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LEISHMANIASIS: RAINBOW OF PRESENTATIONS UNVEILED ON BONE MARROW

Pathology

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ABSTRACT

Context: Leishmaniasis (VL) is a chronic infection presents with fever, weight-loss, and splenomegaly. It's a mimicker of many infectious and noninfectious illnesses.

Aims: We will highlight both the clinical and hematological spectrum of Leishmaniasis in the immunocompetent patients including their unusual presentations.

Settings and Design: During evaluation, amastigote forms of Leishmania in bone marrow were diagnosed. In all such cases serological assay of rK39 was done which was well correlated.

Methods and Material: We performed a retrospective study of 09 months at a Tertiary Health & Research Centre of Northern India. The patients presented with variable clinical features ranging from infectious etiology to malignant ones.

Statistical analysis used: Being a limited number of cases, no statistical software was used.

Results: Among total six patients, four presented with splenomegaly, one with renal failure and one with unilateral pleural effusion. Three and two of such patients presented with long and short duration of fever respectively and one was afebrile. Outcome of all cases was good due to timely diagnosis and management with intravenous liposomal amphotericin B.

Conclusion: Diagnosis of visceral leishmaniasis can be challenging due to atypical clinical presentations at times, therefore an early investigation and prompt treatment is recommended.

KEYWORDS

Visceral Leishmaniasis (VL), Kala azar, Bone marrow aspiration (BMA), rK39 antigen, Leishmania donovani (LD) bodies, Amastigote

INTRODUCTION:

Leishmaniasis refers to a diverse spectrum of clinical syndromes caused by infection with protozoan parasites of the genus *Leishmania.* Three forms of Leishmaniasis comprises of visceral, cutaneous and mucocutaneous. Visceral Leishmaniasis (VL) or Dumdum fever is commonly seen in Indian subcontinent, China, Southern Europe, Mediterranean region, East Africa, South and Central America.[1] According to one study, there are approximately 90% of the total 2.5 million cases of VL in Indian subcontinent like India, Bangladesh, Nepal, Brazil and Sudan.[2,3] Usual manifestations of VL include fever, loss of appetite, splenomegaly, however at times atypical manifestations in form of pneumonia, portal hypertension, gastrointestinal symptoms and absence of splenomegaly also noted.[4,5] The aim of the index study is to create awareness of VL because of the difficulty in recognizing it; especially in the settings of fewer, varied and mimicking symptoms with other disease.

SUBJECTS AND METHODS:

A retrospective analysis of bone marrow aspirates, positive for Leishmania donovani (LD) bodies was carried out for nine months duration at a Tertiary Health & Research Centre of Northern India. Bone marrow slides were stained with May-Grunwald-Giemsa stain. Two slides were stained in all cases except for one case in which all slides were stained due to hemodilution. Detailed history along with demographic, clinical and hematological profiles were noted for each patient. Serological test for recombinant kinesin antigen (rK39) by ELISA assays was done in all cases. The outcomes of these patients were compared and analyzed.

RESULTS:

A summarized clinical and hematological profile of all the patients has been depicted in table 1 and 2 respectively. Leishmaniasis seems to affect patients of all ages ranging from 2 months to 62 years with immunocompetent status. In our study 5 males and 1 female was included with variable clinical differential diagnosis. All these patients were from North India, 4 of them belonged to Bihar. All patients have variable clinical presentation of 5 being febrile and 1 nonfebrile during course of illness, the later did not have splenomegaly. Hematological evaluation revealed anemia in all five patients while additional leucopenia and thrombocytopenia in three cases. Bone marrow aspiration studied showed few to marked intracellular/extracellular LD bodies in all cases with hemophagocytosis in four cases. After that rK39 was performed, that was positive in 100% cases.

Table 1: CLINICAL PROFILES OF PATIENTS

PARAMETERS	CASE 1	CASE 2	CASE 3	CASE 4	CASE 5	CASE 6
AGE/ SEX	40/M	02/M	48/M	38/M	62/M	60/F
RESIDENCE	East Uttar Pradesh	Bihar	Himachal Pradesh	Bihar	Uttarakhand	Bihar
PAST HISTORY	Post thymectomy	Failure to thrive since 3 months	Anemia	Not signficant	Not signficant	Not signficant

FEVER DURATION	20 days	Yes	No	8 days	2 months	3 months
LOSS OF APPETITE	No	Yes	No	Yes	Yes	Yes
ONSET	20 days	02 months	2 months	08 days	02 months	03 months
IMMUNOCOMPROMISED	No	No	No	No	No	No
SPLEEN	Yes	Yes	No	Yes	Yes	Yes
ORGAN FAILURE	No	No	No	Renal Failure	No	No
CLINICAL DIAGNOSIS	Myaesthenia gravis	Storage disease	Multiple Myeloma	Fever	Tuberculosis	Non-Hodgkin's Lymphoma
OUTCOME	Good	Good	Good	Good	Good	Good

Table 2: HEMATOLOGICAL PROFILES OF PATIENTS

PARAMETERS	**CASE 1**	**CASE 2**	**CASE 3**	**CASE 4**	**CASE 5**	**CASE 6**
HB (g/dl)	9.5	8.2	7.2	7.3	10.6	7
ROULEAUX	Yes	Yes	Yes	No	Yes	Yes
NRBCS (per 100 WBCs)	Yes	Nil	Nil	Nil	Nil	No
TLC /Ml	4200	2100	5200	800	4100	900
PLATELETS /Ml	1.2 lacs	40,000	1.5 lacs	50,000	1.2 lacs	43,000
BONE MARROW ASPIRATION FINDINGS						
PLASMA CELLS (%)	11	08	12	15	07	07
HEMOPHAGOCYTOSIS	Yes	Yes	Yes	No	Yes	No
LD bodies	Few	Few	Marked	Few	Marked	Marked
rK39	Yes	Yes	Yes	Yes	Yes	Yes

DISCUSSION:

Visceral Leishmaniasis, or Kala-azar (named due to hyperpig mentation of the skin), is a vector borne visceral disease transmitted by Phlebotomus aregentipes and caused by obligate, intracellular protozoa; Leishmania donovani and Leishmania infantum (Leishmania chagasi). Kala-azar is unique being anthroponotic in nature as humans are only reservoir of infection. Kala-azar is a major public health problem with >90% cases are reported from Eastern Bihar in India.[2]

The incubation period is usually 2-6 months with insidious or subacute onset of symptoms in the form of slow progression of malaise, fever, weight loss, and splenomegaly. Asymptomatic infection to clinically manifest disease ratio ranges from >30:1 in Europe to 4:1 in Bangladesh, may be attributable to differences in human genetic patterns, parasite virulence, environmental and nutritional factors. VL is a mimicker of variable disease spectrum ranging from infectious to malignant ones, as noted in the index study. Clinical differential diagnosis of VL includes malaria, histoplasmosis, tuberculosis, multiple myeloma and lymphoma. Splenic size increases gradually depending upon duration and chronicity of the disease, therefore splenomegaly may be absent in initial stages. Hepatomegaly and lymphadenopathy are rare; similar findings have been observed in index study. As the parasite multiplies in the reticuloendothelial system, therefore maximum parasite loads accumulate in the spleen, liver, and bone marrow. VL commonly reveals normochromic anemia, leukopenia, thrombocytopenia, rouleaux formation; similar findings of anemia and thrombocytopenia in all six cases, pancytopenia and leukopenia in three cases were observed in the present study. Severe anemia can be attributable to bone marrow suppression, hemolysis, and splenic sequestration.

VL may lead to immunosuppression that further increases the risk for secondary bacterial infections. In our study one of the patient developed secondary pneumonia. Similarly another study from Brazil on VL, observed that 60 percent patients developed bacterial infections.[6]

VL may cause mild renal impairment which is reversible with appropriate treatment.[7] In our study also; one patient had acute kidney injury, but after initiation of liposomal Injection Amphotericin, the renal function tests became normal in next 10 days. Pathophysiology of renal injury is probably attributed to immune-complex interstitial nephritis, though in an animal study, it was suggested due to cellular inflammatory responses.[8] VL is lethal in most cases if treatment is not initiated.[3] Timely diagnosis on bone marrow aspirates followed by prompt treatment led to well recovery in all our patients.

VL is also associated with systemic illness in the form of Hemophagocytic Lymphohistiocytosis (HLH).[9-11] In our study we found HLH in 5 out of 7 cases. Clinically HLH and VL are indistinguishable, as they share similar symptoms including fever, hepatosplenomegaly, and pancytopenia. It is important to look for LD bodies in diagnosed cases of HLH as most of patients with HLH secondary to VL respond to antileishmanial treatment alone if detected early; otherwise delayed diagnosis also requires adjunctive therapy.[10] Hemorrhagic and neurological manifestations were not observed in any of our patients.[12,13]

Diagnostic tests include Napier's aldehyde test, indirect fluorescent antibody tests, indirect hemagglutination test, enzyme linked immunosorbent assays (ELISA), PCR-ELISA and direct agglutination test. As a positive serological test is not definitive proof of active VL, therefore such results must be interpreted in the context of clinical and epidemiological information. The parasite can be detected through direct evidence from peripheral blood, bone marrow aspirate (60-85% sensitive), liver and splenic aspirates.[14] The most sensitive method is splenic aspirates (95% sensitivity), however is associated with high risk of potentially life-threatening hemorrhage.[14] Cytomorphological differentials of LD bodies include Cryptococcus and Histoplasma, their features have been described/compared. [Table 4] In Wright-Giemsa stained preparations, the amastigote cytoplasm appears blue with a relatively large, eccentrically located red nucleus. Amastigotes have a distinct, rod-shaped, red-staining structure called kinetoplast, a specialized mitochondrial structure that contains substantial amount of extranuclear DNA arranged as catenated minicircles and maxicircles. Visualization of the kinetoplast under oil-immersion microscopy confirms the diagnosis of leishmaniasis.

In vivo culture of the tissue on Novy-MacNeal-Nicolle (NNN) medium can be used to identify the difficult Leishmania species. Immuno-flourescent antibody test (IFAT), in which parasite antigen labeled with fluorescent dye is conjugated with serum antibodies and seen under fluorescent microscope has also been widely used. Montenegro skin test (leishmanin skin test) for delayed type hypersensitivity or T cell mediated immunity specific to leishmaniasis can be performed, has limited role, being negative in acute cases and positive in cured ones.

rK39 is a simple, rapid nitrocellulose dipstick test based on the recombinant kinesin antigen (rK39; a 39-aminoacids cloned in Escherichia Coli). K39 is an epitope apparently conserved on amastigotes of *Leishmania* species that cause visceral infection. The recombinant kinesin antigen (rK39) is a useful antigen in ELISA assays as well as in immunochromatographic strip format as a rapid test. This is highly sensitive, specific and predictive of onset of acute illness. In the Indian subcontinent, sensitivity of 92.8 to 100% and specificity of 96% has been reported.[15] In our study all the cases showed rk39 protein positivity, thus confirming its 100% sensitivity. In Post-Kala-azar Dermal Leishmaniasis (PKDL), the sensitivity of rK39 was also observed nearly 100%. This test is comparable to parasitology in terms of their sensitivity and can replace parasitology as the basis for a decision to treat visceral Leishmaniasis at peripheral health centers in endemic '*Kala-azar* areas. Other immunodiagnostic serological tests alongwith have been mentioned in Table 3.[14]

Table 3: IMMUNODIAGNOSTIC SEROLOGICAL TESTS FOR LEISHMANIASIS

Test	Principle	Sensitivity %	Specificity %	Limitations	References
Napier's aldehyde test	Jellification of serum globulins to the formaldehyde	34.7-74.6	Nonspecific	False positive in tuberculosis and liver cirrhosis Becomes positive after 3 months of infection till after 6 months of cure	Parvin R, Rahman ME, Islam MN, Uddin SN, Khaleque MA, Choudhury AM. Diagnostic efficacy of Aldehyde test in late cases of Kala-azar. Mymensingh Med J. 2007 16:160-4.
Indirect hemagglutination test (IHA)	Antigen-antibody reaction	47-100	100%	Not available in remote areas	17
Enzyme linked immunosorbent assay (ELISA)	Soluble antigen of promastigotes binds with antibodies	80-100	72-95%	Not available in remote areas	17
Direct agglutination test (DAT)	Antigen-antibody reaction	91-100	72-100		17
PCR-ELISA test	Uses primer	75	90-97	Not available in remote areas	17
rK39	Nitrocellulose dip stick	92.8-100	96		15

Clinical differential diagnosis of VL includes malaria, histoplasmosis, tuberculsosis, and lymphoma. Cytomorphological differentials of LD bodies include Cryptococcus and Histoplasma, their features have been described/compared. In Wright- and Giemsa-stained preparations, the amastigote cytoplasm appears blue with a relatively large, eccentrically located red nucleus. Amastigotes have a distinct, rod-shaped, red-staining structure called kinetoplast, a specialized mitochondrial structure that contains substantial amount of extranuclear DNA arranged as catenated minicircles and maxicircles. Visualization of the kinetoplast under oil-immersion microscopy confirms the diagnosis of leishmaniasis. [Table 4] [Figure 1]

Table 4: Cytomorphological Diffrential Diagnosis Of Ld Bodies

Features	Histoplasmosis	Cryptococcosis	LD bodies (Amastigote form)
Size	5-6 µm	15-20 µm	3-4 width µm and 4-5 µm length
Shape	Oval	Oval	Round to oval
Morphology	Budding, cup-saucer forms, intra and extracellular	Budding, narrow based, extracellular forms	No budding, both intra & extra cellular forms. Kinetoplast and nucleus present
Capsule	Pseudocapsule	True capsule	No capsule
Budding	Present	Present	Absent
Special stains	Gomari Methamine Silver, Periodic Acid Schiff	Gomari Methamine Silver, Periodic Acid Schiff	-

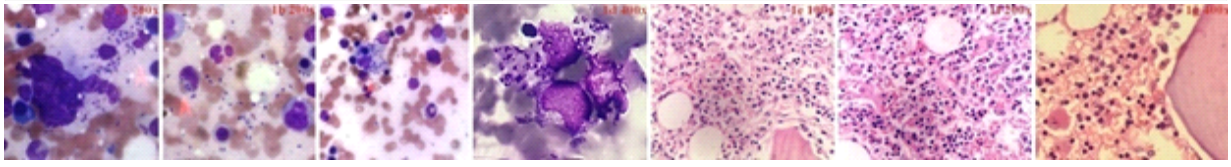

Figure 1; Microphotographs of MGG stained bone marrow aspirates 200x show numerous extracellular (a) and intracellular (b) amastigote forms of Leishmania spp Each amastigote form comprises of bluish cytoplasm, a large eccentric nucleus and distinct rod shaped kinetoplast, (c,d) Hemophagocytosis in the form of engulfment of mononuclear inflammatory cells by the macrophages 200x (c) and 400x (d), (e, f, g) Hematoxylin & Eosin stained bone marrow biopsy at 100x (e), 200x (f) and 400x (g) exhibiting numerous intra/extracellular amastigote in the histiocytes.

Since patients diagnosed to have VL requires parenteral treatment and strict monitoring especially in the settings of HLH and organ failure; their hospitalization becomes essential. Currently recommended drugs for treatment of VL are pentavalent antimonial and liposomal Amphotericin B. While antimony was considered to be the first choice for VL, there is increased cases of resistance have been demonstrated in countries like Sudan, Kenya, and India. In our cases, all patients responded well to liposomal amphotericin B with dosage of 3mg/kg 1-5, 14 and 21 days and were discharged with normal counts and in afebrile condition. In view of variable clinical presentation, diagnosis of visceral leishmaniasis may be challenging therefore an early investigation and appropriate management is the key of patient survival.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Ethical clearance: The present study is in compliance with Ethical Standards.

Funding resources: No funding was obtained from any external source.

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