Original Article

Microscopic Detection of L.D. Bodies in Splenic Aspirates at Rajshahi Medical College Hospital

M A Salam¹, I Ahmed², M S Ali³, S M A Hossain⁴ S Begum⁵

Abstract

Leishman stained smears prepared from ninety-nine (99) splenic aspirates and eleven (11) slit skin were examined under light microscope in order to detect L.D. bodies at the department of Microbiology, Rajshahi Medical College from 2002 to May 2003. L.D. bodies were found in 46 (46.46%) and 01 (9.09%) samples of splenic aspirates and slit skin smears respectively. Smear positivity and concomitant higher parasitic load was noted among comparatively younger age groups with slight male preponderance. Demonstration of parasitic by direct microscopical examination in splenic aspirates for the diagnosis of visceral leishmaniasis or kala azar and in slit skin smears for Post kala azar dermal leishmaniasis (PKDL) is reliable method.

Introduction

Leishmaniasis results from an infection with the protozoan parasite Leishmania spp. The organism is transmitted to humans by the bite of an insect vector Phlebotomine sandfly. Humans are usually accidental hosts; natural hosts include a variety of rodents, small mammals and dogs¹. Leishmaniasis countries a diverse collection of human diseases ranging in severity from a spontaneously healing skin ulcer to overwhelming visceral disease. Geographically and ecologically the disease is widespread occurring in tropical and subtropical regions on all countries except Australia². Worldwide 1,00,000 new cases occur each year and at least 200 millions people are at risk of infection³. Kala azar or visceral leishmaniasis (VL) is considered to be an important public health problem in the Eastern and Northern part of Indian subcontinent including Bangladesh⁴⁵. The annual incidence of leishmaniasis ia around 15,000 in Bangladesh.

Proper diagnosis of leishmaniasis is a prerequisite to treat the infected person as well as to control the disease. The diagnosis is based on either parasitological or immunological evidences. The amastigote or tissue form of the parasite is called L.D. body, which can be demonstrated in spleen, bone marrow, lymph node of blood. While serodiagnosis for the detection of antibody includes aldehyde test (AT), Antimony test, Complement Fixation Test (CFT), Enzyme Linked Immunosorbent assay (ELISA), Indirect Fluorescent Antibody Test (IFAT), Direct Agglutination Test (DAST), Indirect Hemagglutination Assay (IHA) and Counter current Immuno Electrophoresis (CIE)⁷. More recently molecular diagnosis like DNA hybridization and PCR are also available. Direct

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evidence by detecting the causative agent of any
microbial disease including leishmaniasis is
always preferred and reliable than serological test
or other indirect evidences for the same.

We have undertaken this study to direct L.D.
bodies in suspected cases of leishmaniasis by
direct microscopical examination at Rajshahi
Medical College Hospital (RMCH) which is a
good centre for kala azar because of its location in
the endemic zone of kala azar in Bangladesh.

Study population and Methods

Patients included in this study are of both sexes
having different age groups from RMCH covering
a period of January 2002 to May 2003. Suspected
cases of kala azar were all admitted patients in
different adult and Pediatric medical wards while
cases of suspected PKDL attended the
Dermatology outpatient department of RMCH.
Experienced and trained persons collected splenic
tissues through aspiration following standard
procedure and precautions. After preparation of
smears at bed site all slides were sent to the
department of Microbiology, Rajshahi Medical
College with a request to detect L.D. bodies. Slit

<table>
<thead>
<tr>
<th>Specimen</th>
<th>No. Positive (%)</th>
<th>No. Negative (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Splenic aspirates</td>
<td>46 (46.46%)</td>
<td>53 (53.54%)</td>
<td>99 (100%)</td>
</tr>
<tr>
<td>Slit skin smear</td>
<td>01 (9.09%)</td>
<td>10 (90.91%)</td>
<td>11 (100%)</td>
</tr>
</tbody>
</table>

Table 2: Age & Sex distribution of Kala azar patients (n=46)

<table>
<thead>
<tr>
<th>Age groups (in Years)</th>
<th>Sexes</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 10 yrs.</td>
<td>M: 08; F: 02</td>
<td>10 (21.74)</td>
</tr>
<tr>
<td>11-20 yrs.</td>
<td>M: 07; F: 05</td>
<td>12 (26.09)</td>
</tr>
<tr>
<td>21-30 yrs.</td>
<td>M: 05; F: 06</td>
<td>11 (23.91)</td>
</tr>
<tr>
<td>31-45 yrs.</td>
<td>M: 07; F: 06</td>
<td>13 (28.26)</td>
</tr>
</tbody>
</table>

(Figures in the parentheses indicate percentage)

Table 3: Grading of amastigotes (L.D. bodies) detected in splenic aspirates

<table>
<thead>
<tr>
<th>Age groups (in Years)</th>
<th>Grading of amastigotes</th>
<th>Interpretations of Grades</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 20</td>
<td>4+ &amp; 5+</td>
<td>5+ : 10-100 amastigotes/field</td>
</tr>
<tr>
<td>21-30</td>
<td>3+ &amp; 4+</td>
<td>4+ : 1-10 amastigotes/field</td>
</tr>
<tr>
<td>31-45</td>
<td>2+ &amp; 3+</td>
<td>3+ : 1-10 amastigotes/10 fields</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2+ : 1-10 amastigotes/100 fields</td>
</tr>
</tbody>
</table>

* Using 10x eyepiece and 100x oil-immersion lens

Although invasive but demonstration of the
parasites in spleen, bone marrow or lymph gland
aspirates (in order of sensitivity) is the most
definitive in the diagnosis of leishmaniasis and
is also a prerequisite of treatment. As stated
earlier, there are many serological tests available
for the diagnosis of leishmaniasis and some of

Results

Out of 99 splenic aspirates, L.D. bodies were
detected in 46 (46.46%) cases and 01 (9.09%) slit
skin smear was found to be positive for L.D.
bodies among 11 suspected cases of PKDL (Table
1). Table 2 depicts number of positive VL cases
shown according to different age and sex groups.
It is evident from these figures that the
parasitological positive cases are mostly (72%) young people within 30 years of age. There is
slight male preponderance M:F=1.42:1) in sex
distribution of L.D. bodies positive cases. Table 3
shows the average parasitic density in different
age groups expressed in grading according to
WHO Criteria. Again as a whole increased
parasitic load was noted among comparatively
younger age groups.
recently introduced tests like DAT or dot ELISA can be performed in the peripheral health centres and under field condition. But because of obvious limitations of any serological test these methods are not always satisfactory for diagnosis. Further serological assays for VL may well cross-react with other diseases including African trypanosomiasis, Mucocutaneous leishmaniasis, Malaria, Tuberculosis, Leprosy and Amoebiasis which are co-endemic in some parts of the world\textsuperscript{9,10,11,12}.

We have examined 99 samples of splenic aspirates from clinically suspected kala azar patients microscopically and were able to detect L.D. bodies in 46 (46.46\%) cases. The rate of parasitological positivity in this study is high in comparison to other studies\textsuperscript{13,14}, because of selection of patients from a tertiary level hospital located in endemic zone of kala azar in Bangladesh. Further the sensitivities for demonstration of the causative parasites in the splenic aspirates that we have examined is quite high as compared to bone marrow or lymph node aspirates\textsuperscript{15}. The reasons we could not be able to detect L.D. bodies in 53.54\% cases can be explained by the fact that some of the splenic aspirates probably were not representative tissue sample or in a few cases there might have been faulty staining technique that obscured positive findings. Furthermore the scanty parasitic load in a few splenic aspirates might have been missed and also some selected suspects may have had other diseases clinically simulating kala azar. Regarding the age of kala azar patients, it has been found that comparatively younger age groups are the victims with 72\% cases having age within 30 years. This finding is in accordance with others\textsuperscript{5}. There is slight male preponderance of VL cases in our study. This could well be due to our males have more outdoor exposure for obvious reasons and as a consequence there is more chance of infection in comparison to their counter sex group. We have also noted the parasitic load in the splenic aspirate smears and according to WHO criteria different grades of amastigotes were observed in different age-group. Again it was found that comparatively younger age groups have higher grades indicating maximum parasitic load.

In our microscopical examination series we also included 11 cases of suspected Post kala azar dermal leishmaniasis (PKDL) with slit skin smear positivity in one case only. PKDL is a cutaneous presentation and is regarded as complication of VL characterized by macular, maculopapular and nodular rash in patients who has recovered from VL and who is otherwise well. In Indian subcontinent, 5-10\% treated cases of VL terminate into PKDL, where Leishmania donovani is the causative agent\textsuperscript{16}. The poor detection rate of PKDL in suspected cases is probably due to its inaccurate provisional diagnosis and many differential diagnosis of PKDL such as tropical ulcers, impetigo, infected insect bites, leprosy, lupus vulgaris, tertiary syphilis yaws, blastomycosis, skin cancer etc.

In conclusion we would like to state that our centre does not have the facilities of molecular diagnostic methods for leishmaniasis which are very sensitive and specific and high tech immunodiagnostic tests are also not available. In such a context, although invasive the confirmatory diagnosis of leishmaniasis is rested upon the parasitological evidences like detection of L.D. bodies in splenic aspirasus.

References


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