could detect parasitemia a few weeks before the appearance of any clinical signs or symptoms.⁹⁹ The specificity of PCR on bone marrow aspirates has been reported to be up to 100% and the sensitivity 80–93.3%, as reviewed earlier.² Also a modified form of the PCR, the nested PCR, was found positive in 27 of 29 (93%) samples, while only 20 of 29 (69%) PKDL samples were positive in the primary PCR assay.¹⁰⁰

Molecular techniques are also very useful for genotyping and speciating the causative strain of *Leishmania*. Lukes et al.⁹⁷ and Quispe-Tintaya et al.⁹⁹ used a combination of PCR with fluorescence resonance energy transfer/melting curve analysis (FRET/MCA) and multilocus enzyme electrophoresis (MLEE) as molecular advancements in genotyping the *Leishmania* spp and redefining the taxonomy and evolutionary history of *Leishmania*. These authors could successfully genotype *L. donovani* complex into 25 genotypes and postulated that indeed *L. donovani* complex has only two species: *L. donovani* and *L. infantum*. They also suggested that *L. donovani* originated from South America but diversified only after migrating to other parts of the world. The molecular techniques can also be used for tracking the source of infection and for the purposes of molecular epidemiology. Researchers have used kDNA restriction fragment length polymorphism (RFLP) for fingerprinting the clinical isolates. Such applications are most useful in contact tracing and fingerprinting of the disease-causing pathogen.¹⁰³

In recent years further advances in molecular biology have taken place, and highly sensitive and quantitative real-time PCR assays have been developed that can be used for assessing drug efficacy¹⁰⁴ and the disease outcome in HIV-infected patients.¹⁰⁵ The sensitivity of PCR in tegumentary leishmaniasis (CL and MCL) has been reported to be between 71% and 100%.¹⁰⁶ Various types of specimen can be used, such as skin biopsies and dermal scrapings from the bottom of the ulcer, as well as exudates and syringe-sucked fluid taken from ulcerative lesions and the blood.^{107–109} Multiplex PCR can be used whenever infections with more than one species are suspected.⁴⁶ PCR can also be used for species, strain, and genotype identification.^{110,111}

10. Which test to opt in HIV–*Leishmania* co-infected patients?

Various immunological, parasitological, and molecular techniques available for the diagnosis of HIV–*Leishmania* co-infection have been evaluated by Deniau et al.¹¹² These authors reported that PCR is the only promising tool in HIV–*Leishmania* co-infections. Bossolasco et al.¹¹³ reported that real-time PCR was the most useful tool not only for the diagnosis but also for the follow-up of patients co-infected with HIV-1 and *Leishmania* spp. These authors found that *Leishmania* DNA levels correlated with the clinical course and their measurement before, during, and after treatment, and were useful in the clinical management of co-infections particularly in the HAART era. In another study, Cruz

et al.⁴⁶ reported that a Leishmania-specific nested PCR (Ln-PCR) done on blood samples was the technique of choice for diagnosis, monitoring the success of treatment, and predicting relapses in patients with HIV–Leishmania co-infection. A PCR assay using the SSU rRNA gene target was assessed for post-therapeutic follow-up, and the detection of relapses could be predicted in 97% of cases with peripheral blood and 100% of cases with bone marrow samples from HIV-infected patients.¹⁰⁰

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