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Research Article

Bone marrow smear VS Blood smear in diagnosis of Kala-azar

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ABSTRACT

Background and Objectives: Bone marrow specimen is considered as superior to the blood in the laboratory diagnosis of Kala-azar. The main objective of this study is to compare these two methods of diagnosis and determine the usefulness of the diagnostic techniques.

Material and Methods: This prospective cross sectional study was conducted at Janakpur Zonal Hospital, Janakpur which was aimed to determine the usefulness of the bone marrow specimen and blood specimen in the laboratory diagnosis of Kala-azar. Bone marrow aspirate and venous blood was collected aseptically from the cases were processed simultaneously. The results of these two cultures were compared.

Results: Total 60 cases of Kala-azar were included in the study of which 32 were male and 28 were female. Amastigote form of Leishmania donovani were detected in 56 (93.33%) samples with high titre of parasitemiae and 119 (18%) in the blood sample with low parasitemiae. Sensitivity and Specificity of the test was calculated of the bone marrow sample test have more sensitivity (98%) and specificity (100%) over the sensitivity (90%) and specificity (96%) of blood smear test.

Conclusion: Bone marrow specimens were found to be more useful than the blood sample in the laboratory diagnosis of Kala-azar.

Key Words: Kala-azar, Amastigote, Bone marrow

INTRODUCTION

Kala-azar is a protozoal disease caused by a blood parasite, *Leishmania donovanii* transmitted by the bites of a sandfly, Phlebotomus argentipus. It is the most common vector borne disease of Terai region of Nepal. Large number of people infected during the summer season in each year. Among them, mostly children are infected.

The disease ia characterized by remittent type of fever, massive enlargement of spleen and generalised weakness [2]. There are different techniques available for the laboratory diagnosis of Kala-azar. Diagnostic spectrum is changing over the time because of advent of the sophisticated instruments and techniques. Various non-invasive test with various specifications and sensitivities are available for the diagnosis of Kala-azar [9,10]. Parasite detected by this method is gold standard [1,3]. This study was aimed to determine, the appropriate choice of method in the laboratory diagnosis of Kala-azar between these two methods.

MATERIAL AND METHODS

This study prospective cross sectional study was conducted at Janakpur Zonal Hospital, Janakpur which is located in Dhanusha district of Nepal. This district is the endemic region of Kala-azar. Each year more than thousand people were admitted to the Zonal Hospital Janakpur suffering from Kala-azar. Due to geographical location, open border, open sewage, poor hygiene and sanitation leads to the endemic for Kala-azar.

Total of 60 cases of Kala-azar confirmed by clinical symptom and formal gel test were included in the study. In all the cases clinical findings were recorded and patients were informed about the procedure. Bone marrow aspirator sample and blood sample collected aseptically were processed by Giemsa stain as per standard staining protocol [10]. After staining the both slide were observed microscopically for the detection of Donovan body and findings were noted. The finding data were tabulated and analyzed statistically.

RESULTS

Total 60 cases of Kala-azar were included in the study of which 32 were male and 28 were female. Amastigote form of *Leishmania donovanii* were detected in 56[93.33%] samples with high titre of parasitemiae and 11[18%] in the blood sample with low parasitemiae. It was found that in most of the cases bone marrow sample were positive while blood smear showed negative result.

All the blood smear positive cases were found to be positive in bone marrow smear also but most of the case diagnosed positive with Formal gel test and bone marrow smear examination were found to be negative in blood smear examination. Sensitivity and Specificity of the test was calculated of the bone marrow sample test have more sensitivity [98%] and specificity [100%] over the sensitivity [90%] and specificity [96%] of blood smear test. The examination was found to be equal in both male and female while load of parasites were found higher in the child of age less than 8 than of the higher age (table1, 3 and 4).

Table 1 : Total numbers of positive samples

Case	Number	Blood smear positive	Bone marrow smear positive
Male	32	04(12.5%)	30 (93.75%)
Female	28	07 (25%)	26 (92.8%)
Total	60	11(18%)	56(93.33%)

Table 2 : Age and Sex wise distribution

Age Group	Male	Female	Positive in blood smear		Positive in bone marrow	
			Male	Female	Male	Female
<15	10	08	03 (30%)	01 (12.5%)	09 (90%)	07 (87.5%)
15-30	09	05	02 (22.22%)	00	09 (100%)	05 (100%)
30-45	07	10	01 (14.2%)	02 (20%)	07(100%)	08 (80%)
>45	06	05	01 (14.7%)	01 (20%)	06(100%)	05 (100%)

Table 3: Sensitivity and specificity of the test.

Blood smear sample

Test	Disease	No Disease	Total
Positive	09	02	11
Negative	01	48	49
Total	10	50	60

Sensitivity of the test: 9/10 x100 = 90%
Specificity of the test: 48/50x 100 =96%

Table 4: Bone marrow sample

Test	Disease	No Disease	Total
Positive	56	00	56
Negative	01	03	04
Total	57	03	60

Sensitivity of the test: 56/57 x100 = 98%
Specificity of the test: 3/3 x100 = 100%

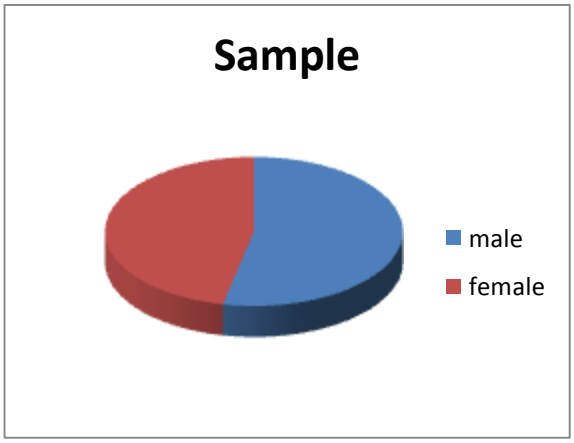

Fig. 1: Number of Samples

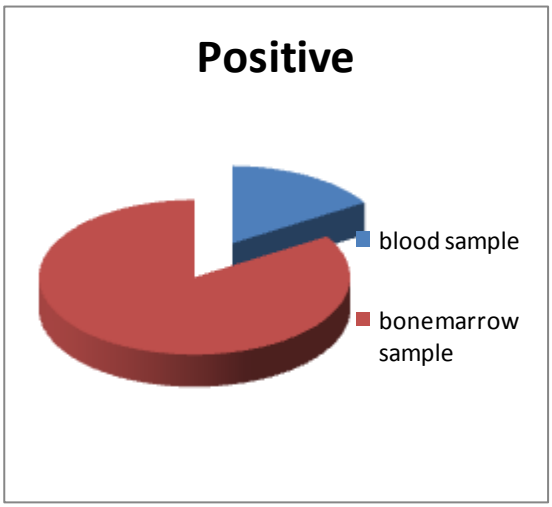

Fig. 2: Number of Positive Samples

DISCUSSION

In present study, it was found that bone marrow sample were more preferable

than the blood sample. Most of the studies done regarding the sensitivity of these two samples shows similar result. In a study by Haq et al, sensitivity of the bone marrow sample was found 30.23% than the blood sample 5.34%. In a study by Farouqi et al, orgamism isolated from the bone marrow in all cases of kala-azar is 83.0% .this is similar to the finding of this study shown in table 2 and 3 [7,8]. Bone marrow culture could confirm positive diagnosis in patient with negative biood culture[5]. One of the reasons for blood culture being negative and bone marrow beining positive in kala-azar may be due to antibiotic treatment before collection of sample. An injudicious antibiotic regimen may diminish or eliminate organism from the blood[6,7]. Leishmania beining an intracellular organisms persist in the reticulo-endothelial system including bone marrow[9].

The intracellular location of the organism protect them from conventional chemotherapeutic measures. In a study by John et al, the number of organism in blood but not bone marrow was correlated inversely with the duration of fever. Thus with increasing duration of illness the ratio of bone marrow to blood increased. This study provided proof that the concentration of Leishmania in bone marrow is higher than the blood. Bone marrow contained over 10 times moe organism than the blood. Leishmanias are transmitted by sandflies and cause the following main forms of leishmanioses in warm regions: visceral leishmanioses (VL), cutaneous leishmanioses (oriental sore) (CL), and mucocutaneous leishmanioses (MCL). In Central Europe, leishmaniosis is of significance as an imported disease and as an HIV-associated infection. In central Europe, leishmaniosis deserves attention as a travelers' disease, especially the VL imported from Mediterranean countries. Major VL epidemics have occurred recently in various parts of the world, e.g., in southern Sudan with 100 000 deaths in a population of <1 million [1,2].

CONCLUSION

It was found that in most of the cases bone marrow sample were positive while blood smear showed negative result. All the blood smear positive cases were found to be positive in bone marrow smear also but most of the case diagnosed positive with formal gel test and bone marrow smear examination were found to be negative in blood smear examination. Sensitivity of the bone marrow examination was found to be equal in both male and female while load of parasites were found higher in the child of age less than 8 than of the higher age.

An etiological diagnosis of VL is made by means of direct parasite detection in aspirate material from lymph nodes or bone marrow. A bone marrow examination including culture is important part of investigaton in kala-azar. A good clinical history, radiological, haematological evaluation including bone marrow culture and serology would yield diagnosis in a high proportion of cases. Yield of bone marrow culture is more than blood culture in kala-azar. Cultivation and PCR have about the same

high level of sensitivity. Antibodies are detectable in nearly all immunocompetent patients [2,4].

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