Hematological changes in Visceral Leishmaniasis

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Abstract

Visceral Leishmaniasis (VL) is caused by *Leishmania donovani*, and is transmitted by the bite of female phlebotomine sandfly. This disease is widely prevalent in the world, and is endemic in a few states of India. Clinical features of this disease include fever, anemia, weight loss, lymphadenopathy, hepatosplenomegaly. Hematological features in VL include anemia, neutropia, lymphocytosis and thrombocytopenia. Bone marrow examination shows reticuloendothelial cell hyperplasia, erythroid hyperplasia, reduction of neutrophils, eosinophilic myelocytes and plasma cells. The ratio of granulocytes to non-granulocytes is decreased. The ability of megakaryocytes to form platelets is also decreased.

Keywords: Visceral Leishmaniasis, Hematological changes, Bone marrow

Introduction

Visceral leishmaniasis (VL) is an infectious disease, which is caused by a protozoa *Leishmania donovani* (LD), belonging to genus Leishmania, which was created by Ross in 1903. This parasite was discovered by two scientists simultaneously. While Sir William Leishman discovered it in spleen smears, Charles Donovan identified the same parasite in spleen biopsy specimen. (1) It is an obligate intracellular parasite, and is transmitted by female phlebotomine sand fly. Although VL is endemic in sixty countries, including Southern Europe, North America, the Middle East, Central and South America, and the Indian subcontinent, but according World report, about 90% of new cases occurred in six countries namely Brazil, Ethiopia, India, Somalia, South Sudan and Sudan in 2014. Recently, epidemics with high disease mortality have been reported in East Africa (Ethiopia, Kenya, South Sudan and Sudan). (2,3) In Indian subcontinent, this disease is endemic in the states of Bihar, West Bengal, some parts of Himachal Pradesh, and North-West India. (4)

VL is a disease of reticuloendothelial system. The parasite LD has two forms aflagellate or amastigote form and flagellate or promastigote form. Amastigote form of this parasite
proliferates in the mononuclear phagocytic system (MPS) of the body, mainly in spleen, liver and bone marrow. Thus proliferation of MPS leads to enlargement of these organs. Bone marrow becomes hyperplastic and infiltrated by reticulo-endothelial cells. (4) Splenic enlargement is more prominent than hepatomegaly. Grey discoloration of skin over hands, feet, abdomen and face is frequently seen. Therefore, this disease is also known by the name of Kala azar, which means "Black disease". (5) The important clinical features of this disease are anemia, hepatosplenomegaly, leukopenia and hypergammaglobulinemia, fever, weight loss and cough. Due to release of acute phase reactants, ESR is almost always raised. Complications of VL include bleeding, dysentery, pneumonia, cancrum oris, agranulocytosis and anasarca.

Leucopenia, anemia and thrombocytopenia occur in almost all cases in later stage of disease. (6,7) Studies show that duration of disease and splenomegaly positively correlates with main hematological abnormalities in VL, but studies have failed to find correlation between bone marrow abnormalities and degree of parasite load. Based on these findings, researchers have suggested that sequestration in spleen and ineffective erythropoiesis are the mechanisms by which bone marrow and peripheral blood changes occur in this disease. (8, 9) It is also observed by researchers that splenomegaly is the most common and prominent sign, and also the most important cause of abdominal distension in these patients. The prevalence of splenomegaly in VL has been found to be as high as 85% to 100% in various studies. (7, 10)

**Hematological Changes Seen in VL**

Anemia is almost always present in VL, which is frequently of normochromic normocytic type. Patients of VL generally have hemoglobin levels in the range of 7 to 10 gm/dl. Anemia is more severe in children affected by this disease. (4, 11, 12) In most of the patients, hemoglobin is reduced in proportion to the reduction in erythrocyte count. (13) Hemolysis is the most important cause of anemia in these patients. Various mechanisms of red cell destruction have been suggested by researchers including splenic sequestration, increased sensitivity to complement, changes in erythrocyte enzymes resulting in change in red cell membrane permeability, and presence of cold agglutinins. (14,15) Deficiencies of iron, folate and vitamin B12 may also have some additional role. (16) Studies have not shown any role of ineffective erythropoiesis in development of anemia in these patients. Most studies have shown that there is a mild increase in reticulocyte count, not exceeding 4% in most of the cases. (13,17) Iron stores are greatly increased, but plasma iron levels are often decreased, suggesting that there is retention of iron in macrophages along with hyperplasic reticuloendothelial system. Hypersplenism is the primary mechanism by which red blood cells are destroyed in LV.

Leucopenia is an important abnormality seen in VL. It occurs early in the course of the disease. As early as in the beginning of twentieth century, researchers stated that, clinicians should suspect kala-azar if the proportion of leukocytes to red blood cells is decreased to around 1:1500. In the two large studies conducted in the past, the total leucocyte count was 2800/cm³ and 4000/cm³ respectively. The leucopenia in VL is mainly due to neutropenia. The percentage and absolute number of neutrophils in VL are remarkably decreased, and there is a shift towards left in case of juvenile neutrophils. The main cause of neutropenia is thought to be hepersplenism. While absolute number of lymphocytes is mildly decreased, there is relative lymphocytosis. The number of eosinophilis is decreased significantly, or they completely disappear from peripheral blood. While the percentages of monocytes are increased, there is no consensus among various authors about the absolute number of lymphocytes in VH. (5, 4, 18, 19)

Thrombocytopenia is a relatively late manifestation of VL. Splenic sequestration and immune mediated mechanisms are mainly thought to be responsible for thrombocytopenia, while the role of anti-platelet antibody has not been shown in research. Because of decreased platelet count, bleeding manifestations are very common. (9, 12) In the study conducted by Rai et al, the incidence of thrombocytopenia was reported to be as high as
In their study, Dube et al, reported that in cases of VL there is not only reduction in platelet count, but platelet function disorder are also present. In that particular study, the prevalence of thrombocytopenia was reported to be as high as 92%, and in 44% of the patients, platelet count was lower than 60,000. It was also reported that platelet adhesive index was less than 30% in most of the cases. Moreover, platelet aggregation time was prolonged with ADP and adrenaline. One of the interesting findings of this study was the positive correlation between platelet adhesiveness and platelet factor III availability. (20)

Many studies have reported pancytopenia in the late stages of VL. There is variation in the frequency of pancytopenia reported by various researchers. The cause of pancytopenia is thought to be due to sequestration of blood cells in the spleen. The presence of reticulocytes and immature blood cells in peripheral blood helps in differentiating pancytopenia due to VL from aplastic anemia. The clinical picture in these patients can mimic leukemia, especially in the presence of fever, hepatomegaly and lymphadenopathy. In those cases bone marrow examination is very useful for making accurate diagnosis. (21, 22, 23)

Bone marrow

Bone marrow examination in VM reveals erythroid hyperplasia and increased number of plasma cells. The erythroid cells increase to around 36% from normal value of 22%. Amastigote form of parasite (intracellular form) may be seen. Often erythroid cells show megaloblastosis, and deficient iron stores are found. The severity of hematological changes depends upon the disease duration and severity of splenomegaly, and is generally not linked to the degree of parasitemia. (23, 24) It was known from the early days that the bone marrow in VL is hypercellular, and shows hyperplasia of both reticuloendothelial, and bone forming cells. There is a positive correlation between the number reticuoloeial cells in marrow, and duration of the disease, but no relationship exit between number of reticuloendothelial cells in marrow, and severity of anemia, thrombocytopenia or leukopenia.

Leukopenia is one of the most consistent and early changes in peripheral blood. It appears even before any significant growth of reticuloendothelial cells in the marrow. There is reduction in the number of polymorphonuclear neutrophils to around 8% from normal of about 20% in bone marrow. This is the earliest change which takes place in the marrow. This occurs at the same time when leukopenia starts to appear in peripheral blood. The lymphocytes and monocytes are normal in number, but the ratio of granulocytes to non granulocytes is reduced to about 1.6:1 from the normal value of around 5:1. The percentage of plasma cells is increased to about 2.8% form normal percentage of about 0.4%. In the late stage of the disease, the eosinophilic myelocytes and mature eosinophils are greatly reduced. The leukocyte-erythrocyte ratio is changes to around 1.8:1 from the normal of about 3.5:1, and the granulocyte-erythrocyte ratio is decreased to about 1.2:1 from the normal of about 3:1. It has been observed that the decrease in leukocyte-erythrocyte ratio is directly proportional to the increasing size of spleen in VL. (4, 7)

Although, studies have shown that number of megakaryocytes in patients of VL is variable in different patients, some abnormalities were consistently observed. The cytoplasm of granulocytes becomes less granular, and their nuclei become a little degenerated. The platelet formation from megakaryocytes is also reduced significantly. Normally around 75% of megakaryocytes have platelet like bodies attached to their peripheral cytoplasm, but in VM this number is reduced to around 32%. Moreover, the average number of platelets attached to a megakaryocyte cytoplasm is reduced form an average of 60 to a lower lever (Between 10 to 50). (4, 24)

After starting treatment, reticuloendothelial cells infected by parasites rapidly disappear and the percentage of reticuloendothelial cells also comes down slowly. In the bone marrow, shift to right occurs in respect to the leukocytes.
Eosinophilic myelocytes and adult eosinophils are also decreased significantly. The number of plasma cells decreases to a normal level, and lymphocytes are basophilic myelocytes becomes more numerous. The leucocyte-erythroid ratio is increased, thereby nucleated cells becomes less in number. The relative number of megakaryocytes per million nucleated cells increases, and the number of platelets attached to each megakaryocyte is also increased significantly. The percentage of megakaryocytes forming platelets increases to normal levels within 6 to 8 days of treatment response. (4, 5, 8, 23)

Conclusion

The principal changes in peripheral of patient with VL are reduced number of red blood cells, reduction in leukocytes and decreased platelet count. Although, the leukopenia is a result of reduction of all cell types, but there is more marked reduction of neutrophils. The first change to occur in the peripheral blood is occurrence of leukopenia, which is followed by anemia. Thrombocytopenia occur after these two changes. With the progress of the disease, these changes become more marked and, spleen becomes larger in size. The splenic enlargement is in proportion to the degree of anemia, leukemia and thrombocytopenia. It has been shown that bone marrow in VL is infiltrated by reticulo-endothelial cell, and this mechanical destruction of bone marrow has been suggested as a reason for hematological changes in VL by many researchers. But it has also been observed that occurrence of leukemia in VL occurs, even before the infiltration of marrow by reticulo-endothelial cells to any significant level, moreover researchers have failed to link the degree of anemia to the level of infiltration of marrow. Rather, some researchers have pointed out that in anemia of VL, there is increase of skeletal red marrow, instead of any decrease. (11) Bone marrow examination shows reduction in polymorphonuclear neutrophils and eosinophils, while erythroid cells are increased in number. There is also a marked reduction in the ability of the megakaryocytes to form platelets.

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