Case Report

A rare case of pancytopenia due to leishmaniasis - A case report

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Abstract

Visceral Leishmaniasis (VL) or Kala Azar is a chronic protozoal infectious disease caused by the Leishmania donovani complex which causes a variety of hematologic manifestations. It is manifested by fever, hepatosplenomegaly, weight loss, pancytopenia and hypergammaglobinemia. In India it is mainly seen in the states of Bihar and West Bengal. Patients with VL can present to the hematologist for variety of hematological presentation even before the diagnosis of VL is made. Anemia is the most common hematological manifestation of VL. VL may also be associated with leucopenia, thrombocytopenia, pancytopenia, hemophagocytosis and disseminated intravascular coagulation. Hematological improvement is noted with the treatment for VL. Relapses are rare. In this case report, we present a rare case of leishmaniasis with pancytopenia, which is rare in Hyderabad, Telangana, India.

Key words

Pancytopenia, Visceral Leishmaniasis, Kala Azar.

Introduction

History

The British medical doctor Ronald Ross in November 1903 commented on the discovery of the ovoid bodies found by Leishman and Donovan in spleen pulp of patients with chronic pyrexia and splenomegaly [1]. He concluded that the ovoid bodies were not degenerated trypanosomes but a novel protozoan organism and that the clinical picture of the cases resembled that of kala azar [2]. In a follow-up paper, Ross concluded that these ovoid bodies belonged to a new genus and proposed to name them Leishmania donovani [3].

The parasite

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 [4, 5] 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 [6, 7]. 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 [8].

Pathology

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 [9]. 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.

Clinical manifestations

The spleen, in particular, becomes massively enlarged. About 90% of VL patients do not acquire the disease's characteristic symptoms and are classified as subclinical or asymptomatic. When an infection advances to illness, it results in spleen and liver enlargement [10]. 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. The splenic sequestration and ineffective hematopoiesis appear to be the main etiopathogenetic factors in the emergence of bone marrow changes and peripheral cytopenias [11].

Materials and methods

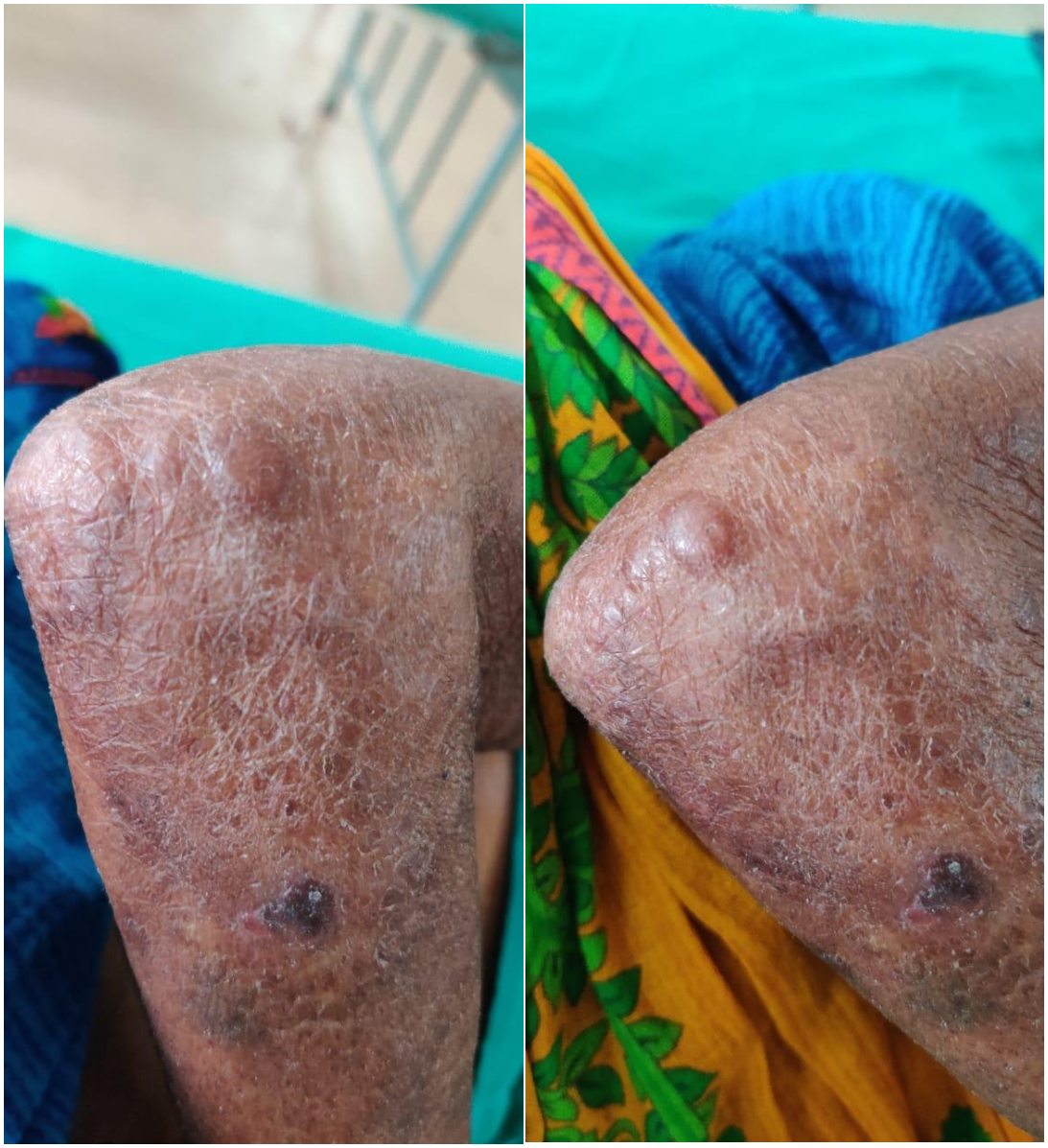
A 62 year old female patient, a native of Samastipur Bihar, came with chief complaints of abdominal discomfort after partaking food of 7 days duration and bilateral swelling of lower limbs of 5 days duration.

History of present illness - Patient was apparently asymptomatic 7 days back then she developed abdominal discomfort on partaking food along with retrosternal burning sensation. She complains of breathlessness on exertion and easy fatiguability of 7 days duration. She complained of swelling of both lower limbs initially up to ankle which gradually progressed to knee since 5 days. She has no history of bleeding manifestations, chest pain, palpitations, decreased urinary output, vomiting and diarrhoea. She has history of cutaneous lesions all over the body for the past 40 years, for which she took ayurvedic and homeopathic treatment. She is not a known case of hypertension, diabetes mellitus, epilepsy, asthma, CVA and CAD. She has 10 children and the last 4 children died during childhood with history of high fever for more than 1 month duration. She was tubectomised 26 years back and attained menopause 14 yrs ago. There was no history of similar complaints in the family.

On examination – The patient was of thin built, short stature, her skin has peculiar gray discoloration when compared to her children who are of fair complexion. She has multiple nodules all over the body as shown in **Figure - 1, 2**. Her abdomen was distended and pitting type of oedema up to knee in both lower limbs. Patient had no icterus, no cyanosis, no clubbing, no koilonychia, no lymphadenopathy. Her temperature was 101 ºF, Pulse Rate 116/min, BP 110/70 mm Hg. Cardiovascular System -

examination revealed soft systolic murmer at left parasternal region. JVP was raised by 10 cm H_2O. 'A' waves were prominent Respiratory system examination - revealed coarse crepitations at basal areas. Per abdomen examination revealed soft and distended abdomen. Massive Splenomegaly and hepatomegaly were present. CNS examination was normal.

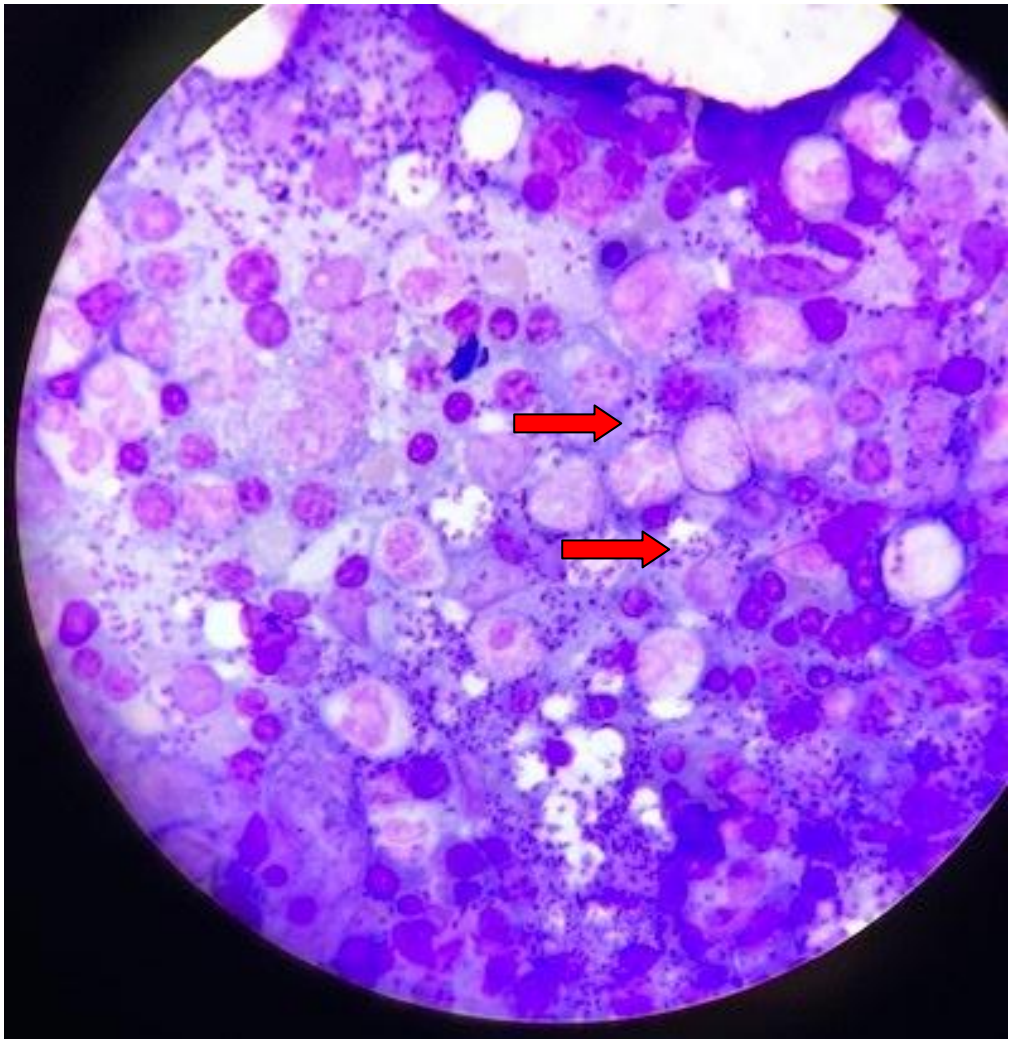
Figure - 1, 2: Skin Nodules.



On investigations - Complete blood picture: Hb-5.9 gm%, RBC Count - 2.0 mill/cumm, WBC Count-1,100 cells/cumm, Neutrophils - 38%, Lymphocytes - 50%, Eosinophils - 4%, Basophils - 0%, Monocytes - 8%; Platelet Count - 0.80 lakhs/cumm; Blood Picture – Normocytic, normochromic to Normocytic hypochromic with erythropenia, leucopenia with relative lymphocytosis, thrombocytopenia; Reticulocyte count – 1.7%; ESR – 110 mm/hr; Random Blood Sugar – 77 mg/dl; B. Urea – 41 mg/dl, S. Creatinine - 0.7 mg/dl, LFT - Total Bilirubin - 1 mg/dl, Direct Bilirubin - 0.8 mg/dl, Indirect Bilirubin - 0.2 mg/dl, AST – 102 U/L, ALT - 25U/L, ALP – 266 U/L, Total Proteins – 7.2 g/dl, S. Albumin-1.1 g/dl, S. Globulin-6.1g/dl, A/G ratio-0.18. S. Electrolytes-Na+ - 140 mEq/L; K + - 4.1 m Eq /L; Cl- - 106 mEq/L;); HIV, HBsAg,

HCV – Negative, CUE- Normal; Thyroid Profile - T3- 1.91 ng/dl, T4-5.17 µg /dl, TSH–2.77 µIU/ML; S. Iron – 31.3µg/dl; TIBC - 125 µg/dl; ANTI CCP Ab-59.3 (POSITIVE) – (Negative – < 5U/ml Positive- > 5U/ml.) Anti ds DNA- 12.9 (NEG) (NEG- <20 IU/ml Equivocal - 20-25 IU/ml; Positive - > 25 IU/ml)., Chest X- ray- NAD; BARIUM SWALLOW –WNL; ECG - WNL; USG Abdomen and Pelvis – Mild hepatosplenomegaly, Massive splenomegaly with minimal ascites. Bone marrow aspiration cytology (**Figure - 3, 4**).

Figure – 3: Arrows showing Leishmania Donovani, at the amastigote stage of development, are seen extracellularly.



Revealed hypercellular marrow; M : E ratio = 1;1, ERYTHROID SERIES- mild erythroid hyperplasia with normoblastic maturation. All series of maturation noted.; MYELOID SERIES- relatively suppressed with all series of maturation noted with slight increase in eosinophils; MEGAKARYOPOISIS- adequate and active.; PLASMA CELLS- present and morphologically appears normal.; R-E CELLS - present and morphologically appears normal; Lymphocytes- present and morphologically appears normal. PARASITES- Leishmania Donovani, at the amastigote stage of development, is seen within macrophages as well as extracellularly. Normoblastic Erythroid Hyperplasia associated with parasitic Infection of leishmania donovani.

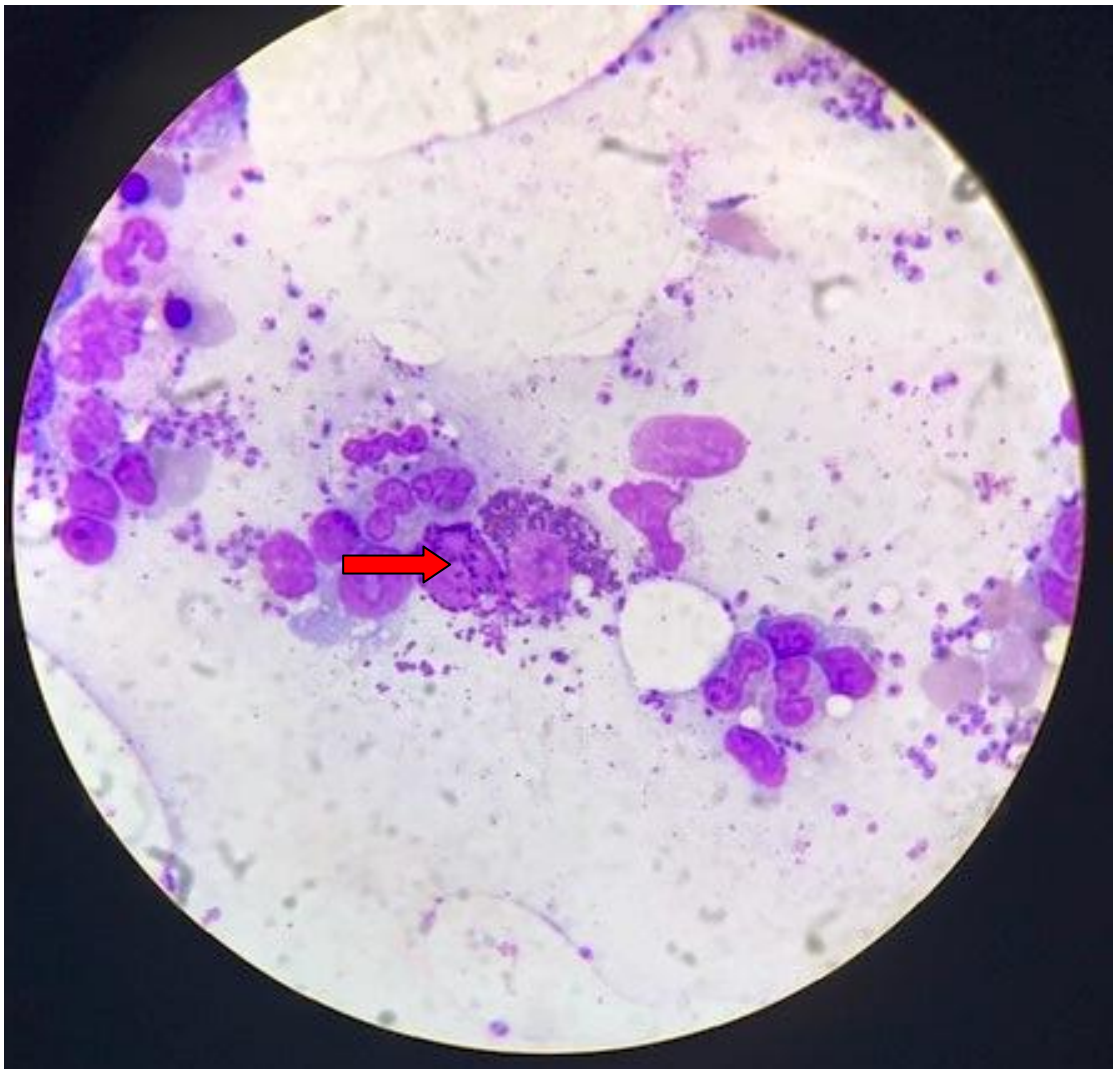
Diagnosis

Visceral Leishmaniasis with Pancytopenia

Treatment given

1. Cap IMPAVIDO (Miltefosine) 50 mg 1 BD for 28 days

Figure – 4: Arrow showing Leishmania Donovani, at the amastigote stage of development, are seen within macrophages.



Discussion

Hematologists play a leading role in diagnosing the hematological disorders in VL . Hematologists need to ensure a high standard of suspicion for VL and include it in the differential diagnosis of patients who expressed fever, hepatosplenomegaly, anemia, leukopenia, thrombocytopenia, pancytopenia, and DIC, especially in endemic areas [12].

Hematological changes

Hematological profile dysregulation is linked to VL patients, and this leads to substantial source of mortality and morbidity. The most common hematological symptoms include anemia, leukopenia, thrombocytopenia, and pancytopenia. As a result of thrombocytopenia and neutropenia, changes in hematological profiles have been linked to bleeding disorders as well as increased host susceptibility to bacterial infection [13].

Anemia

The cause of anemia 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 [14, 15, 16]. 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 [15]. This may limit the marrow response to hemolysis. In Mediterranean population a very rapid onset of anemia with hemolysis is commonly observed [17].

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 [14, 15], although nutritional deficiencies of iron, folate and vitamin B12 may play further contributory role [18].Other mechanisms suggested include increased sensitivity to complement [19], inhibition of erythrocyte enzymes [20], production of hemolysin by the parasites [21]and presence of cold agglutinins [22].

Besides amastigote, the parasite's intracellular stage proliferates in mononuclear phagocytic systems, where a large amount of iron is stored. A ligand on the surface of amastigotes binds hemin with high affnity and can also exploit iron from heme and Hb for nutritional purposes. This ligand may participate in intracellular heme transport, resulting in iron depletion for erythropoiesis [23]. Furthermore, the parasite directly scavenges iron from macrophage iron pools, which is critical for them to avoid oxidative stress in the host, as iron is a cofactor for the antioxidant enzyme superoxide dismutase (Fe-SOD). The inactivation of Fe-SOD has an impact on their virulence and intracellular survival [24].Since amastigote lives and proliferates in the mononuclear phagocytic system, namely the spleen, liver, and marrow, the severity of hematological abnormalities is determined by the duration of the disease and the size of the spleen. This causes hyperplasia of the mononuclear phagocytic system, resulting in phagocyte-bearing organs such as the spleen becoming enormously expanded and resulting in hematological symptoms [16].

Leucopenia

Leucopenia is an early and striking manifestation of VL. The main cause for its development has been attributed to hypersplenism. 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 16].

Thrombocytopenia

Platelets counts are usually affected after long duration of illness [11]. 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. In general, thrombocytopenia may be caused by bone marrow suppression and hepatosplenomegaly as a result of disease progression. Furthermore, because one-third of platelets are stored in the spleen, abnormalities in this organ result in a decrease in platelet count [16]. The leishmania parasite invades and multiplies in macrophages, potentially triggering an infammatory response. During the acute and chronic phases of the infammatory process, neutrophils and monocytes are the main players, with the involvement of other types of WBC that may be destructed as a result of the infammatory process [16].

Pancytopenia

Pancytopenia of varying degree of frequency and severity has been reported by several group of workers [18, 25, 26]. Pancytopenia is the most common haematological abnormality (85%) [27], similar to the findings by Hamid, et al.; Uzair, et al.; (80%) [28, 29], but Chakrabarti, et al., reported pancytopenia in 58.3% of cases [30]. Agarwal et al., reported bicytopenia (40%) as the most common finding [31].

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.

Leishmaniasis may mimic autoimmune cytopenias like Evans syndrome or be associated with viral infections but it may also lead to and mimic less frequent diseases, such as macrophage activation syndrome (MAS) [32, 33] or systemic lupus erythematosus [34]. MAS is characterized by T cell and macrophage activation, leading to cytokine overproduction resulting in inflammation. Due to increased macrophage activation, blood cells may be phagocytosed consecutively leading to cytopenia [32].

In a recent study, it is shown that autophagocytosis may play an important role in the immune response to Leishmania. Whereas other infections may trigger macrophage activation and T cell response, an infection with vital and dead Leishmania at the same time may induce autophagocytosis in cells infected with dead Leishmania resulting in a hampered adaptive immune response, so vital Leishmania will not be killed at all. In cells only infected with vital Leishmania, the immune response is not hampered due to the missing induction of autophagocytosis. According to these study results, an infection solely with vital Leishmania will not lead to autophagocytosis and the immune response kills the entered Leishmania [35]. Several investigators have revealed the presence of autoantibodies against cellular and humoral components as well as against nuclear antigens [36, 37] in visceral leishmaniasis. Many different autoantibodies/laboratory values might be altered, such as antinuclear antibodies (ANA), rheumatoid factor, anti-cardiolipin antibodies, cryoglobulins, Coombs test, hypergammaglobulinemia, anti-smooth muscle antibodies (ASMA), protoplasmic-staining anti-neutrophil cytoplasmic antibodies (pANCA), anti-extractable nuclear antigens antibodies (anti-ENA), anti-myeloperoxidase antibodies (anti-MPO), anti-Smith antibodies (anti-SM), anti-Sjögren's-syndrome-related antigen A (anti-SS-A)/anti-Ro antibodies (anti-Ro), anti-SS-B/anti-La, antiribonucleoprotein antibodies (anti-RNP), and decreased C3 and C4 [36, 37, 38].

Liberopoulos, et al. showed that all autoimmune laboratory findings normalized only 3 months after therapy [38]. The presence of these unspecific autoantibodies may be explained in part by polyclonal B-cell-activation, which may lead to hypergammaglobulinemia [36, 39, 40].

Bone Marrow 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 [41] 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. VL related hemophagocytic lymphohistiocytosis (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. 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 [16].

Immunocompromized hosts

In the early years of the HIV/AIDs epidemic, visceral leishmaniasis emerged as an important opportunistic infection in HIV-positive patients, especially those with AIDS [42, 43]. In co-infected patients, clinical signs and symptoms of VL usually develop in late-stage AIDS. CD4 counts are usually less than 50 and almost always less than 200.

Autoimmune manifestations

The hallmarks of VL may mimic symptoms of autoimmune diseases, especially of SLE. The presenting autoimmune symptoms of VL patients generally include pancytopenia, hypergammaglobulinemia, and the production of autoantibodies, such as ANA, and others. Polyclonal activation of B cells is considered to be the main reason of immunoglobulin and autoantibody formation [36, 44]. Extensive cross-linking of membrane immunoglobulins by microbial antigens or cytokines produced by other cells can stimulate the polyclonal activation of B cells [45]. In addition, Leishmania parasites, themselves, may cause tissue destruction, thus releasing self-antigens, which act as B-cell mitogens [44]. In clinical practice, VL patients often present with increased levels of serum immunoglobulins and increased production of autoantibodies, which could easily lead to a misdiagnosis as SLE. Recently, Santana, et al. [34] reviewed original studies of cases of VL-infected patients, in order to identify the clinical and laboratory manifestations of VL mimicking SLE [34]. The literature review identified 18 cases of VL mimicking SLE. The most common manifestations in VL patients were reported as intermittent fever, pancytopenia, visceromegaly, and increased acute phase reactants. The most common laboratory results were positive ANA (17 cases, 94%), positive RF (10 cases, 56%), and positive direct Coombs' (9 cases, 50%) tests. Even for tests generally considered highly specific for SLE, such as the anti-dsDNA test, the prevalence of a positive result in VL patients was 16% [37, 46]. Other autoimmune antibodies, such as RF, anti-dsDNA, anti-Sm and ANCA, were detected in 24.4–63%, 4.5%, 6%, and 25% of VL patients, respectively. The results of direct and indirect Coombs' tests were positive in 13% and 6% of VL patients, respectively, and some studies showed decreased C3 and C4 levels in VL patients [37].

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 costeffective 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) [47]. 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 mm 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).

Treatment

The drugs include pentavalent antimonial compounds, amphotericine b, paromomycin and miltefosine. 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 [48]:

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) [48]:

It is 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 [16].

Accordindly, patients with visceral leishmaniasis had improved hematological profiles after treatment.

The effect of treatment

The effect of treatment on parasite proliferation and concentration within visceral organs, in which the parasite load could directly affect the patient's hematological profiles, may be associated with the change in hematological profiles [5]. Improvements in hematological profile are expected within 2 weeks of starting anti-leishmanial medicines, with complete recovery taking 4 to 6 weeks. Treatment methods for leishmaniasis vary, with a broad range of first-line medications including Sodium Stibogluconate (SSG) and Paromomycin in combination, Sodium Stibogluconate or Meglumin Antimoniate as Monotherapy, and Liposomal Amphotericin B. Second-line medications, on the other hand, include Liposomal Amphotericin B (AmBisome), Miltefosine, and Paromomycin. In the treatment of leishmaniasis patients, a total dose of 20 mg/kg was found to be beneficial [49].

Visceral leishmaniasis is very rare in Hyderabad, Telangana, India. This patient migrated to Hyderabad from Bihar for various reasons. The diseases which are common at the patient's native district should be kept in mind while considering differential diagnosis. This patient had cutaneous lesions of more than 40 yrs duration. The cutaneous lesions may be because of leishmaniasis, but Biopsy of the nodule is not done as the patient did not give consent. She has 10 children and the last 4 children died during childhood with H/O high fever for more than 1 month duration, may be that they had fever of leishmaniasis.

This patient had pancytopenia. In literature, pancytopenia of varying degree of frequency and severity has been reported by several group of workers [18, 25, 26]. Pancytopenia is the most common hematological abnormality (85%) [27]. In our case, serum globulins were also raised, but

we have not done serum immunoglobulin electrophoresis. However in clinical practice, VL patients often present with increased levels of serum immunoglobulins and increased production of autoantibodies, which could easily lead to a misdiagnosis as SLE [34]. We found in our case Anti CCP Ab is Positive, and Anti ds DNA is Negative, it is in consonance with, several investigators who have revealed the presence of autoantibodies against cellular and humoral components as well as against nuclear antigens [36, 37].

Conclusion

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Leishmaniasis is to be included in the diagnostic considerations in patients presenting with persistent pancytopenia [50], and a positive travel history to endemic areas, as an effective therapy of this potentially life-threatening disease is available.

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