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RESEARCH ARTICLE

Identification of *8kda* specific protein fraction from patients of *Visceral Leishmaniasis* and Reference Strain

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Abstract

Visceral Leishmaniasis (VL) is a vector borne anthrozoonotic disease caused by a protozoan, *Leishmania donovani*, of Trypanosomatidae family. It is an endemic disease of warmer parts of the world & covers 88 countries (16 developed and 72 developing) with a total of 350 million people at risk and 12 million cases of infection. This disease is fatal unless treated. Incidence of VL is rising with urban migration of people from rural areas (WHO, 2002) and increasing overlap of the distribution of VL and human immunodeficiency virus (HIV) has led to the emergence of Leishmania / HIV co-infection which has so far been reported from 34 countries (WHO, 2002; Desjeux, 2004; Alvar et al., 2008). Because of the diversity of epidemiological situations, no single diagnosis treatment, or control will be suitable for all (Philipee et. al., 2002).

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. Recently PCR based diagnosis and a latex agglutination test (KATEX) has been developed for the detection.

The current research develops a new idea for the diagnosis of Visceral Leishmaniasis. This study is based on the comparison of different diagnostic technique with healthy controls, diagnosed patients and cured cases of VL. Protein estimation of promastigotes carried by Lowry's method and followed by polyacrylamide gel electrophoresis. after completion of PAGE, 35Kda, 27Kda, 19 Kda, 15Kda, 10Kda, 9.5Kda and 8 Kda protein bands were obtained, it provide specific clue for diagnosis of VL in patients.

Introduction

Visceral Leishmaniasis (VL) also known as Kala-Azar (Hindi: kala means black, azar means sickness), was first described by Leishman and Donovan in 1903. They separately demonstrated VL parasites in stained smears prepared from the spleen of patients suffering from a malaria-like illness.

Visceral Leishmaniasis (Leash'ma NIGH a sis) is a vector- borne anthrozoonotic disease caused by obligate intracellular macrophage protozoan, *Leishmania* a genera within the family *Trypanosomatidae*. The protozoan parasites of the genus *Leishmania* are the causative agents of a group of diseases called Leishmaniasis. The causative organism of VL in the Indian subcontinent and Africa is *L. donovani*.

Leishmaniasis, because of its importance, was selected by WHO out of six most precarious diseases under its special programme for research and training

in tropical diseases. It ranks next to malaria among the human diseases caused by protozoan. The disease is endemic, covering almost the five continents, in 88 countries (16 developed and 72 developing or 66 countries are in the Old World while 22 countries located in the New World) with a total of 350 million people at risk and 12 million cases of infection.
Out of 500, 000 cases of visceral Leishmaniasis, more than 90 percent are reported from India, Bangladesh, southern Sudan, Nepal and northeast Brazil. The actual number is believed to be at least five times great to the officially estimated number. Despite the widespread geographic distribution in Bihar, Leishmaniasis is very common within an endemic area, leading to 'hotspots' of disease transmission.

Material and Methods

This prospective study is based on the 100 patients diagnosed as VL infected, 20 normal control and 20 patients, which have previous infection history. From these patients, splenic aspirates also collect for the in-vitro culture of Promastigotes. Aseptic condition is very necessary for the culture of promastigote because any contamination/inhibitor can diminish the growth of promastigote. Growths regularly checked by prepare a smear in every 48 hours. Similarly Reference Strain *MHOM/IM/80/Dd8 WHO* was also cultured.

Media and culture conditions:

Diphasic Culture media NNN (Novy, & Mac Neal, 1904; Nicoll, 1908) medium (Liquid phase) was used for subculture of standard strain and for culture of bone-marrow aspirates (Mackie & McCartney, 1989). The parasite were maintained in NNN' media at 26ºC by sub culturing after every 7-10 days.

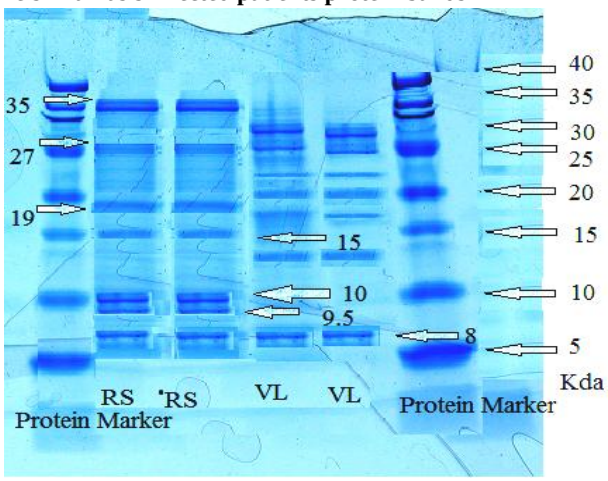
Protein extraction and Poly Acrylamide Gel Electrophoresis:

Protein estimation of promastigote isolate done by Lowry's method and found that protein content of *reference strain MHOM/IM/80/Dd8 WHO* strain was found to be 1000 mg/ml, and the protein content of isolates obtained from patients, was measured between 300-950 mg/ml. it represents that protein content of isolate obtained from patients have low due to inhibition of growth by some factors.
Protein contents (both reference strain and patients strain) obtained by Lowry's method run over Polyacrylamide Gel electrophoresis slab containing Commassie Brilliant Blue (Colouring Agent). The polyacryalmide gel electrophoresis was carried out by **Davis, B. J., 1964** method in slab gel system using running gel of 7.5% polyacrylamide at pH 9.5 and 2.5% stacking gel have been used for polyacrylamide.

Result

After the completion of overall process 7 dark coloured bands found on the gel, representing different molecular weight (Kda). The range of bands obtained from *Reference strain MHOM/IM/80/Dd8 WHO* was as follow -35Kda, 27Kda, 19 Kda, 15Kda, 10Kda, 9.5Kda and 8 Kda. Similarly isolate obtained from culture of aspirates also develops a specific band pattern after Poly acrylamide gel electrophoresis.
Comparison of bands after completion of PAGE, Standard Protein Marker, protein isolated from reference strain and other protein, shows that one protein with Molecular weight 8 kda was similar among all the protein.

Fig 1: Polyacrylamide gel electrophoresis, 1 & 6 lane represent Standard Protein Biomarker: RS stands for Reference Strain protein bands, VL: Visceral leishmaniasis infected patients protein bands



Discussion

It represent that 8 kda proteins was present in all VL infected patients. When these bands were transfer on nitro cellulose paper (NCP) for the hybridization with patient's serum, which contained the specific antibodies for visceral leishmaniasis specific promastigote antigens.
This was quite surprising to know that ,when these bands were treated with VL infected patient serum, it shows that serum of the VL patients react with only one protein content of the reference Strain i.e. 8 Kda

and none of the protein band shows agglutination reaction with patient's serum.

The serum of cured patients doesn't react with this protein content. It was also surprising, that this 8.0 Kda protein fraction was also present in the electrophoresis bands of isolates obtained from VL Patients along with other bands. This band was also present in the bands observed in all 95 isolates tested including DD8.

Antigenic fractions of 8Kda of these isolates were electro-transferred on to the surface of NCP and subsequently immune-blotted for further experiments.

Conclusion

On the basis of study, identification of antibody in VL infected patients against 8kda protein fraction of promastigote can be an effective tool for the diagnosis of *Visceral Leishmaniasis***.**

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