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Research Article

Hematological Profile of Newly Diagnosed Patients with Visceral Leishmaniasis from a Non-Endemic Hilly Region of India

Sujeet Raina[1]* and Rashmi Kaul Raina[2]

[1]Department of Medicine, Dr. Rajendra Prasad Government Medical College, India
[2]Department of Pathology, Dr. Rajendra Prasad Government Medical College, India

*Corresponding author

Sujeet Raina, C-15, Type-5 Residences, Dr. R.P. Government Medical College Campus, Tanda Kangra (HP), 176001, India, Tel: 91-9418470708; Fax: 01892-267115; Email: sujeetrahmishera@yahoo.co.in

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Keywords

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Abstract

Aim: Visceral Leishmaniasis (VL) may lead to variable haematological responses like cytopenias, hemophagocytosis, dyserythropoiesis and increase in plasma cells. The purpose of this study was to investigate the hematological manifestation of VL and associated changes that may be observed in bone marrow aspirate smears.

Materials and methods: A 12-year hospital based cross section observational study was conducted in the Department of Medicine and Pathology in two tertiary care centres of Himachal Pradesh, located in the Northern India. In all 18 patients who were diagnosed as visceral leishmaniasis on the basis of demonstration of amastigotes, Leishman Donovan (LD) bodies in bone marrow aspiration smears were included in the study. Detail clinical history, examination and investigation were carried in each case as per protocol.

Results: Anemia was present in all the cases. Pancytopenia was observed in 61.1% of cases. Normocytic normochromic and dimorphic picture was the most common finding on peripheral smear. Bone marrow was normocellular in 83.3% of cases. Erythropoiesis was normoblastic in 33.3% of patients. Increased plasma cells, lymphocytes and histiocytes were seen in 50%, 33.3% and 22.2% of cases respectively.

Conclusion: In non-endemic region where clinical suspicion is low, bone marrow findings can be a strong indicator for VL and pathologist should look for LD bodies in the bone marrow to prevent the unnecessary delay in diagnosis.

INTRODUCTION

Although Visceral Leishmaniasis (VL) is endemic in various parts of India, mainly Bihar, West Bengal, eastern Uttar Pradesh, and neighboring countries such as Nepal and Bangladesh, it has been rarely reported from the hilly areas of India [1-3]. Himachal Pradesh is a small hilly state in India (30^0-33^0N, 70^0-75^0E) situated in the northwestern Himalayas. The topography is mostly mountainous and a number of snow fed perennial rivers namely Chenab, Ravi, Beas, Sutlej and their tributaries drain the region [1,2]. Visceral leishmaniasis was not known to occur in this state but a few sporadic cases were detected after 1979 [1-3]. However, details of hematological profile of such patients are insufficient from this region. Therefore, this study was planned and conducted in two tertiary care hospitals providing health services in Himachal Pradesh, India. Since the disease is rare in this region, initial failure of recognizing it causes a diagnostic delay.

MATERIALS AND METHODS

This is a hospital based cross sectional observational study. Eighteen cases above the age of 18 years were included. All the cases were diagnosed between 2004 and 2015 by demonstration of amastigotes, Leishman-donovan (LD) bodies in bone marrow aspiration smears. The study has been conducted in Indira Gandhi Medical College, Shimla and Dr. Rajendra Prasad Govt. Medical College, Kangra the two tertiary care teaching hospitals of Himachal Pradesh located in northwestern Himalayas. Detailed information was collected on the clinical presentation, examination findings and epidemiological features in all the patients. Details of travel and places of residence were recorded in all the patients. All the patients had a baseline hemogram including peripheral blood film. Detailed examination of bone marrow aspiration smears was done. The diagnosis of anemia and assessment of severity was based on WHO criteria [4]. Leucopenia was defined as total leucocyte count below 4000/cmm and thrombocytopenia as platelets count below

1,50,000/cmm. Current cut-offs were used for the diagnosis of other hematological abnormalities. The study was approved by the institutional ethics committee. The data, thus collected, was analyzed on Microsoft Excel sheet 2010 and percentages were calculated.

RESULTS

All the patients were original natives of the state and none of the patients had ever visited known endemic area for VL. Out of eighteen patients, fourteen were males and four were females. The patients were in the age range of 18-65 years with the median age of 33 years. Ten patients lived in the higher alpine zone of the Lesser Himalayan and Trans Himalayan region along the Satluj River and include the tribal district of Kinnaur and adjacent area of Shimla and Kullu districts. Six patients were native of Ravi river valley area of Chamba district which is bounded by the Dhauladhar and Zanskar ranges, south of inner Himalayas. Two patients belonged to Beas river valley area of Kullu and Mandi districts (Figure 1). The altitude of these areas ranges between 924 and 2,960 meters above mean sea level. The temperatures remain low throughout the year with subzero temperatures during the winter months.

All the patients reported fever. The duration of illness varied from three weeks to one year with the median period being three months. Five patients had epistaxis. Splenomegaly was found in all the cases and massive splenomegaly was noted in 15 cases. Hepatomegaly was detected in eleven patients. Lymphadenopathy was seen in eight cases. Significant axillary lymphadenopathy was noted in seven patients, cervical lymphadenopathy in three and retroperitoneal in four patients. LD bodies were isolated on aspiration cytology from lymphnodes in two patients and on splenic aspirate in one. Lymphnode aspiration was performed in all the eight cases with lymphadenopathy and splenic aspiration was done in only one patient. One of the patients had jaundice and none had hyper pigmentation though all were fair skinned.

All the patients were anemic. The details of abnormalities of blood parameters and peripheral smear morphology are shown in Table (1). Very high erythrocyte sedimentation rate (> 100 mm in 1st hr) was observed in 38.8% of patients. The myeloid: erythroid (M:E) ratio was normal in fourteen patients and four had erythroid hyperplasia. Bone marrow myelogram revealed normal neutrophilic series and megakaryocytic in all the patients. Bone marrow aspiration findings are shown in Table (2). Six patients died during the treatment in hospital. The causes of deaths were possibly either due to the side effects of chemotherapeutic agents or disease processes itself or both. The comparative results of hematological parameters and bone marrow findings from other non endemic regions of India have been shown in Table (3) and Table (4) respectively.

DISCUSSION

Visceral leishmaniasis (VL) is focally endemic in four eastern states of India, Bihar, West Bengal, Jharkhand and eastern Uttar Pradesh. Sporadic cases have been reported from natives of non endemic sub-Himalayan region of Uttarkhand and Himachal Pradesh [1-3,5-8]. Survey of phlebotomid sandfly from the Himalayan region has established presence of *Phlebotomus longiductus* and *Phlebotomus major* in Himachal Pradesh and *Phlebotomus argentipes* in Uttarkhand region [9-11]. Presence of the strains of *Leishmania donovani* and *Leishmania tropica* has also been established in the state of Himachal Pradesh [12]. We diagnosed 18 cases of VL from 2004 to 2015 and report in this study. The clinical presentation was classical in majority of cases with fever, pallor and splenomegaly present in all the cases.

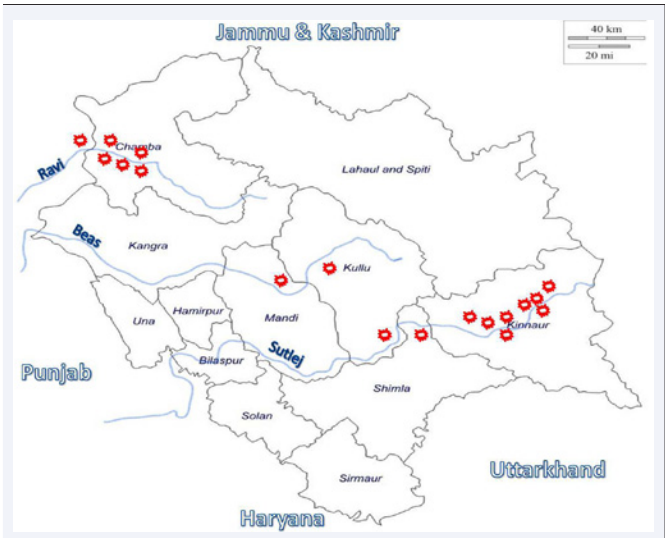

Figure 1 Map of the state showing geographic distribution of patients (red spots) from various districts.

Table 1: Hematological findings of patients.

Parameter	Number of cases (%)
Anemia	
Mild	1(5.5)
Moderate	2(11.1)
Severe	15(83.3)
RBC Morphology	
Normocytic normochromic	7(38.8)
Dimorphic	7(38.8)
Macrocytic	3(16.6)
Microcytic hypochromic	1(5.5)
Leucopenia	12(66.6)
Thrombocytopenia	11(61.1)
Lymphocytosis	4(22.2)
Monocytosis	29(11.1)
Pancytopenia	11(61.1)
Bicytopenia	12(66.6)

Hematological changes are generally determined by the duration of the disease, degree of parasitization and the grade of splenomegaly [13,14]. Anemia was present in all the patients and is a universal feature. Normocytic normochromic and dimorphic picture was the leading RBC morphology observed. Dimorphic blood picture was the leading finding in 61.1% on peripheral smear examination by Chakrabarti et al, [15]. The mechanism of anemia is hypersplenism, haemolysis, and utilization of erythrocyte enzymes, increased sensitivity to complement and nutritional deficiencies of folate, vitamin B12 and iron [13]. Leucopenia, thrombocytopenia, bicytopenia and pancytopenia were observed in two thirds of our patients. Varying degree of frequency and severity has been reported by several groups of workers from both endemic as well as non endemic areas of

Table 2: Bone marrow findings of patients.

Bone marrow findings	Number of cases (%)
Cellularity	
Normocellular	15(83.3)
Hypocellular	3(16.6)
Hypercellular	0
Erythropoiesis	
Normoblastic	6(33.3)
Micronormoblastic with normoblastic	6(33.3)
Megaloblastic	3(16.6)
Normoblastic with megaloblastic	2(11.1)
Micronormoblastic	1(5.5)
Dyserythropoiesis	2(11.1)
Hemophagocytosis	2(11.1)
Increased Plasma cells	9(50)
Increased lymphocytes	6(33.3)
Increased macrophages	4(22.2)

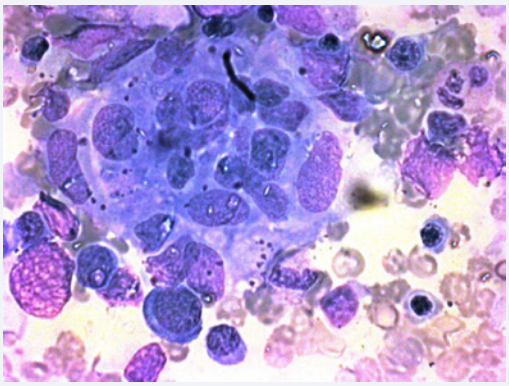

Figure 2 Photomicrgraph showing hemophagocytosis and intracellular Leishman Donovan (LD) bodies. (Giemsa, X 1000).

India [7,14-19]. Higher prevalence has been reported in other series [18-20]. Though none of these studies have mentioned cut off values in their methodologies so these differences are possibly due to variable reference values used for defining hematological abnormalities. Hypersplenism is the main reason for various cytopenias. Duration of illness is a determinant for the variable frequencies of different cytopenias [13]. Associated with neutropenia is relative lymphocytosis.

In bone marrow the cellularity was predominantly normocellular. Hypercelularity has been regularly reported though we did not observe in our patients. Similar finding has been observed by Agrawal et al from a study in Nepal [21]. In a series from Uttarkahand all the patients observed had hypocellular marrow [7].

Erythroid hyperplasia was observed in 22.2% of cases which otherwise is a common finding. Erythropoiesis observed was dimorphic reaction (micronormoblastic with normoblastic and normoblastic with megaloblastic), normoblastic, micronormoblastic as well as megaloblastosis. Dyserthropoiesis with megaloblastic change was noted in two patients. Similar

Table 3: Comparison of hematological parameters from other non endemic regions of India.

Parameter study	Sud et al, [18].	Chandra et al, [19].	Chufal et al, [20].	Present study
Study area	Chandi-garh	Dehradun Uttarakhand	Kumaon Uttarakhand	Himachal Pradesh
Number of patients	57	27	20	18
Anemia(%)	95	100	100	100
RBC Morphology(%)				
Normocytic normochromic	-	-	35	38.8
Dimorphic	-	-	-	38.8
Macrocytic	-	-	10	16.6
Microcytic hypochromic	-	-	55	5.5
Leucopenia(%)	86	96.2	100	66.6
Thrombocytope-nia(%)	75	96.2	85	61.1
Lymphocytosis (%)	-	-	100	22.2
Monocytosis (%)	-	18.5		11.1
Pancytopenia(%)	57.9	96.2	85	61.1
Bicytopenia(%)	30	-	-	66.6

Table 4: Comparison of bone marrow findings from other non endemic regions of India.

Bone marrow findings	Sud et al, [18].	Chandra et al, [19].	Chufal et al, [22].	Present study
Cellularity(%)				
Normocellular	51	-	35	83.3
Hypocellular	-	-	15	16.6
Hypercellular	49	26	35	0
Erythroid hyperplasia	80.7	81.4	-	22.2
Erythropoiesis(%)				
Normoblastic	-	-	-	33.3
Micronormoblastic with normoblastic	-	-	-	33.3
Megaloblastic	91.2	-	10	16.6
Normoblastic with megaloblastic	-	-	-	11.1
Micronormoblastic	-	-	55	5.5
Dyserythropoiesis(%)	24.5	-	10	11.1
Hemophagocytosis(%)	-	70.3	-	11.1
Increased Plasma cells(%)	-	96.2	70	50
Increased lymphocytes(%)	-	-	70	33.3
Increased macrophages(%)	-	100	70	22.2

findings have been reported by Dhingra et al and Chufal et al [20,17]. Erythropoiesis is variable in different studies and one type predominates over the other. Variable degree of megaloblastosis has been reported in 91% of patients by Sud et al. [18].

Moderate to severe degree of megaloblastosis was observed in 50% patients by Merwaha et al. [14].

Agrawal et al., reported megaloblastic changes in greater than 50% of cases [21]. It was reported in 27.8% of cases by Chakrabarti et al, [15]. The erythropoiesis is determined by the chronic inflammatory state, nutritional status and associated deficiency [13]. Hemophagocytosis was seen in two of our cases (Figure 2). It has been reported as a complication of VL in various other reports [17,19,22].

It is emphasized that in patients diagnosed with hemophagocytic syndrome, a careful search for the etiologic agent including LD bodies should be performed [13].

Plasma cells were increased in 50% patients and are similar to reports published [14,17,20, 21].

CONCLUSION

In nonendemic areas, a high index of suspicion is required and careful search for LD bodies is warranted particularly with low parasitic load. Since the area is not an endemic area, even the classical clinical picture is always confused with more commonly occurring diseases and without the suspicion of VL there is a high chance of missing LD bodies on bone marrow examination. It is therefore important that with the bone marrow findings like hemophagocytic syndrome or increased plasma cells with or without erythroid hyperplasia, the pathologist should look for LD bodies in the bone marrow to prevent the unnecessary delay in diagnosis of cases of VL from nonendemic areas.

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