Diagnosis & management of leishmania/HIV co-infection

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Accepted January 4, 2005

Leishmaniasis, a globally prevalent parasitic disease occurs in three forms viz., visceral, cutaneous and mucocutaneous, transmitted by the bite of infected female Phlebotomus sandflies. Visceral leishmaniasis (VL) has 100 per cent fatality rate, if left untreated. India has the largest burden of this disease. HIV infection is also increasing worldwide and several reports indicate rising trend of VL/ HIV co-infection, modifying the traditional anthroponotic pattern of VL transmission. Both VL and HIV tend to lower the cell mediated immunity (CMI) resulting in poor drug response and opportunistic infections involving gastrointestinal, cutaneous, respiratory tract and central nervous system (CNS) may occur. Diagnosis of such co-infected cases is quite difficult. However, newer tests like nested PCR, rk39 immunochromatographic test etc., can be of help. Response to different antileishmanial drugs like sodium antimony gluconate (SAG), amphotericin B is far from satisfactory. However, a new oral drug miltefosine has been found to be promising. Highly active antiretroviral therapy (HAART) need to be given for management of HIV infection along with treatment of other opportunistic infections.

Key words Co-infection - HIV - Leishmania - opportunistic infections

The leishmaniases is a group of insect-transmitted parasitic diseases which are most misunderstood and least studied of endemic diseases. Three parasitic varieties are the primary culprits: Leishmania donovani causing visceral leishmaniasis (VL), Leishmania tropica causing cutaneous leishmanisis (CL) and Leishmania braziliensis causing mucocutaneous leishmaniasis (ML). The disease is prevalent worldwide, considered to be endemic in 88 countries: 72 of which are developing countries and 13 are among the least developed countries. It is believed that 350 million people are at risk, and 12 million people are affected by leishmaniasis worldwide. Of this, 1.5 - 2 million new cases are estimated to occur annually of which only 600,000 cases are officially reported1-3.

It is estimated that of the 600,000 new cases of VL that occur annually, 90 per cent of these occur in five countries, viz., Bangladesh, Brazil, India, Nepal and Sudan (WHO Fact Sheet No. 116, Rev. May 2000)4. Three types of VL are recognized worldwide namely African kala-azar, mediterranean or infantile kala-azar and Indian kala-azar. African kala-azar, generally affecting older children and young adults, is found in eastern half of Africa from the Sahara in the north to the equator. Mediterranean or infantile kala-azar is found in mediterranean area, China and Latin America for which dogs, jackals, foxes and rats are the potential reservoirs. For Indian kala-azar, humans are the only reservoir. Ninety per cent of CL cases occur in seven countries namely Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia and Syria (WHO Fact Sheet No.116, Rev. May 2000)4.
The global burden of leishmaniasis was 2-4 million disability adjusted life years (DALYs) lost and 59,000 deaths in 2001. India has the largest burden of this disease in the world. The disease affects the poor who cannot afford the expensive investigations and treatment. It is estimated that 80 per cent of the patients suffering from VL earn less than $2 a day. Because host defence against this intracellular infection is T-cell dependent, VL has joined the list of AIDS related opportunistic infection in endemic areas.

Though it is well known that the advent of HAART (highly active anti retroviral therapy) has modified the natural history of HIV infection and related opportunistic infections and neoplasms, the benefits of HAART are only available to 5 per cent or less of the HIV infected patients in the world at present. There is no available data regarding HIV/VL co-infection in developing countries; it may be increasing worldwide.

Although the geographical distribution of Leishmania infection is restricted to the area of distribution of Phlebotomus sandflies, HIV infection may modify the traditional anthroponotic pattern of VL transmission. Very rarely Leishmania transmission has been described by alternative means that are also shared by HIV transmission, including blood transfusion, congenital transmissions and laboratory acquired.

Worldwide, VL mainly occurs in HIV negative individuals more so in paediatric patients. The association of Leishmania with HIV has lead to a significant shift in the age of people at risk. In South West Europe, 75 per cent of HIV seronegative and 80-83 per cent of HIV positive patients seen with VL were men. In general, overt clinical leishmaniasis occurs in profoundly immunosuppressed HIV infected patients. Mean CD4 counts were 200 cells/mm³ in 62-90 per cent and 50 cells/mm³ in 42 per cent of co-infected patients. In a majority of HIV positive individuals, L. donovani and L. infantum cause VL. The co-infection by HIV and Leishmania both causes enhanced immunological disturbances. Both infections switch the predominantly cellular immunological response from Th1 to Th2 through complex cytokine mediated mechanisms leading to a predominantly humoral response. There is a defect in lytic capacity of macrophages, which cannot eliminate intracellular Leishmania amastigotes through the nitric oxide pathway. HIV related CD4+ T cells depletion leads to a further decrease of T cells and stimulates β-lymphocytes. This leads to an oligoclonal β cell response, which explains the elevated frequencies of false negative Leishmania serology. HIV mediated inhibition of proliferative response to Leishmania favours the dissemination of leishmaniasis. Conversely, Leishmania infection increases HIV replication mainly due to chronic immune activation. There is also an increased secretion of TNF-α, IL2, IL4, IL6 and IL10. The induction of HIV expression has been suggested by the observation of progressive increase in HIV-1 RNA loads in co-infected patients in parallel to increase in IL4, IL6 and IL10 levels.

Clinical presentation

In cutaneous leishmaniasis, the systemic symptoms are usually absent. Initially, the lesions are small red papules up to 2 cm in size. The papule ultimately ulcerates and there could be exudates formation or it can become dry with a crusted scab. Sources are usually found on the exposed areas of the skin, especially the extremities and face. Regional adenopathy, satellite lesions, and subcutaneous nodules can be present. Untreated sores can leave de-pigmented retracted scars. Lesions can be pruritic and painful.

Mucocutaneous leishmaniasis presents as single or multiple cutaneous lesions which become painful gradually. Mucosal lesions often develop after the primary lesion has healed. These lesions can progress to involve the entire nasal mucosa which can lead to nasal obstruction and bleeding. The palates can also become deformed along with ulceration of the nasal septum and lips. Other signs can include gingival oedema, periodontitis and adenopathy. Secondary infection plays a prominent role in the size and persistence of ulcers.

Visceral leishmaniasis generally presents with bouts of fever, of which double peak is characteristic.
Hepatosplenomegaly with pancytopenia, wasting and weakness, and darkening of the skin are among the other prominent features.

*Leishmania*/HIV co-infection is emerging as a serious new disease pattern and is becoming increasingly frequent. Of the 1700 cases of co-infection reported to the World Health Organization from 33 countries worldwide up to 1998, 1440 cases were from South-West Europe: Spain (835), Italy (229), France (259) and Portugal (117). Of 965 cases retrospectively analyzed, 83.2 per cent were males, 85.7 per cent were young adults (20-40 yr) and 71.1 per cent were intravenous drug users. Most co-infections in the Americas are reported from Brazil where the incidence of HIV has risen from 0.8 cases per 100,000 inhabitants in 1986 to 10.5 cases per 100,000 inhabitants in 1997. In India, the *HIV/Leishmania* co-infection has not been extensively studied. The risk of visceralization for HIV positive person, infected with *Leishmania* species typically associated with cutaneous disease, is not much of a problem, since VL is several thousand times more common than CL in India. However, this may be a very serious issue in the Mediterranean countries where CL is very common.

A majority of HIV/Leishmania co-infected cases, show classical features of VL. These co-infected patients may also have other features, *viz.*, atypical location due to decreased cell mediated immunity (CMI), parasitic dissemination to skin, cutaneous and reticulo-endothelial system (RES), a chronic and a relapsing course, poor drug response and lack of anti-lesishmanial antibodies.

The incubation period is variable and may be age related. Other concomitant opportunistic infections are diagnosed in 42-68 per cent of HIV-positive patients. Fever, pancytopenia, hepatosplenomegaly and hypergammaglobulinaemia are common. Classically, splenomegaly may be less in HIV positive patients. Constitutional symptoms (asthenia, anorexia, weight loss *etc.*) are seen in 50-70 per cent of patients and lymphadenopathy in 15-60 per cent of patients. VL with HIV infection may present as pyrexia of unknown origin (PUO). Other opportunistic infections like mycobacterial infection, cytomegalovirus (CMV), pneumonias and AIDS related neoplasms may also occur.

Gastrointestinal (GI) symptoms are among the most frequent complaints. LD bodies have been identified in up to 50 per cent of such patients. The commonest site of involvement is the jejunum. Endoscopy and routine biopsy are important tools in the diagnosis. The symptoms may include diarrhoea, malabsorption, and hypoalbuminaemia and weight loss. There may be erosive gastro-duodenitis, ulcers and colonic lesions.

Cutaneous involvement may appear in the skin with Kaposi sarcoma, Herpes simplex or Zoster. Leishmaniasis may be associated with dermatofibroma, psoriasis, Reiter’s syndrome, bacillary angiomatosis, cryptococcosis and oral aphthous ulceration. It may also present as dermatomyositis like eruption.

Respiratory tract involvement occurs in alveoli and pulmonary septa in 75 per cent of patients with VL. They could present with pulmonary tuberculosis and pneumonia, more commonly *Pneumocystis carinii* pneumonia (PCP). The symptoms could be cough, breathlessness, haemoptysis and excessive sputum production. Renal involvement can occur. Glomerulonephritis with mild proteinuria, haematuria and even acute renal failure have been reported. Tubulointerstitial damage can also occur.

Central nervous system (CNS) involvement is very common in the late stages. Pandey *et al.* reported cases in which HIV-Leishmania co-infection was associated with pulmonary tuberculosis and tuberculoma in the brain neurocysticercosis and tuberculous meningitis. AIDS dementia complex occurs in the late stages and may lead to early death. In such cases, CD4 count has been reported to be as low as <50 cells/mm³.

Pancreatic, pulmonary, pleural, laryngeal, adrenal, pericardial, myocardial and lingual leishmaniasis have also been reported. Mucocutaneous leishmaniasis appears in 2-3 per cent of VL-HIV co-infected patients.
Diagnosis

Diagnosis is quite difficult as only 40-50 per cent of VL/HIV co-infected cases have a positive leishmania serology\(^56\). This percentage is inversely proportional to CD\(_4\) cell depletion. Anti-leishmania antibodies in HIV-positive patients are 50 times less than those in HIV-negative patients\(^57\). Therefore, there may be many false negative tests. Immunochemical results may show a sensitivity of 70 per cent and specificity of 73 per cent\(^58\). However, there is substantial non-specific cross reactivity\(^59\). The direct examination of amastigotes in the splenic and bone marrow aspiration has been the gold standard. Amastigotes in the peripheral blood occur in 50 per cent of the co-infected patients and in the Novy-McNeal-Nicolle (NNN) medium culture the sensitivity goes up to 70 per cent\(^60,61\). Detection of *Leishmania* antigens by Western blot in the urine samples as well as by rk39 strips in being tried. The rk39 strips have a sensitivity and specificity of close to 95 per cent\(^62\). PCR techniques requiring blood and tissue samples are very time consuming\(^63,64\). However, when used in combination with ELISA and Direct Agglutination Test (DAT) the results are very encouraging. Nested PCR assay has a sensitivity of 95 per cent in the peripheral blood and 100 per cent in bone marrow/splenic aspirates\(^65,66\).

Treatment

Certain issues are significant in the management of HIV/Leishmania co-infected patients. Firstly, optimal duration of treatment is to be given. Secondly, the dose has to be monitored and thirdly, there is frequent relapse.

Sodium antimony gluconate (SAG) has developed resistance and low cure rates of 30-50 per cent have been reported\(^67\). In such patients, the treatment with SAG has to be given for a longer period\(^68\). However, the longer duration of therapy may lead to cardiotoxicity. With SAG, there are frequent relapses as seen often in Bihar\(^69\). Amphotericin B has also been tried and response rate of 60 per cent has been observed but 25-60 per cent of the patients treated with amphotericin B, are likely to have relapse during the first year after completion of treatment\(^70,71\). HIV infected individuals are more likely to suffer from drug related adverse events. In almost all the patients, depending on the CD\(_4\) counts (<200/ml) HAART should be given\(^72\). Amphotericin B can be given at a dose of 1 mg/kg for 15 days. Although lipid formulations are less toxic, they are very costly\(^73\). Pentamidine is usually not effective and should not be used due to its toxic effects. Oral miltefosine\(^74,75\) is a promising alternative at a dose of 2.5 mg/kg for 28 days and has been tried by Thakur *et al*\(^76\) in six co-infected patients with good results. Besides treatment of VL and administration of HAART, other secondary infections like tuberculosis of the chest, oral candidiasis, CMV infections, *Pneumocystis carinii* pneumonia (PCP), toxoplasmosis, Kaposi sarcoma also need to be treated\(^77\).

Usually, when patients relapse, the management of the case becomes very difficult. Relapses have been treated with either antimonials or amphotericin B in the standard dosages for a more prolonged duration. Since there are no specific comparative studies, the selection of regimen for treatment should be determined by the toxicity profile of the drug as well as the patient’s co-morbidity.

The drug-drug interactions between antiretrovirals and antileishmanials have not been extensively studied. However, in some of the co-infected patients with tuberculous infection, antituberculosis therapy has to be given. The antituberculosis drugs like rifampicin and rifabutin can decrease the levels of protease inhibitors like indinavir, saquinavir, ritonavir, nelfinavir and non-nucleoside reverse-transcriptase inhibitors like efavirenz by inducing cytochrome P450 drug metabolizing enzyme system in the liver\(^78-80\). The best approach would be to avoid simultaneous use of rifampicin or rifabutin together with protease inhibitors and non-nucleoside reverse-transcriptase inhibitors.

Conclusion

*Leishmaniasis per se* is a mysterious disease as far as its epidemiology is concerned. Nevertheless, adequate information has been contributed for its diagnosis and management. In India, visceral form
of leishmaniasis is of prime concern. VL and HIV infection both are attributed to the lowering of CMI and hence this co-infection has aggravated the threat to the VL endemic population.

Various indirect serological methods for diagnosing VL cases include ELISA, dot ELISA, DAT, rk39 but have not been satisfactorily validated. The clinicians have to eventually rely on the gold standard method of directly visualizing parasites in the bone marrow/splenic aspirates. This is not feasible in the field setting level i.e., at the primary health centre (PHC) level in the current scenario. Hence, there is an utmost need for development of highly specific and sensitive diagnostic tool for VL that can be in practiced at the field level. Nested PCR can be of some help in the diagnosis. However, it takes a longer duration, is costly and difficult to perform under field conditions.

For the treatment of VL, the first line of drug is SAG, which has low response rate in several endemic districts of Bihar. Pentamidine is another drug of second line but due to its side effect in the form of insulin dependent diabetes, it has not found favour amongst the doctors. VL being the disease of neglected people belonging to low socio-economic strata of the population, the other antileishmanial drugs like amphotericin B, amphotericin B lipid complex are not cost effective. A new clinically tried oral drug, miltefosine, is very promising for treating VL cases, as it is very efficacious and easy to administer at the field level.

HAART is to be administered in co-infected patients for HIV infection along with management of other opportunistic infections but it is very expensive and beyond the means of poor patients. There is a dire and urgent need to develop cheaper and effective methods for diagnosis and management of both VL and HIV infection.

Acknowledgment

The authors wish to acknowledge Shri Rakesh Bihari Verma, Technical Officer and Shri Brijnath Prasad, LIA, RMRIMS for help in the preparation of the manuscript.

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