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

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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.

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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 dogs[1]. 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 Australia[2]. Worldwide 1,00,000 new cases occur each year and at least 200 millions people are at risk of infection[3]. 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 Bangladesh[4,5]. 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 duisease. 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 mode of blood. While swrodiagnosis 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 (CIF)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 out patient department of RMCH. Experienced and trained persons collected splenic tissues through aspiration following standard procedure and precautions[2]. 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 skin smears were prepared from cases of suspected PKDL at the department of microbiology by trained personnel. Microscopical examination of Leishman stained smears of both splenic aspirates and slit skin samples were examined for L.D. bodies at the department of Microbiology.

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[2]. Again as a whole increased parasitic load was noted among comparatively younger age groups.

Table-1: L.D bodies detected by Microscopical examination

Specmens	No. Positive (%)	No. Negative (%)	Total
Splenic aspirates	46 (46.46)	53 (53.54)	99 (100%)
Slitt Skin smear	01 (9.09)	10 (90.91)	11 (100%)

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

Age groups	Upto 10 yrs.	11-20 yrs	21-30 yrs.	31-45 yrs.
No. of cases	10 (21.74)	12 (26.09)	11 (23.91)	13 (28.26)
Sexes	M : 08; F : 02	M: 7; F: 05	M: 05; F: 06	M: 07; F: 06

(Figures in the parentheses indicate percentage)

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

	Age groups (in Years)			Interpretations of Grades
	Upto 20	21-30	31-45	5+ : 10-100 amastigotes/field*
				4+ : 1-10 amastigotes/field
Grading of amastigotes	4+& 5+	3+& 4+	2+& 3+	3+ : 1-10 amastigotes/10 fields
				2+ : 1-10 amastigotes/100 fields

* Using 10x eyepiece and 100×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[8]. As stated earlier, there are many serological tests available for the diagnosis of leishmaniasis and some of

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 coendemic in some parts of the world[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 parasitilogical positivity in this study is high incomparison to other studies[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 mode aspirates15. 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 others6. 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 incomparison 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 agegroup. Again it was found that comparatively younger age groups have higher grades indicating maximum parasitic load.

In our microscopical examination series wealso 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 agent16. 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 asporasus.

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