CLINICOPATHOLOGICAL FEATURES OF CHILDHOOD VISCERAL LEISHMANIASIS IN AZAD JAMMU & KASHMIR PAKISTAN

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Background: In Pakistan visceral leishmaniasis (VL) is endemic in Azad Jammu & Kashmir, Northern Areas and Northwest Frontier Province; the areas which lack adequate diagnostic facilities. This study describes the clinical and laboratory features in 61 cases of childhood VL. **Methods**: All the children below 12 years of age who were managed as indoor cases from 1st Jan 1999 to 31st Dec 1999 were included in this study. The diagnosis of VL was established by demonstration of leishmania parasites in bone marrow aspiration. The demographic information, physical signs at presentations and results of complete blood picture and formol gel test were recorded. **Results**: Median age of the patients was 18 months. Eighty four percent children were malnourished. Mean duration of fever before diagnosis was 45 days. Hepatosplenomegaly was present in all cases with mean enlargement of spleen and liver 6.8 and 3.2 cm respectively. Mean haemoglobin level, WBC and platelet counts were 6.7 g/dl, 4.8 x 109 /l and 70 x 109 /l respectively. Absolute neutrophil count was <1.5 x 109 /l in 61% cases. Mean reticulocyte count was 6.2%. There was significant negative correlation (p= 0.014) between haemoglobin level and spleen size. Formol gel test was positive in all cases. Mean hospital stay to established diagnosis was 8.6 days. **Conclusion**: The clinical and laboratory features of childhood VL in Azad Jammu and Kashmir are similar to Mediterranean type of disease caused by leishmania infantum. Cytopenia with high or normal reticulocyte count provides a useful clue to diagnosis in a febrile patient with hepatosplenomegally in an endemic area.

Key words: leishmaniasis,visceral childhood, clinicopathological

INTRODUCTION

Visceral leishmaniasis (VL) is caused by the parasites of Leishmania genus and is seen in 47 countries of the world, most of them being developing countries. Mean annual incidence of the disease is 0.5 million.[1,2] In Pakistan the first case was described in 1970s from Sub Himalayan region and since then it is frequently seen in Northern areas, Kashmir and North West Frontier Province and is believed to be endemic in these araes.[3-5]

It is a parasitic disease of reticuloendothelial system characterized by malnutrition, fever, visceromegaly and cytopenia of varying severity. Children are at greater risk of developing VL than adults in endemic areas.[6] Diagnosis of VL is not always easy and requires a high index of suspicion combined with adequate laboratory support. Since most of the clinical features are shared by other common diseases, the diagnosis is established by demonstration of parasites from spleen and bone marrow aspirates or by serological techniques.[7] Although parasites can be cultured in vitro but it is rarely needed in clinical practice.

VL is a disease of rural areas, where trained manpower and adequate diagnostic facilities are lacking, the diagnosis can be easily missed or delayed for months to years. The disease is a 'slow killer' since 90% of untreated cases have fatal outcome.[1] We studied the clinical and laboratory features of childhood VL at Azad Kashmir Combined Military Hospital Muzaffarabad and this study describes our experience of the disease in this area.

MATERIAL AND METHODS

Children below 12 years of age who were admitted in paediatric ward of Azad Kashmir Combined Military Hospital Muzaffarabad (AK CMH Mzd) from 1st Jan 1999 to 31st Dec 1999 were included in this study. The AK CMH Mzd is referral hospital for whole of the population of AJ& K. Diagnosis of VL was established by demonstrating leishmania parasites in bone marrow aspirate. Demographic information, weight, duration of fever at the time of admission, physical signs and time taken to establish diagnosis after admission were recorded.

Two-ml EDTA blood was analyzed on automatic haematology analyzer (Abacus-16 parameter) and white blood cell count, absolute neutrophil count (ANC), haemoglobin (Hb) level, reticulocyte and platelet counts were recorded. Bone marrow aspiration was done from tibial tuberosity or posterior iliac spine. Slides were stained with Leishman and brilliant cresyl blue using methods described by Dacie and Lewis.[8] Formol gel test was performed by mixing 1 ml of serum with two drops of 40% w/v formalin. Whitening and gelling of serum within 30 minute was taken as positive test.[9] Bone

marrow aspirates were examined for cellularity and presence of parasites.

Statistical Package for Social Sciences (SPSS) computer software was used to enter and analyse the data. Pearson correlation (2-tailed) was used to find out correlation between various parameters.

RESULTS

Sixty one cases were diagnosed as childhood VL during the study period. Forty were males and 21 females. The median age was 18 months (range 9-60). Mean weight was 8.4 ±1.3 kg and 84% children were malnourished as per WHO road to health chart (weight for age). All patients had fever at presentation and mean temperature was 102.8°F (max 106°F). Forty-nine cases had temperature of ≥102°F at presentation. Mean duration of fever before diagnosis was 45 days (range 5-99). Forty-seven (77%) patients had pallor. Splenomegaly and hepatomegaly was present in all the cases with mean enlargement of spleen and liver 6.8 and 3.2 cm respectively. Distribution of different clinical features is given in table 1. Mean WBC and platelet counts were 4.8 x 10^9 /l (±2.5) and 70 x 10^9 /l (range 2-298) respectively. Sixty one percent cases had absolute neutrophil count less than 1.5 x 10^9 /l. Mean haemoglobin concentration was 6.7 g/dl (±1.63) and mean reticulocyte count 6.2% (±1.7). Formal get test was positive in all cases. Similarly all the patients had normocellular or hypercellular marrow aspirates. Distribution of cases according to blood counts is given in table 2. Mean hospital stay before the diagnosis could be established was 8.6 days whereas median of total hospital stay was 17 days(range7-42).

Table1: Clinical features of cases with visceral leishmaniasis (n=61)

Features	Number of cases
Mean duration of fever at presentation	
≤ 2 weeks	8
≤ 4 weeks	24
≤ 8 weeks	18
≤ 12 weeks	8
> 12 weeks	3
Hospital stay before diagnosis (days)	
≤ 7	21
≤ 10	48
≤ 15	59
>15	2
Hepatomegaly (cm)	
1-2	15
3-4	39
>4	07
Splenomegaly (cm)	
2-4	12
5-8	34
9-12	13
>12	02

Table-2: Haematological parameters in patients with visceral leishmaniasis (n=61)

Parameters	Number of cases
Haemoglobin (g/dl)	
≤ 6.0	28
6.1-8.0	26
8.1-10.0	06
> 10.0	01
Absolute neutrophil count (x 10^9/l)	
< 0.5	07
> 0.5 -≤ 1.0	18
> 1.0 - ≤ 1.5	12
> 1.5	24
Platelet count (x 10^9/l)	
≤ 20	07
21-50	20
51-100	24
101-150	06
>150	04

DISCUSSION

Typically patients of childhood VL are malnourished with prolonged history of pyrexia, abdominal distension and cytopenia of varying severity. Most of the studies have described a younger age of the patients especially those from Mediterranean region and Middle East.[5,10-12] Median age in our study was 1.5 years (mean 1.7). Mean age of 2.9 and 4.2 years has been reported in studies from Pakistan and Brazil.[5,10]

Eighty four percent children were malnourished as per WHO road to health chart (weight for age). Malnutrition is both a predisposing factor as well as effect of the disease[1]. Duration of fever at presentation varies from 02 days to as long as over year.[1,10,13] In our study mean duration of fever at presentation was 45 days (range 5-99 days). Campose[14] has reported a period of 1-6 months in 78.6% patients. Mean duration of 4.8 weeks has been reported from Malta.[15] Another study[16] has reported duration of symptoms from 3 months to1.5 years.

VL could be associated with massive splenomegaly.[17] In our study mean size of spleen was 6.8 cm. In a study form Saudi Arabia[13] 85.9% patients had spleen size of more than 5 cm. None of our patient had significant lymphadenopathy and similar finding has been reported in another study from Pakistan.[11] Lymphadenopathy is usually a feature of VL in Africa and India not in Mediterranean type.[13,18]

Ninety eight percent children in our study had anaemia as per WHO criteria (Hb <11 g/dl) with mean haemoglobin being 6.7g/dl (±1.63). Queiroz et al[10] have described anaemia in 98% cases and in 25% of their patients Hb was less than 5g/dl while in our study 23% had Hb ≤ 5 g/dl. Median and mean Hb of 7.6 & 7.9 has been mentioned by Grech et al[15]. They reported minimum Hb of 4.7 g/dl in their patients while minimum value in our study was 4.0 g/dl. In our study mean WBC count was 4.8 x 10^9/l with 61% cases having ANC ≤ 1.5 x 10^9/l. Similar results have been reported by Grech et al.[15] In a study by Maricia et al[10]

mean WBC count was 3.5 x 10^9/l and 74% of their cases had neutropenia. The average ANC in their study was 1.2 x 10^9/l and 16% cases had ANC < 0.5 x 10^9/l in while in our study ANC of less than 0.5 x 10^9/l was seen in 11.4% cases.[10]

Mean platelet count in our study was 70 x 10^9/l (range 2-298) while 83.6% cases had platelet count ≤ 100 x 10^9/l. A study from Saudi Arabia[13] has described platelet count <100 x 10^9/l in 76.8% cases. Haider et al[19] have reported thrombocytopenia in 56% cases. Another study[15] has mentioned a mean platelet count of 121 x 10^9/l in 145 patients suffering from VL. Minimum count in this study was 25 x 10^9/l.

Anaemia in childhood VL is usually profound and multi-factorial. It may be caused by haemolysis, hypersplenism and malnutrition.[10,18] We found a statistically significant negative correlation (p= 0.014) between Hb level and spleen size. A similar correlation of Hb level was seen with degree of malnutrition. The value however, was not significant (p= 0.083). Hb level also had a not significant positive correlation with platelet counts (p=0.089). Goat milk anaemia could be an additional contributing factor[20] as majority of children in this area are fed on goat milk. Anaemia is usually slow to progress as compared to granulocytopenia, which progresses more rapidly.[21]

Mean time spent in hospital before diagnosis could be established was 8.6 days. Combination of pancytopenia with high to normal reticulocyte count is frequent finding in VL.[16] We were able to establish early diagnosis in most of the cases because of this clue on blood counts.

Formol gel is a simple test that could be helpful in screening of VL. In our study all cases had positive formal gel and similar results have been descried by another study from Pakistan.[11] A major limitation of the test is that like Montenegro skin test it can not differentiate between acute and treated cases.

Amastigotes are spread unevenly[7] in tissue smears, warranting laborious microscopy for diagnosis. The diagnostic yield of bone marrow (60-85%) is less than splenic aspirate (95%)[7,16]. Despite having better diagnostic yield the splenic aspiration is less commonly used due to certain contraindications like severe thrombocytopenia, restless child and pregnancy. Moreover it is associated with risk of fatal haemorrhage.[22]

CONCLUSION

Childhood visceral leishmaniasis in Azad Jammu Kashmir is seen in children of less than 5 years of age and has typical presentation. The cytopenia with high or normal reticulocyte counts provides a useful clue to diagnosis. Bone marrow aspiration is a safe diagnostic method, while formol gel is useful screening test in areas where facilities for bone marrow examination are not available.

REFERENCES

1 Dedet JP, Pratlong F. Leishmaniasis. In: Cook G, Zumla A Manson's Tropical Diseases. 21st ed. London: Saunders;2003. 1339-64.
2 World Health Organization. 1998. Life in twenty first century: a vision for all world health, World Health Organization, Geneva Switzerland.
3 Rab MA, Iqbal J, Azmi FH, Munir MA, Saleem M Visceral leishmaniasis: a seroepidemiological study of 289 children from endemic foci in Azad Jammu and Kashmir by indirect fluorescent antibody technique J Pak Med Assoc 1989;39:225-8.
4 Rab MA, Evans DA. Leishmania infantum in Himalayas. Trans R Soc Trop Med Hyg 1995; 89: 27-32.
5 Rathore MH, Buksh D, Hassan M. Visceral leishmaniasis in Pakistani children. South Med J 1996; 86: 491-3.
6 Kafetzis DA. An overview of paediatric leishmaniasis. J Postgrad Med 2003; 49: 31-8.
7 Sundar S, Rai M. Laboratory Diagnosis of Visceral Leishmaniasis. Clin Diagn Lab Immunol 2002; 9: 951-8.
8 Dacie SJV, Lewis SM,. Preparation and staining methods for blood and bone-marrow films. In: Practical Haematology. 8th ed. Edinburgh: Churchill Livingstone,1994; p: 83-96.
9 Appendix 6 to WHO/Leish/96.40 page 59.
10 Queiroz MJA, Alves JGB, Correia JB. Visceral leishmaniasis: clinical and epidemiological features of children in an endemic area. J Pediatr (Rio J) 2004;80: 141-6.
11 Rahim F, Rehman F, Ahmad S, Zada B. Visceral leishmaniasis in District Dir, NWFP. J Pak Med Assoc 1998; 48: 161-2.
12 Addadi K, Dedet JP. Epidemiology of leishmaniasis in Algeria. Survey of clinical cases of infantile visceral leishmaniasis from 1965 to 1974. Bull Soc Pathol Exot Filiales 1976;69:68-75.
13 Al-Orainey IO, Gasim IY, Singh LM, Ibrahim B, Ukabam SO, Gonchikar D et al. Visceral Leishmaniasis in Gizan, Saudi Arabia. [serial online] 1994 [cited 1994 Sep]; 14(5). Available from URL:http://www.kfshrc.edu.sa/annals/145/93166. Html
14 Campos Jr D. Clinical and epidemiological features of Kala Azar in chidren. J Pediatr (Rio J) 1995; 71: 238-40. [Abstract]
15 Grech V, Mizzi J, Mangion M, Vella C. Visceral leishmaniasis in Malta-an 18 year paediatric, population based study. Arch Dis Child 2000; 82: 381-5.
16 Mehabresh MI, el-Mauhoub MM. Visceral leishmaniasis in Libya-review of 21 cases. Ann Trop Paediatr 1992; 12: 159-63.
17 de Gorgolas M: Leishmaniasis. Medicine International (Pakistan ed). 1997;11:32-7.
18 Ravanbod M. Kala Azar in Adults: A Case Presentation and Review. Sheraz E Medical Journal 2001;2:
19 Haidar NA, Diab AB, El-Sheik AM. Visceral Leishmaniasis in children in the Yemen. Saudi Med J 2001;22:156-9.
20 Schwartz E. Megaloblastic anemias. In: Behrman RE, Kliegman RM, Jenson HB, (edi). Nelson Text Book of Pediatrics. 16th ed. Philadelphia: WB Saunders Company, 2000; p: 1041-4.
21 Parsad LSN. Kalazar. Indian J Pediatr 1999;66:539-46.
22 Guerin P, Olliaro P, Sundar S, Boelaert M, Croft, Desjeux P, et al. Drugs for the Treatment of Visceral Leishmaniasis: Current Status, Needs, and a Proposed R & D. Agenda. Available from URL: http://www.neglecteddiseases.org/1-2.pdf.

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