Original Article

Visceral Leishmaniasis – A study of 42 cases

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Background: Visceral leishmaniasis (VL) is prevalent in Azad Kashmir and some areas of Northern Pakistan. Many of these patients seek medical treatment in hospitals of Rawalpindi-Islamabad, and majority of them present in advanced stage of the disease.

Objective: To document the clinical and laboratory presentation of visceral leishmaniasis in patients presenting from the northern areas of Pakistan

Study Design: Retrospective Chart Review

Place and Duration: Department of Pathology, Holy Family Hospital, Rawalpindi from Jan 2002 to December 2007

Materials and Methods: 42 biopsy diagnosed patients with VL out of 1165 bone marrow biopsies done were studied on clinical and laboratory parameters.

Results: It was found that most of the patients (2/3rd) belonged to Azad Kashmir, rest of patients belonged to Murree. Sign and symptoms included fever (95.2%), Nausea/vomiting/diarrhea (66.7%), Weight loss (33%), Pallor (95.2%), hepatomegaly (85.5%), splenomegaly (81%), and enlarged Lymph nodes (50%). Regarding hematological tests done, mean TLC was found to be (4.3%) with range from 1.4-7.8. Mean Neutrophil count was (24.5%), Lymphocytes (69.9%), monocytes (4.0%), eosinophils (0.52%), blast cells (0.86) and Platelets (55 x (103/ul). Average hemoglobin was found to be 6.57g.dl (range 3.4 – 9.9), RBC count (3.2 x 109/L), PCV (21.8), MCV (68.1 fl), MCH (26.7 pg), MCHC (27.4). Anisocytosis (95.2%) with most common distribution in + (38.1%) and ++ (52.4%) followed by poikilocytes (95.2%) with majority (61.9%) reported as "+". Microcytosis in (91%), hypochromia (90.2%), and dimorphic Blood picture (33.3%). In bone marrow examination, aspirate was found Hyper cellular in (86.7%). Myeloid precursors were immature in (57.5%). No blast or fungi were seen (0%).There were increased hemophagocytic and histiocytic cells (72.2%).

Conclusion: Patient from northern areas with varied symptomatology of fever, NVD, anemia and hepatosplenomegaly should be suspected of VL for early detection of disease and to adopt treatment options.

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Introduction

Visceral Leishmaniasis (VL), also known as kala-azar and black fever, is the most severe form of leishmaniasis. This disease is caused by Leishmania donovanii, a protozoa transmitted by the bite of the sandfly, Phlebotomus. The insect vector of Leishmania is found throughout the world's inter-tropical and temperate regions.[1] It is estimated that there are 500,000 new cases of symptomatic VL each year, resulting in up to 5000 deaths annually. More than 90 percent of the cases are reported from Bangladesh, India, Sudan and Brazil, where there have been epidemics over the past 10 years.

Visceral leishmaniasis is characterized by irregular bouts of fever, substantial weight loss, enlargement of the spleen and liver, and anemia (occasionally severe). If left untreated, the fatality rate in developing countries can be as high as 100% within 2 years.[1] The peak age for developing symptoms from VL varies in different geographic regions. Laboratory findings in visceral leishmaniasis (VL) include pancytopenia and hypergammaglobulinemia. Anemia, neutropenia, and thrombocytopenia are associated with a variable degree of splenomegaly.[2]

Visceral leishmaniasis is endemic in Azad Kashmir and in some areas of NWFP. It is found in population in asymptomatic state but often diagnosed in very advanced stage when it has already spread to liver, spleen or bone marrow, etc. This study was conducted in an urban area of Pakistan where patients are referred from different parts of the country including Azad Kashmir, hilly areas around Rawalpindi and even NWFP.

Materials and Methods

This retrospective study was performed in the department of Pathology, Holy Family Hospital Rawalpindi. The record of a total of 1165 patients who underwent bone marrow biopsy from the year 2002 to 2007 was looked into, and 42 patients were found to having been diagnosed as "Visceral Leishmaniasis". All these cases were included in the study. The patients who yielded a dry tap on marrow aspiration and those who had non diagnostic marrow were excluded from the study.

Information relating to their demographic data, residence, symptoms, signs, blood parameters viz complete blood picture, white cell count, differential leucocyte count, red cell indices and bone marrow biopsy findings was recorded in a proforma. The data were analyzed using SPSS version 16.

Results

In a total of 1165 patients who underwent bone marrow biopsy during the period of study, 42 were found to have visceral leishmaniasis. Age of patients ranged from 0.6-11years with mean $\pm$ SD of 2.57 $\pm$ 2.51years. Twenty eight patients were females, and 14 males, with a female: male ratio of 2: 1. The patients belonged to rural areas of Azad Kashmir (74%) and Murree (26%). Not even a single case was reported from urban areas.

Table I: Clinical Symptoms (n=42)

Symptom	Number (%)
Fatigue	8 (19)
Nausea, vomiting, Diarrhea	28 (66.7)
Bleeding	6 (14.3)
Fever	40 (95.2)
Weight loss	12 (28.6)
Abd. Pain	8 (19)
Bruising	2 (4.8)

The commonest presenting feature was fever (95.2%) followed by nausea, vomiting and diarrhea (66.7%).

Table II: Clinical Signs (n=42)

Pallor	40(95.2)
Spleen (palpable)	34(81%)
Petechiae	4(9.5%)
Palpable lymph nodes	22(52.4)
Liver palpable/ enlarged	36(85.5%)

Weight loss was reported in one third of patients. Fatigue, abdominal pain and bleeding were reported in a very small percentage of cases. Amongst the clinical signs, pallor was the most consistent (95.2%), followed by hepatomegaly (85.5%) and splenomegaly (81%), respectively (Table II).

As shown in table 1, Lymph nodes were enlarged in about half of the patients (52.4%); the distribution of lymphadenopathy is shown in table 3.

Table III: Distribution of Lymph node involvement (n=42)

Lymph node group	Number (%)
Axillary nodes only	4 (18)
Cervical nodes only	12 (54.5)
Inguinal nodes only	-
Generalized lymphadenopathy	6 (27.3)

Various hematological parameters including white cell count, differential leucocyte count, red cell indices etc have been detailed in table 4 and 5.

Table IV: Hematological Parameters (n=42)

	Range	Mean
Hemoglobin g/dl	3.4-9.9	6.57
RBC count $x10^9$/l	1.9-4.2	3.2
PCV	12.2-27.4	21.8
MCV fl	47.3-92.1	68.1
MCH pg	13.2-36.2	26.7
MCHC	19.9-32.4	27.4

Bone marrow findings:- Bone marrow was found hypercellular in most of the cases (85.7%) with erythroid series as mostly hypercellular (85.7%) and myeloid series was found hypercellular in 2/3rd of cases with rest either normo or hypocellular. Iron was absent in almost half of cases (52.4%). Plasma cells were found slightly increased in 57.1% of cases. Hemophagocytic cells and histiocytes were found in (72.7%) of cases.

Table V: Hematological parameters (n=42)

Haematological parameters	Range	Mean
TLC x 10^3/ul	1.4-7.80	4.3
Neutrophils %	9-67.2	24.5
Lymphocyte%	28.7-85	69.9
Monocytes	1-16	4.0
Eosinophils %	0-2.0	0.52
Basophils %	0-0	0
Metamyelocyts	0-0	0.14
Platelets x 10^3/ul	5.0-95	55.28

Discussion

Although a rare disease, visceral leishmaniasis can be found in increased number of patients from northern areas of Pakistan.

In our study, clinical features of VL were diverse. Fever, nausea, vomiting and diarrhea, weight loss being most common symptoms can be easily conceived for a different disease by general practitioner in these remote areas. Also, pallor, hepatosplenomegaly and lymphadenopathy can point to many diagnostic possibilities. These diverse set of symptoms along with lack of proper diagnostic modalities available in these remote areas delay the diagnosis.

Patients were found anemic which ranged from mild to severe anemia. Mean values of all red cell parameters were found to be low. Few of the patients were neutropenic, with mild to severe thrombocytopenia was noticed in almost all the patients. Studies show that laboratory findings in visceral leishmaniasis include pancytopenia and hypergammaglobulinemia. Anemia, neutropenia, and thrombocytopenia are associated with massive splenomegaly.[2] Anemia might be from combination of certain factors including hemolysis, hypersplenism, blood loss and bone marrow suppression. Eosinopenia and neutropenia was also noted in most of the patients, with few showing neutrophils and eosinophils within normal limits.

Based on clinical features and hematological studies done on these patients, it was found that, most of patients presented to our hospital when disease was fairly advanced. As mentioned earlier this is in part due to diversity of clinical features of this disease and in part due to lack of knowledge of practitioner and lack of diagnostic modalities available.

This signifies the importance of measures that need to be taken in the region like methods of controlling and preventing disease and early detection of disease once it occurs. Treatment has to be started early to prevent the lethality of the disease.

Diagnosis of Cutaneous Leishmaniasis involves skin scrapings / aspirate but most commonly used specimen for visceral leishmaniasis is bone marrow aspirate. The gold standard for diagnosis is visualization of the parasite in splenic aspirate or bone marrow aspirate. This is a technically challenging procedure that is usually unavailable in areas of the world where visceral leishmaniasis is endemic, like northern areas of Pakistan. Although splenic aspirate is more specific but it is associated with risk of splenic rupture. In one study it was seen that among 87 patients with VL, the sensitivity of bone marrow aspirates was 40.2%, 65.5%, 89.7%, 92%, and 95.4% at 1, 5, 20, 30, and 60 minutes, respectively. The sensitivity of spleen aspirate examination was 93% for 114 patients.[3,4] In another study bone marrow biopsies have been reported to have approximately 60 to 80 percent sensitivity. [5]

Other tests done to diagnose VL include DAT freeze-dried antigen, antigen detection in urine (of special interest in immunosuppressed VL patients) and dipsticks K39/K26.The dipstick K39 for serological diagnosis was successfully used in Ethiopia, India, Nepal, and Sudan. 1 Serological testing is much more frequently used in areas where leishmaniasis is endemic. The K39 dipstick test is easy to perform, and village health workers can be easily trained to use it. Latex agglutination test (KAtex) is currently being tested in Asia and Africa. Another potential test that detects leishmania is erythrosalicylic acid [6].

There is no vaccine to prevent the acquisition of infection, although attempts are in progress [7]. A number of other approaches have been tried to control of leishmaniasis. These include vector control with insecticides or by reducing breeding sites; control of animal reservoirs; personal protective measures against sandfly bites; and early diagnosis and treatment of cases. However, each of these approaches has limitations[8]. The use of residual insecticides is limited by cost, environmental concern and potential development of resistance among sand flies [9].

In our study all the patients belonged to pediatric group. In another study done in same region similar thing was observed[10].

Conclusion

VL is common in northern areas of Pakistan. VL can manifest itself with symptoms which are fairly common for other diseases. Lack of proper equipment and personnel of diagnosis leads to delay in detection and treatment. That is why most patients present in advanced stage of disease. Patients from these areas with varied symptomolgy of fever, NVD, anemia and hepatosplenomegaly should be suspected of VL and referred for early detection of disease to the centers where facilities for its diagnosis and treatment are available.

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