Kala-Azar - New Developments in Diagnosis and Treatment

Praveen Aggarwal, Rohini Handa, Sarman Singh and Jyoti Prakash Wali

Department of Medicine, All India Institute of Medical Sciences, New Delhi

Abstract: Kala-azar is an endemic disease in many parts of India. Traditionally, diagnosis of this disease was based on demonstrating the parasites in various tissues like bone marrow or splenic aspirates. However, lack of high sensitivity of these methods led to the use of various immunodiagnostic methods in the diagnosis of kala-azar. Antigen detection and polymerase chain reaction to detect parasitic DNA have been found to be useful in patients with an underlying immunosuppressive disease like AIDS. For treating kala-azar, pentavalent antimonial compounds are still the first-line agents. However, due to increasing resistance to this agent, many patients at present require other drugs including amphotericin B and pentamidine. Toxic effects of these second-line agents have led to development of drug delivery systems like liposomal amphotericin B, which has shown uniform efficacy in clinical trials. Combining stibogluconate with either paromomycin or interferon-γ has also been shown to be useful in many patients with drug-resistant kala-azar. (Indian J Pediatr 1999; 66: 63-71)

Key words: Kala-azar; Visceral leishmaniasis; Direct agglutination test; Pentavalent antimony.

Kala-azar or visceral leishmaniasis (VL) is caused by protozoa belonging to the genus Leishmania. In India, L. donovani is responsible for this disease. VL is widespread in tropical areas and has emerged as an opportunistic infection in HIV-positive patients. The incidence of HIV infection has been rapidly increasing in India, which may lead to increasing number of kala-azar patients. This makes rapid diagnostic and effective management strategies essential so as to reduce the burden of this disease. In this article, the newer methods of diagnosis and treatment of kala-azar have been discussed.

DIAGNOSIS OF KALA-AZAR

A diagnosis of kala-azar should be considered in patients who present with fever, anorexia, weight loss and discomfort in the left upper abdomen, and have pallor and splenomegaly. However, a definite diagnosis of kala-azar is based on the demonstration of the causative organisms in bone marrow or splenic aspirates, liver biopsies, or isolation of organisms in culture. These tests are invasive in nature, and collecting and processing these specimens require extensive facilities and experience. Further, the sensitivity of these techniques is low. The splenic aspirate detects around 70-90% active cases whereas bone marrow aspirate can detect only 50-75% cases. Therefore, immunodiagnostic tests have been studied by several workers which permit clinically conclusive, albeit indirect, diagnosis of VL. Several serologic tests have been developed to detect circulating antibodies to L. donovani. Tests have also been developed to detect circulating antigenic components.
of the parasite, or to detect the parasitic DNA in the tissue samples.

**Immunodiagnostic Tests**

Several immunodiagnostic methods have been developed which are less invasive and are useful in community surveillance studies. The skin test to demonstrate delayed-type hypersensitivity is positive only in patients with cured kala-azar. The present-day immunodiagnosis of kala-azar patients is based on various serologic tests that detect either antibodies or parasitic antigens.

*Antibody detection*: In patients with kala-azar, antibody production is vigorous and rapid. Various techniques of serodiagnosis of kala-azar are based on polyclonal stimulation of B-cell (non-specific tests) or clonal stimulation of B-cell (specific tests).

(a) **Non-specific tests**: Infection by *L. donovani* stimulates production of immunoglobulins by B-cell. In contrast, production of albumin is hampered leading to reversed albumin-globulin ratio. This increased production of immunoglobulins is used quite frequently at less-equipped, peripheral laboratories. Some of these tests are Napier’s aldehyde test and Chopra’s antimony test. These tests are easy to perform but have a high false-positive rate due to overproduction of immunoglobulins in many other diseases. Since these tests fail to detect cases of early leishmaniasis, the sensitivity of these tests is around 85% only.

(b) **Specific tests**: Specific serological techniques are based on the demonstration of antibodies produced against the circulating parasitic antigens. The specificity of various tests depends on the antigen or its epitome used in the test, as the parasite will stimulate several antibody-producing B-cells including group and genus-specific (polyclonal) as well as species-specific (monoclonal) cells. Therefore, the sensitivity may depend on the test and its methodology but the specificity will depend on the antigen rather than the method used.

The conventional methods used for antibody detection include gel diffusion, complement fixation test (CFT), indirect hemagglutination test (IHA), indirect fluorescent antibody (IFA) test, and counter current immunoelectrophoresis (CIEP). However, besides practical difficulties at peripheral centres, the sensitivities and specificities of these tests are poor.

Direct agglutination test (DAT) is a promising test as it is simple, quick, cheap and specific and can be applied in the field conditions. Allain and Kagan first described DAT for the diagnosis of visceral leishmaniasis. It was later modified and simplified to increase its sensitivity and specificity. Since then, this test is being used widely in India and other endemic countries for diagnosis of VL. In this test, the trypsinized whole promastigotes are fixed in formalin and then stained with a vital dye. Serum from a suspected case of kala-azar is incubated with the antigen and antigen-antibody agglutination is observed the next day. This test has been found to have a sensitivity of 96.5-100% and specificity of 91-95%. It has also been shown to be highly sensitive and specific in the early diagnosis of kala-azar patients. The drawback of this test is that it remains