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REVIEW

Hematologic Changes in Visceral Leishmaniasis/Kala Azar

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Abstract Visceral Leishmaniasis (VL) or Kala Azar is a chronic infectious disease caused by parasites of the *Leishmania donovani* complex that can cause various hematologic manifestations. It is characterized by fever, enlargement of liver and spleen, weight loss, pancytopenia and hypergammaglobinemia. It is endemic in the Indian subcontinent, mainly seen in the states of Bihar and West Bengal. Patients with VL can present to the haematologist for various haematological problems prior to receiving the diagnosis of VL. Anaemia is the most common haematological manifestation of VL. VL may also be associated with leucopenia, thrombocytopenia, pancytopenia, hemophagocytosis and disseminated intravascular coagulation. Hematological improvement is noted within a week and complete hematological response occurs in 4–6 weeks of treatment. Relapses are rare and increased risk of being diagnosed with hematolymphoid malignancies on long term follow up is not noted.

Keywords Anaemia · Hematological changes · Kala Azar · Pancytopenia

Introduction

Leishmaniasis is a protozoan parasitic infestation, associated with three main types of disease patterns: visceral, cutaneous and mucocutaneous leishmaniasis. Various hematologic manifestations are found in visceral forms. Visceral Leishmaniasis (VL) may present to the hematologist as splenomegaly, hepatomegaly, fever, lymphadenopathy or pancytopenia.

VL or Kala Azar is endemic in more than 60 countries worldwide [1] including Southern Europe, North Africa, the Middle East, Central and South America and the Indian subcontinent. In India, it is largely endemic in the states of Bihar and West Bengal and in small pockets in Himachal Pradesh and North-West part of India [2].

VL is a systemic infection of the reticuloendothelial system caused by protozoa *Leishmania donovani* (LD) of the genus *Leishmania*. Genus *Leishmania* was created by Ross in 1903. Sir William Leishman discovered the parasite in spleen smears simultaneously with Charles Donovan identifying the same parasite in spleen biopsy [3]. The parasite has two forms: aflagellate or amastigote and flagellate or promastigote.

As amastigote it exists and proliferates in the mononuclear phagocytic system (MPS), especially spleen, liver and marrow. This leads to hyperplasia of the MPS with resultant disturbances in phagocyte bearing organs, producing hematological manifestations. Hence, this condition is of interest to hematopathologists, because the reticuloendothelial system is the target of parasitization. The spleen, in particular, becomes massively enlarged. Other clinical manifestations include hepatomegaly, fever, and a peculiar gray discoloration of the skin of the hands, feet, abdomen and face—which gave the name "kala azar," or "black disease," to the condition.

It is observed that the spleen size and duration of illness correlate with some of the main hematological features. Calvo et al. [4] studied the effect of parasitaemia on bone marrow ultrastructure but found no significant correlation

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between degree of parasitaemia and both the rate and structural abnormalities of bone marrow in patients with VL. Thus splenic sequestration and ineffective hematopoiesis appear to be the main etiopathogenetic factors in the emergence of bone marrow changes and peripheral cytopenias [5].

A recent article by Tripathi et al. [6] has provided new insights into the basic immunological mechanisms controlling leishmaniasis and suggested a crucial role of IL-10 produced by leishmania-parasitised macrophages in the disease initiation and progression. It appears that the Th1/Th2 paradigm of resistance/susceptibility may be an oversimplification of a far more complicated network of regulatory/counter-regulatory interactions seen in these patients. However better understanding of immune response to the parasite would pave the way for development of prophylactic and therapeutic strategies against it.

This review will summarize the associations of VL with both common and uncommon conditions that may be of interest to hematologists so as to provide guidance to the appropriate means of investigation to aid in identifying VL in a timely fashion.

Hematological Changes Seen in VL

Profound involvement of the hematological system in the form of bone marrow and peripheral blood changes are consistently seen in VL and include the following.

Anemia

Normochromic normocytic anemia is a frequent and clinically significant feature of VL and hemoglobin levels of 7–10 g/dl are commonly found. The average hemoglobin levels reported in two large series of patients were 8.3 and 7.8 g/dl [7, 8]. It is more severe in pediatric patients. Al-Jurrayan et al. [9] reviewed 94 patients with VL and found that all patients were anemic. Marwaha et al. [5] reviewed 23 patients with VL and found all patients to be moderately to severely anemic (Hb = 4.3–8.1 gm/dl). In this study children had slightly lower Hb values (mean = 6.4 vs 7.3 g/dl) as compared to adult patients. The cause of anemia seen in these patients is multifactorial: sequestration and destruction of red blood cells (RBC) in enlarged spleen, immune mechanism and alterations in RBC membrane permeability have been implicated. Red cell survival and ferrokinetic studies have suggested that hemolysis is the major cause of anemia in VL [10, 11] though there may also be plasma volume expansion associated with massively enlarged spleen. However ferrokinetic studies have shown very little evidence of ineffective erythropoiesis. Reduced plasma iron level in the presence of greatly increased iron stores suggests that the reticuloendothelial hyperplasia is accompanied by abnormal iron retention by macrophages, typical of anemia of chronic diseases [11]. This may limit the marrow response to hemolysis. In Mediterranean population a very rapid onset of anemia with hemolysis is commonly observed [12]. Occasionally both IgG and complement components are found on red cells, but this finding is not consistent and its significance remains to be determined. However, in most instances there is no evidence of immune hemolysis, and it appears that non sensitized red blood cells are destroyed in the macrophages that are recruited to the spleen and liver as part of inflammatory response to parasite. Hypersplenism is another primary pathogenetic mechanism [10, 11], although nutritional deficiencies of iron, folate and vitamin B12 may play further contributory role [8]. Other mechanisms suggested include increased sensitivity to complement [13], inhibition of erythrocyte enzymes [14], production of hemolysin by the parasites [15] and presence of cold agglutinins [16].

Leucopenia

Leucopenia is an early and striking manifestation of VL. There is relative lymphocytosis with neutropenia, the differential shows an almost complete absence of eosinophils and the presence of significant numbers of eosinophils rules out the diagnosis of VL. The mean TLC reported in two large series [7, 8] is 2.8×10^9/l and 4×10^9/l respectively. However, a lower TLC (2.4×10^9/l) was reported in a series of VL patients studied at our centre [5]. About 75% patients with VL have been shown to have leucopenia in various studies [5, 7]. The main cause for its development has been attributed to hypersplenism.

Thrombocytopenia

Platelets counts are usually affected after long duration of illness. Marwaha et al. [5] reported in their study that the average duration of illness was significantly longer in thrombocytopenic patients as compared to non-thrombocytopenics (9.2 ± 3.4 vs 4.2 ± 1.8 months). Mean platelet count has been found to be $109 \pm 82.3 \times 10^9$/l and an incidence of 55–65% has been recorded in various studies [5, 9]. Splenic sequestration is possibly the main contributory factor and immune mechanisms are believed to be non-contributory as anti-platelet antibodies have not been recorded in any study on VL.

Pancytopenia

Varying degree of frequency and severity has been reported by several group of workers [8, 17, 18]. It is usually seen after prolonged duration of illness. This occurs

because of splenic sequestration of blood cells. In such cases, the peripheral blood picture does in fact resemble aplastic anemia, but the presence of reticulocytes and young white cells indicates continuous regeneration of the blood and helps in differentiation from aplastic anemia. When pancytopenia is associated with fever, hepatosplenomegaly and lymphadenopathy, the clinical picture resembles that of leukemia, however bone marrow examination differentiates easily between the two.

ESR

Regular increase in erythrocyte sedimentation rate in VL is always noted, probably because of release of acute phase reactants.

Bone Marrow (BM) Changes

Common findings include erythroid hyperplasia, increased plasma cells and intracellular parasites (amastigote form) in mononuclear phagocytes. Erythroid cells may show moderate to severe megaloblastosis, deficient iron stores or features of dual deficiency depending on the associated deficiency. Granulocytic and megakaryocyte morphology has been reported to be unaltered except for an increase in immature forms in some cases. Also variable degrees of erythrophagocytosis and leukophagocytosis (46%) and granulomatous reaction (25%) may be seen [9]. Mathur et al. [19] reported a case of fatal hemophagocytosis secondary to VL in a 4 year old child. They emphasized the fact that in patients diagnosed with hemophagocytic syndrome, a diligent search for the etiologic agent including LD bodies should be made in order to initiate timely aggressive and effective treatment. Rajagopala et al. [20] also recorded hemophagocytic lymphohistiocytosis (HLH) in a patient with VL and stressed the fact that VL related HLH is often under-recognized because of overlapping features with HLH and negative marrow evaluation at the onset, leading to high mortality rates. Repeated marrow aspiration, blood cultures and serology may be required to establish the diagnosis in suspected cases. Recently we encountered a VL patient showing profound histiocytic hyperplasia, producing syncytium like arrangement on BM examination [21].

Cotterell et al. [22] sought to identify the factors associated with *L. donovani* infection in VL, which regulate hematopoiesis, by studying the interaction between this intracellular pathogen and stromal cells responsible for regulating hematopoietic colony formation. Their results indicated that stromal macrophages are a target for *L. donovani* infection in vivo and in vitro, and as a consequence of the selective induction of GM-CSF and TNF-α production, infected stromal macrophages preferentially support increased levels of myelopoiesis.

The severity of the hematological changes generally depends on the duration of the disease and the size of the spleen rather than on the number of parasitized mononuclear cells.

Coagulation Abnormalities

Liver dysfunction with jaundice, ascitis and deranged coagulation may occur in late stages and has a poor prognosis [23]. Liver dysfunction may be caused directly by protozoa itself or indirectly to the effect related to the immune response of the parasites. Al-Jurrayan et al. [9] recorded coagulation abnormalities in 10 (11%) of the 94 VL patients in their study, in the form of prolonged PT and APTT, with 4 (36%) of these having disseminated intravascular coagulation.

Platelet Function Studies

Dube et al. [24] reported deranged platelet function studies in their report on patients with VL. They conducted platelet function studies on 25 parasitologically positive cases of Indian VL and 25 age and sex matched healthy controls. Thrombocytopenia of variable degree was found in 92% patients; in 44% of patients, platelets were less than 60,000 mm^3. The platelet adhesive index was less than 30% in 70% of patients with VL (normal 31–60%). Platelet aggregation time with ADP and adrenaline was abnormally prolonged compared to the controls. Platelet factor III availability was poor in 40% of cases. They found that there was a fair degree of correlation between platelet adhesiveness and platelet factor III availability in these patients: 50% of patients with poor platelet adhesiveness showed reduced platelet factor III availability.

However, further studies on platelet function are needed to corroborate their findings.

Diagnosis

Serological studies are recommended as the initial diagnostic tests in suspected leishmaniasis. In advance stages of the disease, parasites can be found in phagocytic cells in spleen, bone marrow, lymph nodes and rarely blood. Morphological identification provides an early, specific and cost-effective diagnosis. Culture of bone marrow is, however, a more sensitive diagnostic technique than microscopy. Aspiration specimens are collected aseptically and cultured in Novy–MacNeal–Nicolle medium or in Schneiders Drosophilia medium supplemented with calf serum. Cultures usually begin to show promastigotes in 2–5 days.

Leishmania is diagnosed in the hematology laboratory by direct visualization of the amastigotes (referred to as Leishman Donovan—LD bodies) [25]. Buffy coat preparation of peripheral blood or aspirates from bone marrow, spleen, lymph nodes or skin lesions should be spread on a slide to make a thin smear and stained with Leishman or Giemsa stain for 20 min. Amastigotes are seen within monocytes or, less commonly, in neutrophils in the peripheral blood and in macrophages in bone marrow aspirates. They are small, round bodies 2–4 μm in diameter with indistinct cytoplasm, a nucleus, and a small rod -shaped kinetoplast. Many times extracellular free lying LD bodies also may be seen (released from the disrupted cells).

We evaluated various tests for diagnosis of VL. On the basis of bone marrow aspirate positivity, the sensitivity and specificity of DAT was 100% while that of rk39 strip test and ELISA was 100 and 87%, respectively [26].

Treatment

Antimonial compounds (sodium stibogluconate, meglumine antimonite) form the traditional treatment for leishmaniasis Resistance to the antimonials is prevalent in some parts of the world, and the most common alternative is amphotericin B. Paromomycin is an inexpensive alternative with fewer side effects than amphotericin that The Institute of OneWorld Health has funded for production as an orphan drug for use in the treatment of leishmaniasis, starting in India.

Response to treatment occurs as stated below [27]:

i. Symptomatic improvement occurs generally within a day of starting treatment
ii. Hematological improvement is noted within a week. Complete hematological response occurs in 4–6 weeks
iii. Splenic clearance of parasite occurs in 2–3 weeks
iv. Reduction in splenomegaly occurs within 2 weeks. Very large spleen may take several months to reduce to normal size, but small spleen may become impalpable within a month.
v. Serological reactions and immunoglobulin levels revert to normal over a period of six months.

Monitoring of treatment and follow up:

i. Daily monitoring of temperature and weekly assessment of spleen size clinically
ii. Weekly aspirates to monitor the clearance of parasites from the spleen
iii. Weekly hemograms to assess hematological response
iv. 3 and 12 monthly follow up after course of drugs to detect any relapses [28]

Test of Cure (to discontinue treatment) [27]: defined as absence of parasites from two successive splenic aspirates taken 1 week apart.

To indicate as cured, patient should have absence of fever, clinical and hematological improvement, reduction in spleen size and splenic aspirate score of zero.

Complicating illnesses, such as pulmonary tuberculosis and AIDS should be looked for in all patients in whom response to treatment is delayed with appropriate therapeutic agents.

Conclusion

Hematological abnormalities in VL are common. The pathogenesis is complex and multifactorial. Hypersplenism, hemophagocytosis, chronic inflammation and dietary factors appear to be most important factors. A high degree of suspicion for VL needs to be maintained by the hematologists and it should be included in the differential diagnosis of patients presenting with fever, hepato-splenomegaly, anemia, leukopenia, thrombocytopenia, pancytopenia or histiocytosis and DIC; particularly in geographical areas where the disease is endemic.

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