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RESEARCH ARTICLE

CHALLENGES IN IDENTIFICATION OF KALA AZAR IN INDIA

*** Kumar Rajesh and Kumar Sanjay**

Faculty of Advance Studies in Life Sciences, Chhatrapati Shahu ji Maharaj University, Kanpur, 208024, Uttar Pradesh, India

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ABSTRACT

Visceral Leishmaniasis (VL) is a vector borne anthrozoonotic disease caused by a protozoan, *Leishmania donovani,* of *Trypanosomatidae* family. The most precise methods used for its diagnosis includes the analysis of spleen & liver smears (90%), Bone Marrow smear (80%), sternal or iliac crest puncture but these all are not reliable and cumbersome/painful also. Various serological tests like indirect haemagglutination assay (IHA), countercurrent immuno-electrophoresis (CCIEP), Immunodiffusion (ID), Direct agglutination test (DAT), Indirect fluorescent antibody test (IFAT), ELISA etc. are also used to diagnose VL. In the case of immunocomprised patient, diagnosis of VL is difficult due to low titer of antibody. Recently PCR based diagnosis and a latex agglutination test (KATEX) has been developed for the detection. The current article is devoted to the detection of specific antigen whish are useful in diagnosis of VL in VL patients as well as in immunocomprised patients.

INTRODUCTION

Visceral Leishmaniasis (VL) also known as Kala-azar (Hindi: kala means black, azar means sickness, also known as Assam fever, Dumdum fever, Sikari disease, Burdwan fever, Shahib's disease and tropical splenomegaly) was first described in 1824, in Jessore District of Bengal, what is now Bangladesh. At the turn of the 20th Century VL was observed in many parts of India. In India, kala-azar (Visceral leishmaniasis; VL) affects millions of people, and the state of Bihar accounts for nearly 90% cases, followed by West Bengal and Eastern Uttar Pradesh. In Uttar Pradesh recently few active cases have been seen from eastern districts e.g. Varanasi, Gorakhpur, Gonda, Faizabad etc. where there is under reporting of kala-azar and post kala-azar dermal leishmaniasis in women and children 0-9 years of age. Untreated cases of kala-azar are associated with up to 90% mortality. Although disease is to widely prevalent, the accurate data on the incidence and prevalence of Leishmaniasis is lacking especially from this part of the country.

MATERIALS AND METHODS

The standard strain of Leishmania donovani (*Strain HOM/ 1N/80/Dd8: WHO reference strain originally isolated from human Kala-azar patients from Muzaffarpur, Bihar, India*), is taken for the preparation of various antigens. NNN' Media is used to culture promastigotes. The polyacrylamide gel electrophoresis is carried out by Davis, 1964. Pathological methods are mainly based on demonstrate of parasites in biopsies or aspirated from infected spleen, bone marrow and lymphnodes (Zijlstra *et al*., 1992). Their sensitivities are in the range of 90-95% (Manson Bahr 1987; Zijlstra *et al*., 1992). Parasitological diagnosis remains the gold standard in Leishmaniasis diagnosis, because of its high specificity. Detection of LD bodies in smear of Spleen, bone marrow Painful, cumbersome and some times. Leishmanian test or Intradermal skin reaction or Montenegro reaction (Singh S, 2003) was also used. This is a test for Delayed type hypersensitivity. In this test an injection of 0.5 ml ($5x10^7$ phenol killed promastigotes) in volar aspect of the forearm of the patient leads to the formation of an induration measureable after 48 / 72 hours. Comparing the size of the induration with a control injection with phenol-saline in the other forearm it is possible to determine either an individual is infected or not. But this test not gives satisfactory result**,** because of large number of false positive and negative cases.

Serological Diagnosis: Various serological tests are being used for the diagnosis of the disease but their demerits are that they cross react with other chronic diseases (Chatterjee, 1957; Evans, 1973). Other serological tests which have been used from the diagnosis are as:-

Napier Test: In Napier Test**,** About 1-2 ml of serum from a case of kala azar will be taken in a small glass tube and to it will add a drop or two of 40 % formalin. The aldehyde test procedure is simple. Five cubic millimeters venous blood is drawn from the patient and left for clotting. Serum is separated by removing supernatant after centrifugation. To one ml of serum, 2-3 drops of commercial formalin (40% formaldehyde) is added. Opaque jellification like white of a boiled egg indicates a positive reaction. Jellification time indicates intensify of infection as indicated in the table below.

Corresponding author: skumarbt88@gmail.com

Antimony Test: This also depends upon a rise of serum gamma globulin. In Antimony test, 4% of urea stibamine solution in distilled water will add to whole serum from kala-azar patients in a small tube. A positive test will be indicated by the formation of a profuse flocculent precipitate. A negative result is indicated when the two fluids mix without precipitation. As a diagnostic aid antimony test is less reliable than the aldehyde test.

Complement Fixation Test with W.K.K. Antigen: This test depends upon the presence of certain immune bodies in blood sera of kala-azar patients, the antigen used in the test is prepared from either human tubercle bacilli (Witebsky, Kligenstein & Kuhn: WKK antigen) or from Kedrowsky's acid-fast bacillus. The test is essentially an antigen-antibody reaction in which the antibodies present in the serum are bound to an antigen.

Direct Agglutination Test*:* The Direct agglutination test (DAT) is a highly specific and sensitive test. It is inexpensive and simple to perform making it ideal for both field and laboratory use. The test is based on antigen-antibody reaction.

Indirect Immuno flourescent Assay Test (IFAT): This is a commonly used technique for Kala azar. This is a specific and sensitive test requiring fewer amounts of antigens and detects the low titer of antibodies present in patient serum, which are demonstrated in the very early stage of infection and are undetectable six to ninth months after cure. If the antibodies persist in low titers, it is good indication of a probable relapse. The sensitivity and specificity of this test is 96 % and 98 % respectively.

Enzyme linked Immunosorbant Assay: ELISA has been used as a potential serodiagnostic tool for almost all infectious disease. Enzyme linked immunosorbent assay (ELISA) is also equally sensitive and specific but specificity depends upon the antigen used, requiring less amount of antigen. This is rapid inexpensive and simple to perform and can be used for diagnosis of Kala-azar for epidemiological work.

Rapid Immunochromatographic assay (dipstick or strip test): A promising ready-to-use immunochromatographic strip test based on rK39 antigen has been developed as a rapid test for use in difficult field conditions. The recombinant antigen is immobilized on a small rectangular piece of nitrocellulose membrane in a band form, and goat anti-protein A is attached to the membrane above the antigen band. After the finger is pricked, half a drop of blood is smeared at the tip of the strip, and the lower end of the strip is allowed to soak in 4 to 5 drops of phosphate-buffered saline, placed on a clean glass slide or tube. If the antibody is present, it will react with the conjugate (protein A colloidal gold) that is pre dried on the assay strip. The mixture moves along the strip by capillary action and reacts with rK39 antigen on the strip, yielding a pink band. In the strip of patients who are infected, two pinkish lines appear in the middle of the nitrocellulose membrane (the upper pinkish band serves as a procedural control). Several studies from the Indian subcontinent reported the test to be 100% sensitive).

Western Blot: A new method, the Western blot for detection of anti-leishmania antibodies in the sera is developed which allows specific serodiagnosis of Visceral Leishmaniasis in patients living in non-endemic areas (Salotra, 1999).

Molecular Diagnosis: A variety of nucleic acid detection methods have also been developed. PCR assay can detect presence of presence of parasite DNA or RNA in blood sample of any clinical sign or symptoms. Different DNA-based methods have been used for characterization of isolates of Leishmania (Bernhard Ronacher 2002).

a) **Genus level**: by performing PCR using Leishmania common primers depending on direct identification of the parasite in clinical
b) **Species level:** different methods could be applied, such as PCR which depends on species specific primers (Ibrahim *et al*., 1994), analysis of restriction fragment length polymorphism (RFLP), where the substrate can be whole genomic DNA, kDNA or PCR amplification products, analysis of kinetoplast and nuclear DNA including Southern blot hybridisation with specific DNA probes (Jackson *et al*., 1984; Beverley *et al*., 1987; Barker, 1989; Van Eys *et al*., 1989&1991)
c) **Strain level:** These methods are capable to detect variation among isolates of Leishmania belonging to the same species such as sequencing.

Antigen Detection

Antigen detection is more specific than antibody-based immunodiagnostic tests (De Colmenares *et al*., 1995). This method is also useful in the diagnosis of disease in cases where there is deficient antibody production (as in AIDS patients) (Riera *et al*., 2004). Because of the conditions prevailing in endemic area, any sophisticated method cannot be employed on a wider scale. There is a need for a simple rapid and accurate test with good sensitivity and specificity, which can be used without any specific expertise. A promising ready to use Latex test (KAtex) based on detection of antigen in urine has been developed as a rapid test for use in difficult field conditions (Attar *et al*., 2001).

RESULTS AND DISCUSSION

All above parasitological as well as serological techniques which are used to diagnose VL in India have some limitations and not is useful in field conditions. As we know that parasitological diagnosis is gold standard due to its specificity. Detection of LD bodies in smear of Spleen, bone marrow are invasive Painful, cumbersome and are not conveniently applicable under field conditions (Osman, 1998), but according to Neva & Sacks, 1990; Weiss, 1995, there is no possibility to distinguish between leishmania amastigotes belonging to the different species. *François Chappuis et al, 2007 demonstrated that***,** The accuracy of microscopic examination is influenced by the ability of the laboratory technician and the quality of the reagents used. Leishmanian Skin Test not gives satisfactory result**,** because of large number of false positive and negative cases. LST requires culture facility to produce LST antigens. The different antigen preparation impact test sensitivity and the test did not recognize present or past infections. In the case of gammaglobulin detection (serological diagnosis) in serum of the patients, the serum globulins also increase in a variety of

infections and thus this test is considered to be rather non-specific. A positive reaction may also be seen in diseases like tuberculosis, cirrhosis of liver, malaria, etc. Further, in kala-azar, the test becomes positive only when infection is at least three months old and may remain so even after six months of cure. (Napier & Antimony) are non-specific, and show cross reaction with other pathological conditions, hence are unsuitable for diagnosis. Compliment fixation tests by W.K.K. antigen gives better results However, cross reactions are observed in cases of pulmonary tuberculosis, leprosy and Mycobacterium infections. Most serological tests are non-specific, permitting detection of antigen shared by related organisms. They do not distinguish antigens confined to a single species as group reactions mask specific ones. The major disadvantage of DAT is the relative long incubation time of 18 h and the need for serial dilutions of serum. Also, DAT has no prognostic value. DAT remains positive for a longtime after the disease is cured, and thus cannot be used as a test of cure or for diagnosis of relapses. Immunochromatographic dip stick test rK 39 has a drawback that an individual with a positive rK39 strip test result may suffer from an illness (malaria, typhoid fever, or tuberculosis) with clinical features similar to those of VL yet be misdiagnosed as suffering from VL. Using western blotting, one can find even minor antigenic differences among various organisms and thus detect cross reactive antigens. This process is time consuming, technically cumbersome, and expensive. 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% and specificity of 100%. *Cruz et al. 2006* demonstrated that the detection of antigens in serum is complicated by the presence of high levels of antibodies, circulating immune complex, serum amyloid, rheumatoid factors, and autoantibodies. Total 7 bands were obtained with Dd8 strain which were 35Kda, 27Kda, 19 Kda, 15Kda, 10Kda, 9.5Kda and 8 Kda. Of these 7 bands, 8.0 Kda protein fractions were shown to react with all kala-azar sera. However, sera from cured cases of kala-azar and other disease sera did not show any reaction with this 8.0 Kda protein fraction. This 8.0Kda protein fraction was observed in all 14 isolates tested including DD8. Antigenic fractions of 8 of these isolates were electro-transferred on to the surface of NCP and subsequently immunobloted.

Conclusion

Many of the diagnostic methods / procedures are reported with due course of time but none of them was found completely reliable for the diagnosis of the VL. Review of several diagnostic approaches revealed that antigen detection is most sensitive and better technique to diagnose VL patients including immunocomprised ones. This review article will provide definitely the insight to the readers about the various methods and be helpful for the development of new specific antigens for the diagnosis of VL.

REFERENCES

Adrino Gomes Silva *et al*. (2008). Serological reactivity of different antigenic preparations of Leishmania amazonensis and Leishmania braziliensis complex. *Revista da sociedade Brasileira de Medicino tropical.,* 41 (2): 135-141.

Agrawal, K. (2006). Field Adaptable Tests for Kala-Azar. J. Armed Forces India, 62, 178-179,

Attar, Z.A. *et al*. (2001). Latex agglutination test for the detection of urinary antigens in visceral Leishmaniasis. *Acta Tropica.,* 78, 11-16.

Boelaert M *et al*. (1999). Operational validation of the direct aggultination test for diagnosis of visceral leishmaniasis. *American J Trop Med Hyg.,* 60(1):129-134.

Chappuis, F. *et al*. (2006) Field validity, reproducibility and feasibility of diagnostic tests for visceral leishmaniasis in rural Nepal . *Trop. Med. Int. Health,* 11(1), 31-40.

Das, ML. *et al*. (2007). Use of rk39 for diagnosis of post kala-azar Dermal leishmaniasis in Nepal southeast. *Asian J Trop Med Public Health,* Vol 38 No. 4 919-625.

DeKorte *et al*. (1990). Introduction of an improved direct agglutination test for the detection of Leishmania infantum infection in southern France. Parasitol Res. 6: 526-30.

Edrissian GH and Darabian P. (1979). A comparison of enzyme linked immunosorbent assay and indirect fluorescent antibody test in the serodiagnosis of cutaneous and visceral leishmaniasis in Iran. *Trans R Soc Trop Med Hyg.,* 73:289-292.

El-Safi, S.H. *et al*. (2003). Field evaluation of latex agglutination test for detecting uinary antigens in visceral leishmaniasis in Sudan. Eastern Mediterr. *Health J.*, 9(4), 844-855,

Goyal, RK and TM Mohapatra (2004). Superiority of DAT over ELISA as a diagnostic and Seroepidemiological tool for the diagnosis of Indian Kala-azar. *Indian Journal of Medical Microbiology,* 22 (1):57-60

Harith AEL *et al*. (1988). Improvement of a DAT for field studies of visceral leishmaniasis. *J Clin Microbial*., 26:1321-1325.

Héctor Dardo Romero *et al*. (2009). Comparative study of serologic test for the diagnosis of asymptomatic VL in an endemic area. *Am J Trop. Med. Hug.,* 81 (11). 27-33

Hommel M *et al*. (1978). The micro-ELISA technique in the serodiagnosis of visceral leishmaniasis. *Ann Trop Med Parsitol.,* 72:213-218.

Hommel, M. *et al*. (2001). Katex for the diagnosis of human visceral leishmaniasis. *Med. Trop.* (Mars), 61(6), 503-505

Israel cruz *et al*. (2006). Comparison of New diagnostic tools for management of pediatric. Mediterranean visceral Leishmaniasis. Journal of Clinical Microbiology, 44, 2343-2347.

Marleen Boelaert *et al*. (2007). Evaluation of rapid diagnostic test: visceral Leishmaniasis. *Nature reviews Microbiology,* 530-539

Raj *et al*. (1999). Serodiagnosis of leishmaniasis with recombinant ORFF antigen. *Am J Trop Med Hyg., 61* : 482-7.

Riera, C. *et al*. (2004). Evaluation of a latex agglutination test (KAtex) for detection of Leishmania coinfection: value in diagnosis and post-treatment follow-up. *Eur. J. Clin. Microbiol. Inf. Dis.*, 23(12), 899-904.

Rijal, S. *et al*. (2004). Evaluation of a urinary antigen-based latex agglutination test in the diagnosis of kala-azar in eastern Nepal. *Trop. Med. & Int. Health,* 9, 724-729

Salotra, P. and R. Singh (2005). Rapid & reliable diagnostic tests for visceral leishmaniasis. *Indian J. Med. Res.,* 122, 464-467,

Sarkari, B. *et al*. (2005). A capture ELISA for the Diagnosis of Visceral Leishmaniasis using a Monoclonal Antibody

against a Leishmanial Urinary Antigen. *Iran. Biomed. J.,* 9(3), 117-122,

Schoone *et al***.** (2001). A fast agglutination test (FAST) for the detection of anti-Leishmania antibodies. *Trans, Royal Soc, Trop Med, Hyg. Jul-Aug;* 95(4):400-1.

Selcuk Kilic *et al*. (2008). Evaluation of serological tests for the Diagnosis of VL.Turk J Med. Sci. 38(1) 13:19.

Sherchand Jeevan, B. *et al*. (2003). KAtex Test for the Detection of Urinary Antigens in Visceral Leishmaniasis Patients of Nepal. J. *Nepal Med. Assoc*., 42, 191-195,

Singh, S. *et al*. (2005). Applications of molecular methods for Leishmania control.*Exp. Rev. Mol. Diagn.,* 5(2), 251-265

Singla N *et al*. (1993). Evaluation of the direct Kala-azar in India. Trans R Soc Trop Med Hyg. 87:276-278.

Sinha R and Sehgal S. (1994) Comparative evaluation of serological test in Indian Kala-azar. *J Trop Med Hyg*., 97:333-340.

Srivastava L and Singh VK.(1988). Diagnosis of Indian Kalaazar by dot enzyme – linked immunosorbent assay (dot-ELISA). *Ann Trop Med Parasitol.,* 82:331- 334.

Srivastava L. (1989). Comparative evaluation of serological test in diagnosis of Kala-azar. *Indian J Med Res.,* 89:265-270.

Sunda, S. and M. Rai (2002). Laboratory Diagnosis of visceral Leishmaniasis *Clin. Diagn. Lab. Immunol*., 951-958,

Sundar, S. *et al*. (2005). Detection of Leishmanial Antigen in the Urine of Patients with Visceral Leishmaniasis by a Latex Agglutination test. *Am. J. Trop. Med. Hyg.,* 73(2), 269-271.

Tavares, C.A.P. *et al*. (2003) Molecular diagnosis of leishmaniasis. *Expert Rev. Mol. Diagn.,* 3(5), 657-667

Vilaplana, C. *et al*. (2004) Noninvasive Method for Diagnosis of Visceral Leishmaniasis by a Latex Agglutination Test for Detection of Antigens in Urine Samples. *J. Clin. Microbio.,* 42, 1853-1854

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