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Original Article

Pathology Section

Role of Haematological Changes in Predicting Occurrence of Leishmaniasis- A Study in Kumaon Region of Uttarakhand

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ABSTRACT

Introduction: A number of cases of Leishmaniasis have been reported from non-endemic sub-himalayan regions of India. Due to low clinical suspicion and atypical presentation, cases may go undetected or there may be a delay in diagnosis.

Aim: The aim of the study was to evaluate clinico-haematological parameters and bone marrow findings so that a high degree of suspicion could be made in unsuspected cases of Visceral Leishmaniasis (VL) and Leishman Donovan (LD) body negative bone marrow smears.

Materials and Methods: A retrospective study was conducted at a tertiary care centre serving the kumaon region of Uttarakhand from 2010 to 2014. Forty bone marrow aspirates were included, which were sent on clinical suspicion of VL. Twenty cases were positive for LD bodies. Their clinico-haematological features including bone marrow findings were studied in detail and compared with rest of the 20 LD negative cases. Five LD negative cases were also positive for rk39.

Results: Twenty LD positive cases were evaluated. Splenomegaly was the most common sign present in 17 cases (85%). Anaemia, leucopenia and lymphocytosis were present in all the cases (100%). Pancytopenia was seen in 17 cases (85%). Microcytic hypochromic blood picture was the most common finding in 11 cases (55%). Bone marrow was normocellular in 7 cases (35%), hypercellular in 7 cases (35%). Erythropoesis was micro-normoblastic in 11 cases (55%). Overall, there were 25 cases of VL (20 LD positive, 5 LD negative). Increased plasma cells, lymphocytes and histiocytes were seen in 17 cases (68%) of VL.

Conclusion: In non-endemic region where clinical suspicion is low, bone marrow findings can be a strong indicator for VL even though marrow is negative for LD bodies. If required other ancillary investigations can also be ordered. This study also emphasizes the need for epidemiological work up in this region.

Keywords: Bone marrow, Haematology, Kalazar, Pancytopenia

INTRODUCTION

Leishmaniasis is a group of vector borne diseases transmitted by bite of female phlebotomus sand flies, and occurs in three forms-visceral, cutaneous and mucocutaneous [1,2]. Visceral Leishmaniasis (VL), the most severe form of the disease, is caused by species of obligate intracellular parasite *Leishmania donovani* complex which includes *L.donovani* and *L.infantum*. Around 0.2-0.4 million cases of VL occur globally every year. More than 90% of these cases occur in six countries: India, Bangladesh, Sudan, South Sudan, Ethiopia and Brazil. Leishmaniasis like many other neglected tropical diseases (NTDs) occurs in focal distributions and in remote locations [3]. *L. donovani* is the major cause of VL in India. It is prevalent in India by other names like kala-azar, black fever and Dumdum fever [2]. The disease is a serious problem in eastern regions of India. Majority of the cases in India (90%) occur in the state of Bihar, districts bordering Bihar, in the state of West Bengal and Uttar Pradesh [4]. The Government of India was committed to eliminate and reduce the incidence of VL by 2015 but on the contrary it is reemerging as a major health problem. Various attempts to eliminate it have failed [2]. Recently a number of cases were reported from non-endemic sub-himalayan regions of Uttarakhand [5-7].

Most of the cases reported from this region remain undiagnosed for long periods with prolonged fever [2,6]. Bone marrow aspirates are considered to be simple and safer for the diagnosis of VL [8,9].

AIM

The aim of the study was to evaluate clinico-haematological parameters and bone marrow findings of patients from tertiary care centre serving the kumaon region of Uttarakhand so that a high degree of suspicion could be made in unsuspected cases of leishmaniasis in non-endemic areas.

MATERIALS AND METHODS

A retrospective cross-sectional study was conducted over a five year period from 2010-14 to include 40 bone marrow aspirates which were sent to look for the presence of LD bodies on suspicion of VL. The cases were separated on the basis of presence or absence of LD bodies. There were total 20 cases which were confirmed by the presence of LD Bodies while remaining 20 cases were negative for it. Clinical history and haematological features were reviewed. Bone marrow aspirates were stained with Leishman–Giemsa stain and findings including cellularity, erythropoesis, myelopoesis number of plasma cells, macrophages and histiocytes were recorded by 3 competent pathologists.

RESULTS

Twenty cases were LD positive out of 40 cases. Male to female ratio was 4:1, age ranges between 4 to 81 years with a mean age of 23 years.

Splenomegaly was the most common sign observed in 17 cases (85%), followed by pallor in 15 cases (75%), pyrexia in 14 cases (70%), weight loss in 12 cases (60%), hepatomegaly in 8 cases (40%) and jaundice in 4 cases (20%). Bleeding manifestations were seen in 2 cases (10%). Only one case had lymphadenopathy. Comparison of these clinical features with LD negative (20) cases is shown in [Table/Fig-1].

Biochemical investigations were also reviewed for 20 LD positive cases. There was reversal of Albumin: Globulin ratio in 18 cases, whereas in 2 cases these tests were not ordered. Liver function tests including AST, ALT and Alkaline phosphatase were deranged in 2 cases with patients having features of hepatitis. One patient had features of chronic renal failure with raised serum urea and creatinine.

Among all LD positive cases anaemia and leucopenia with relative lymphocytosis were present in all the cases (100%). Pancytopenia was seen in 17 cases (85%), bicytopenia in 3 cases (15%) while thrombocytopenia was seen in 17 cases (85%). Peripheral blood smear examination revealed microcytic hypochromic (MCHC) blood picture as the most common finding in 11 cases (55%) followed by normocytic normochromic (NCNC) blood picture in 7 cases (35%) and macrocytic blood picture in 2 cases (10%). Similar features were compared with LD negative cases [Table/Fig-2].

Extracellular and intracellular amastigote forms of the parasite (LD Bodies) were found in all the cases. They are small, round to oval, 2-4 micron in size, a nucleus and a small rod shaped kinetoplast appearing as double dots. Number of plasma cells, lymphocytes and histiocytes were increased in 14 cases (70%) [Table/Fig-3]. Antigen testing for rk39 was performed in only 7 cases, all were positive for it.

Bone marrow smears positive for LD bodies were normocellular and hypercellular in 7 cases (35%) each whereas smears were hypocellular in 3 cases (15%). Comment on cellularity was not possible in the remaining 3 cases because smears were hemodluted and there was absence of bone marrow fragments. Erythropoesis was primarily micronormoblastic in 11 cases (55%) while it was normoblastic in 7 cases (35%). Megaloblastic erythropoesis was seen in only 2 cases (10%).

These bone marrow features are compared with LD negative smears in [Table/Fig-4]. The important features noted contrasting with LD positive smears are increase in plasma cells, lymphocytes and histiocytes in only 7 (35%), 6 (30%) and 3 (15%) cases, respectively. Out of these, 3 cases from each of these were actually cases of VL as evidenced by rk39 antigen positivity.

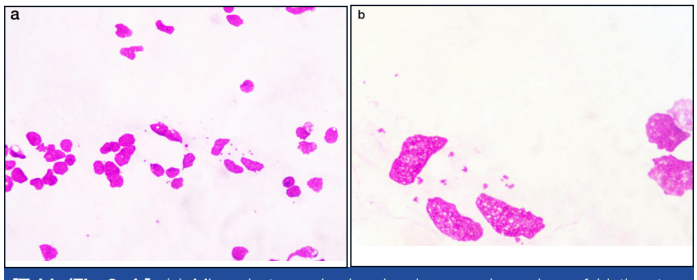
This test was also performed in 8 LD body negative cases, 5 cases were positive for it. So overall there were 25 cases of VL (20 LD positive, 5LD negative). Increased lymphocytes, plasma cells and histiocytes were seen in 17 cases (68%) of VL.

Parameter	LD Positive No.of Cases (%)	LD Negative No. of Cases(%)
Age		
<30 Y	16 (80)	12 (60)
>30 Y	04 (20)	08 (40)
Male : Female Ratio		
MALE	16 (80)	15 (75)
FEMALE	04 (20)	05 (25)
Past History Of Visceral Leishmaniasis		
Present	0	0
Absent	20 (100)	20 (100)
Fever		
Present	14 (70)	17 (85)
Absent	06 (30)	03 (15)
Pallor		
Present	15 (75)	17 (85)
Absent	05 (25)	03 (15)
Spleenomegaly		
Present	17(85)	15 (75)
Absent	03 (15)	05 (25)
Hepatomegaly		
Present	08 (40)	06 (30)
Absent	12 (60)	14 (70)
Jaundice		
Present	04 (20)	0
Absent	16 (80)	20 (100)
Generalized Weakness		
present	15 (75)	18 (90)
Absent	05 (25)	02 (10)
Bleeding Manifestation		
Present	02 (10)	0
Absent	18 (90)	20 (100)
Lymphadenopathy		
Present	01 (5)	0
Absent	19 (95)	20 (100)
Weight Loss		
Present	12 (60)	13 (65)
Absent	08 (40)	07 (35)

[Table/Fig-1]: Clinical findings.

Parameter	LD Positive No.Of Cases(%)	LD Negative No Of Cases(%)
Haemoglobin		
Anaemia Present	20 (100)	20 (100)
Absent	0	0
RBC'S		
Microcytic Hypochromic	11 (55)	05 (25)
Normocytic Normochromic	07 (35)	09 (45)
Macrocytic	02 (10)	05 (25)
Dimorphic	0	01 (5)
WBC'S		
TLC		
Leucopenia		
Present	20 (100)	16 (80)
Absent	0	04 (20)
DLC		
Lymphocytosis	20 (100)	14 (70)
Neutrophilia	0	0
Eosinophilia	0	0
Platelets		
Thrombocytopenia		
Present	17 (85)	18 (90)
Absent	03 (15)	02 (10)
Pancytopenia	17 (85)	14 (70)
Bicytopenia	03 (15)	06 (30)

[Table/Fig-2]: Haematological parameters.



[Table/Fig-3a,b]: (a) Microphotograph showing increased number of histiocytes, lymphocytes with LD bodies (Leishman- Giemsa x100). (b) Higher magnification showing LD bodies appearing as double dots (Leishman- Geimsa x1000).

Parameter	Type	LD Positive No. of Cases(%)	LD Negative No of cases(%)
Cellularity			
	Normocellular	07(35)	07(35)
	Hypercellular	07(35)	04(20)
	Hypocellular	03(15)	07(35)
	Haemodiluted	03(15)	02(10)
Erythropoesis			
	Normoblastic	07(35)	09(45)
	Micronormoblastic	11(55)	06(30)
	Megaloblastic	02(10)	05(25)
Dyserythropoietic Changes	Present	02(10)	04(20)
	Absent	18(90)	16(80)
Plasma Cells	Increased	14(70)	07(35)
	Normal	06(30)	13(65)
Lymphocytes	Increased	14(70)	06(30)
	Normal	06(30)	14(70)
Histocytes and Macrophages	Increased	14(70)	03(15)
	Normal	06(30)	17(85)

[Table/Fig-4]: Bone marrow findings.

DISCUSSION

Kalazar or VL is mostly confined to plains but cases have been regularly reported from the natives of sub-himalayan region of India. The existence of a pocket of disease and vector in Garhwal and Kumaon region of Uttarakhand has already been established [5,6]. There is presence of various species of *Phlebotomus* (*P. argentipes, P. longiductus* and *P. major*) and strains of Leishmania (*L. donovani, L.tropica*) in Himalayan and sub Himalayan regions [7]. Previous study from Kumaon region and same institution reported 10 cases during a period of 6 years (2005-2011) [6]. But from 2010 to 2014, a total of 25 cases of Kalazar were reported in the present study. The increasing incidence of VL in this region can be attributed to climate change due to global warming, forest clearing, colonization and urbanization.

The major signs in our study were splenomegaly (85%) followed by generalized weakness (75%) and pyrexia (70%). Dhingra et al., also reported pyrexia in 70% of the cases in his study of 18 cases with splenomegaly in 100% of cases [10], however studies from other regions showed pyrexia (100%) as the most common finding followed by splenomegaly [11]. Some studies have reported splenomegaly in 100% of cases [7,9,10,12]. Previous study from this region reported fever and hepatosplenomgaly in all the 10 cases [6].

Thus, pyrexia and splenomegaly in our study was absent in 6 (30%) and 3 (15%) cases respectively. In 3 cases, both signs were absent. Such atypical presentation with absence of splenomegaly and fever can result in diagnostic problems [13].

Pallor was seen in 75% of cases similar to the findings by other authors but Dursun et al., Shoaib et al., and Chakrabarti et al., observed pallor in approximately 90 to 95% of cases [9,14,15].

Lymphadenopathy was reported as an unusual characteristic feature in hilly regions in approximately 50% of cases [7,14]. Bleeding manifestations were the least common findings in the range of 10 to 30% from all the studies. Comparison between clinical features from different studies is shown in [Table/Fig-5].

Anaemia, leucopenia and lymphocytosis were the most common haematological features present in all the cases (100%) followed by thrombocytopenia in 17 cases (85%). Research workers from

	Chakraborty et al., [15]	Agarwal et al., [11]	Dhingara et al., [10]	Hamid GA et al., [12]	Dursun et al., [9]	Shoaib et al., [14]	Raina et al., [7]	Previous study [6]	Present study
Location of study	West Bengal	Nepal	Delhi	Yemen	Turkey	Pakistan	Himachal	Kumaon Uttrakhand	Kumaon UttarKhand
No. of cases	36	40	18	64	101	42	18	10	20
Clinical Features									
Fever	100%	100%	70%	100%	99%	95.2%	100%	100%	70%
Pallor	94.4%	75%	72.2%	84.4%	89%	95.2%	75%	-	75%
Spleenomegaly	100%	82.5%	100%	100%	100%	81%	100%	100%	85%
Hepatomegaly	58.3%	65%	44.44%	76.6%	98%	85.5%	61.1%	100%	40%
Bleeding	8.3%	_	22.22%	7.9%	_	14.3%	25.5%	-	10%
Lymphadenopathy	_	_	22.22%	20.3%	_	52.4%	44.4%	-	5%

[Table/Fig-5]: Comparision of various clinical parameters from different studies.

	Chakraborty et al., [15]	Agarwal et al., [11]	Dhingara et al., [10]	Hamid et al., [12]	Dursun et al., [9]	Shoaib et a1., [4]	Raina et al., [7]	Previous study [6]	Present study
Location of study	West Bengal	Nepal	Delhi	Yemen	Turkey	Pakistan	Himanchal	Kumaon Uttarakhand	Kuamon Uttarakhand
No of Cases	36	40	18	64	101	42	18	10	20
Haematological Parameters									
Anaemia	94.4%	90%	94.4%	100%	96%	100%	100%	100%	100%
Leucopenia	61.1%	67.5%	72.2%	67%	74%	100%	38.8%	100%	100%
Thrombocytopenia	83.3%	72.5%	61.1%	93.8%	56%	100%	33.3%	100%	85%
Pancytopenia	58.3%	25%	_	70.3%	33%	_	_	100%	85%
Bicytopenia	41.7%	40%	_	29.7%	_	_	_	_	15%
Lymphocytosis	_	65%	25.5%	_	_	_	44.4%	_	100%
RBC Morphology									
NCNC	_	35%	_	_	_	_	_	100%	35%
MCHC	_	_	_	50%	_	90%	_	_	55%
Macrocytic	8.3%	_	33.3%	20.3%	_	_	_	_	10%
Dimorphic	61.1%	_	_	21.9%	_	33.3%	_	_	_

[Table/Fig-6]: Comparision of haematological paramaters from different regions.

other regions have reported leucopenia and lymphocytosis much lower than our study but anaemia and thrombocytopenia had almost similar findings [Table/Fig-6].

On peripheral blood smear examination, RBCs showed MCHC blood picture in majority of cases (55%) similar to the findings by Hamid et al., [12]. It was a predominant RBC morphology in 90% of 42 cases reported by Shoaib et al., a much higher percentage than other studies [12,14]. The predominance of MCHC blood picture in our cases could be due to undiagnosed long standing infections leading to anaemia of chronic disease. Dimorphic blood picture was common in one study from West Bengal in 61.11% of cases [15], however, Agarwal et al., observed NCNC blood picture and anisocytosis hypochromasia in equal frequency (35%) [11]. NCNC blood picture was seen in 35% of our cases. Macrocytes and microcytes in the form of anisocytosis (25%) was observed by Dash et al., [16]. All the 10 cases (100%) reported from previous study in this region had NCNC blood picture [6].

Pancytopenia is the most common haematological abnormality (85%), similar to the findings by Hamid et al., Uzair et al., (80%) [12,17], but Chakrabarti et al., reported pancytopenia in 58.3% of cases [15]. Agarwal et al., reported bicytopenia (40%) as the most common finding [Table/Fig-6] [11].

Pancytopenia in Kalazar results from splenic sequestration of blood cells as seen in majority of the cases (85%). Three cases in which splenomegaly was absent, could be due to overwhelming infection leading to direct bone marrow suppression as suggested by hypoplastic marrow [18,19].

Bone marrow examination is essential to narrow down the list of exhaustive differential diagnosis of pancytopenia. As most of our patients had pancytopenia or bicytopenia, clinicians and pathologists should also keep in mind the possibility of leishmaniasis while evaluating bone marrow smears. Besides this there is low clinical suspicion for the disease in this non-endemic region due to low prevalence and non specific clinical features [20]. Although clinical presentation may be more or less classical, VL is not the primary diagnosis [2,7,13]. Sometimes patients may have atypical presentation with absence of fever and splenomegaly which is so characteristic of the disease as seen in 3 cases. Such atypical cases and low clinical suspicion can cause diagnostic problems, leading to delayed diagnosis and fatal outcome [13]. There are already reports from Kumaon region of Uttarakhand erroneously diagnosed as opportunistic tubercular infection [2]. So early detection of these cases is necessary to reduce morbidity and mortality associated with complications of the disease. Haematological findings supplemented by other bone marrow features can be a clue to the presence of LD bodies or VL.

LD positive bone marrow smears were normocellular to hypercellular in 14 cases (70%) similar to the findings by Dash et al., and Marwah et al., [16,21]. However, Agarwal et al., reported it in 95% of cases [11]. Hypercellular marrow was also seen in other studies [10,14,15]. But previous study from Kumaon region showed hypocellular marrow in 100% of cases [Table/Fig-7] [6]. Hypercelluar marrow results from induction of GM-CSF and TNF-alpha by stromal macrophages resulting in increased levels of myelopoeisis [18]. Three cases of hypocellular marrow could be the result of direct marrow suppression by the parasite [15,18,19].

Megaloblastic change was observed by Agarwal et al., in greater than 50% of cases [11]. It was reported in 27.8% of cases by Chakrabarti et al., [15]. In our study micro-normoblastic reaction was present in 11 cases (55%), while in only 2 cases (10%) megaloblastic and dyserythropoietic changes were observed similar to the findings by Dhingra et al., (16%) [10]. Megaloblastic change with dyserythropoesis was noted as a prominent finding by Dursun et al. It was used as an indicator of recurrence of Leishmaniasis in 4 cases who were negative for LD bodies. They later on responded to treatment with disappearance of these features [9]. Thus, investigators from other regions had also used other bone marrow features as a clue to the presence of VL in LD negative bone marrow smears.

Plasma cells, lymphocytes and histiocytes were increased in bone marrow smears in 70% of LD positive smears whereas in LD negative smears plasma cells and lymphocytes were increased in 35% and 30% cases respectively. Histiocytes were increased in only 15% of cases. Their number was increased as reported by various other studies [10,14,15]. Histiocytes and macrophages were increased in 50% and 72.7% of cases observed by Dhingra et al., and Shoaib et al., respectively. [10,14]. Previous study from Kumaon region when reviewed revealed increase in all 3 features in 80% of cases [Table/Fig-7].

Among all the 25 cases of VL reported in our study (20 LD positive and 5 LD negative), there was increase in lymphocytes, plasma cells and histiocytes in 17 cases (68%).

LIMITATION

This study is limited by the fact that the number of cases were less, as compared to endemic regions; LD positivity and cellularity were affected by haemodiluted smears in some cases. Besides this rk39 antigen testing could not be performed in all the LD negative bone marrow smears.

CONCLUSION

Thus, in this non-endemic region where clinical suspicion is low, these bone marrow findings can be a strong indicator for leishmaniasis in patients having pyrexia of unknown origin and pancytopenia as well as in patients with atypical presentation. Smears should be thoroughly searched for LD bodies even if haemodiluted or hypocellular. On the other hand the sensitivity of bone marrow for detecting LD bodies is 60-80%. So, if LD bodies are absent, based on associated bone marrow findings

Bone marrow Features	Chakraborty et al., [15]	Agarwal et al., [11]	Dhigra et al., [10]	Shoaib et al., [14]	Previous study [6]	Present Study
Location of Study	West Bengal	Nepal	Delhi	Yemen	Kumaon Uttarakhand	Kumaon Uttarakhand
No of cases	36	40	18	42	10	20
Cellularity						
Hypercellular	63.9%	_	77.77%	87.5%	-	35%
Normocellular	33.33%	92.5%	_	_	-	35%
Hypocellular	_	7.5%	_	_	100%	15%
Megaloblastic Changes	27.8%	55%	16%	_	-	10%
Dyserythropoeitic changes	27.8%	40%	22.22%	_	-	10%
Increased plasma cells	↑	60%	72.22%	57%	80%	70%
Increased Lymphocytes	_	_	72.22%	_	80%	70%
Increased Histocytes	_	-	50%	72.7%	80%	70%

[Table/Fig-7]: Comparision of various bone marrow features from different studies.

and haematological parameters, other diagnostic serological tests can be offered to the patients for anti-Leishmania antibodies using rk39 as antigen.

This study also emphasizes the emerging need for proper epidemiological workup in this region as there is a gradual rise in incidence over the last few years. Haematological and bone marrow features can supplement the epidemiological data by finding these hidden cases.

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