Unique Triad of ‘Pregnancy, Kala Azar and Hemophagocytic Lymphohistiocytic Syndrome from a Non-Endemic Region’

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Abstract

India and neighboring Nepal, Bangladesh along with Sudan and Brazil are the four largest foci of visceral leishmaniasis and account for 90% of the world’s visceral leishmaniasis (VL) burden, with India being the worst affected. High degree of suspicion is usually based on patient presenting from endemic area with features of pancytopenia hepatosplenomegaly. Hemophagocytic lymphohistiocytic (HLH) syndrome also presents with similar clinical features. Visceral leishmaniasis leading to secondary HLH syndrome is in itself a rare entity while both of these presenting in pregnant patient, to the best knowledge of the authors, is yet to be described in literature.

Introduction

Hemophagocytic lymphohistiocytosis (HLH) can be either primary or secondary. Most common causes of secondary HLH are infections or malignancies.¹ ² Visceral leishmaniasis leading to secondary HLH is rare. Though both HLH and visceral leishmaniasis are reported separately during pregnancy, however, co-occurrence of both during pregnancy is not described in the published literature.³ ⁴ In the present case report, we are describing for the first time a case of concurrent visceral leishmaniasis and HLH in a pregnant patient.

Case Report

A 30-year-old pregnant female, a resident of Himachal Pradesh with no known comorbidities, presented to the emergency room of the All India Institute of Medical Sciences (AIIMS), New Delhi in July 2013 with a history of high-grade fever and easy fatigability for the past 10 days along with history of secondary amenorrhea of 6-month period. The fever was associated with chills and rigors, continuous in character with no diurnal variation, with partial response to antipyretics. She also complained of generalized weakness and easy fatigability. There were no associated complaints of cough, expectoration, dysuria, skin rash, joint pains, nausea, vomiting, photophobia, bleeding from any site, pain in abdomen, per vaginal discharge. Her obstetric status was G₂P₁L₁. She had received oral antibiotics and anti-malarial with no response. She was also transfused two units of packed red blood cells for anemia. She was on oral iron and folic acid supplements. She was following-up at antenatal care center at the local hospital. She was strictly vegetarian.

At admission, she was febrile with temperature of 103°F. Pallor was present, however, there was no icterus, clubbing, cyanosis, lymphadenopathy, skin rash or pedal edema. The ocular fundus examination was normal. The sternal tenderness was not present. Cardiovascular system examination revealed normal first and second heart sounds with grade 2 pansystolic murmur in the mitral area with no radiation to other areas that was increasing on expiration. Mild splenomegaly was palpable. Gravid uterus was palpable in the hypogastric area with size corresponding to 24 weeks of gestation. Examination of respiratory and central nervous system revealed no abnormality.

Initial available investigations showed haemoglobin - 4.5 g/dl, total leucocyte count – 1100/mm³, absolute neutrophil count - 450/mm³, and platelet count – 57,000/mm³.

Thus summarizing, we have a 30-year-old pregnant lady (24 weeks of gestation) with no previous comorbidities having ten days history of high grade fever with severe pallor, mild splenomegaly...
and a grade 2 pansystolic murmur present in the mitral area with baseline investigations showing pancytopenia. Possible differentials considered at presentation were enteric fever, infective endocarditis, urinary tract infection with sepsis, viral infections like dengue fever or infectious mononucleosis, complicated malaria, autoimmune conditions like systemic lupus erythematosus and megaloblastic anemia with superadded infection.

Her investigations done on day 1 at AIIMS hospital revealed haemoglobin of 6.4 g/dl, total leucocyte count of 1400/mm³, absolute neutrophil count of 420/mm³, platelet count of 50,000/mm³ and erythrocyte sedimentation rate – 22 mm/1st hr. Peripheral smear showed normocytic normochromic RBC morphology with toxic granulations. Renal functions and electrolytes were within normal limits. Liver functions revealed raised aspartate aminotransferase (AST) 192 IU with normal alanine transaminase (ALT), elevated serum alkaline phosphatase (ALP) 484 IU and reversal of albumin: globulin ratio. She was started on broad antibiotic cover of intravenous piperacillin-tazobactum, clindamycin and azithromycin in view of febrile neutropenia. Packed red blood cells were transfused to keep haemoglobin above 7 g/dl. Patient was monitored periodically for any signs of fetal distress. Level II ultrasound (USG) was done which revealed severe oligohydramnios.

Routine work-up for acute febrile illness in form of urine routine microscopy, urine and blood cultures, Widal test, peripheral smear for malaria and dengue serology were negative. Despite administration of broad spectrum antibiotics she continued to have persistent fever. Transthoracic 2-D echocardiography revealed mild mitral regurgitation; no evidence of infective endocarditis. USG abdomen was suggestive of mild hepatosplenomegaly. Bilateral mild hydroureteronephrosis was also present. Work-up for cause of anaemia revealed low vitamin B₁₂ levels with normal serum folate. She was started on parenteral vitamin B₁₂ supplementation, oral iron and folate supplements were continued. Intravenous vancomycin was added on day 4 to cover Gram positive microorganisms as per febrile neutropenia protocol since she continued to remain febrile (Figure 1A).

On day 6 of admission, she started complaining of respiratory distress. Examination revealed respiratory rate of 32 per minute with bilateral coarse crepitations in bilateral lung fields. Arterial blood gas analysis (ABG) revealed acute respiratory alkalosis with metabolic compensation with features of type 1 respiratory failure (RF) with PaO₂/FIO₂ ratio of 290. Chest X-ray done with a cover of abdominal shield showed bilateral lung infiltrates predominantly in lower and middle zones (Figure 2). This picture was compatible with acute lung injury (ALI). Pancytopenia also showed worsening trend. Based on this progression revised differentials of acute haematological malignancy, disseminated Epstein Bar (EBV)/cytomegalovirus (CMV)/ fungal infection and HLH were considered.

Intravenous amphotericin-B deoxycholate was added as an antifungal agent as per febrile neutropenia protocol. Work-up for EBV/CMV was negative. Bone marrow aspirate was negative for bacterial culture, acid-fast bacilli (AFB) and Leishman-Donovan (LD) bodies. Bone marrow picture was normal. Her serum ferritin levels were markedly elevated (>25,000 ng/ml) with raised triglycerides (442 mg/dl). There are very few differentials for such high levels of ferritin; mainly HLH, adult onset Still’s disease, systemic lupus erythematosus and iron overload states. For diagnosis of HLH, serum ferritin levels > 10,000 ng/ml has about 96% specificity in children, while for adults above-mentioned causes need to be ruled out.¹ Our patient fulfilled five of eight criteria of HLH i.e. fever, hepatosplenomegaly, cytopenias, elevated serum ferritin and metabolic alkalosis.
syndrome characterised by inappropriate activation of macrophages along with lymphocytes. It can be either primary or secondary to underlying infection, malignancy or autoimmune condition. Primary HLH usually occurs in children and it is a hereditary autosomal recessive disease, affecting immune regulation. Defective natural killer (NK) activity and cytotoxic T-cell function lead to the inappropriate uncontrolled accumulation of activated T-lymphocytes and activated macrophages. It is thought that the excessive cytokines are ultimately responsible for multi-organ failure and the high mortality of the syndrome. The major pathogenic role of TH1 hyperactivation is suggested by the presence of high levels of cytokines in HLH. This is evident by the coexistence of HLH TH1-related infections such as tuberculosis and leishmaniasis. This also supports the fact that tuberculosis is one of the leading causes amongst infections leading to secondary HLH in developing countries. The bone marrow in HLH may show the activated macrophages with the engulfed blood cells (hemophagocytic histiocytes). But the finding of hemophagocytosis is not present in all the cases. Also an isolated finding of hemophagocytic histiocytes is not diagnostic of the disease. The occurrence of HLH in pregnancy is also rare. As HLH in adults is mostly secondary further work-up for secondary causes of HLH was done in our case. Viral serology for HIV, EBV, CMV were negative. There were no blasts on bone marrow and no evidence of LD bodies on marrow. It must be noted that the marrow in our patient did not show the hemophagocytic histiocytes. All autoimmune markers were negative.

The goal of therapy in HLH is to suppress life-threatening inflammation by destroying immune cells. As per HLH-94 protocol induction therapy consists 8 weeks of weekly treatment with dexamethasone and etoposide, followed by cyclosporin. In patients with central nervous system involvement intrathecal methotrexate is given. Patients showing improvement are weaned off therapy while those not improving are continued on therapy as a bridge to allogenic stem cell transplantation. Until now we have limited treatment options for HLH in pregnancy. Since high-dose corticosteroids have been used safely in pregnancy, they should be considered one of the treatment options in pregnancy-related HLH. After explaining side-effects of steroids on developing fetus and mother, she was started on intravenous dexamethasone 10 mg/m². On 2nd day of adding steroids her fever subsided (Figure 1B). Cytopenias started improving and respiratory parameters revealed improvement. Further immunosuppression was not considered as patient showed significant improvement with dexamethasone. On day 8th of hospitalization, antibodies to rk-16 antigen were detected. It is a 39-amino-acid protein derived from the C terminus of the kinesine protein of Leishmania donovani from Indian isolates. It has specificity of 99.5% compared to commonly used rk-39 which has 97% specificity, while showing similar sensitivity. Sensitivity of bone marrow to detect LD bodies is still low (65-80%). It is important to note that during initial phase of illness the aspirates from bone marrow are usually normal, therefore, repeat sampling as well as use of sensitive serological method is considered vital in diagnosis of this condition. Visceral leishmaniasis causing secondary HLH is a rare entity. There is an overlap in the clinical presentations of VL and HLH, both of which present with fever, hepatosplenomegaly and pancytopenia. However, VL patients consistently have marked hypertriglyceridemia (Table 1). Based on this, the diagnosis of HLH was considered.

### Hemophagocytic lymphohistiocytosis (HLH)

HLH can be diagnosed if either of the following two are met:

1. A gene mutation consistent with familial HLH is present (these include mutations of PRF, UNC13D and STX11), or
2. At least five out of the eight diagnostic criteria for HLH are fulfilled:
   1. Fever
   2. Splenomegaly
   3. Cytopenia affecting ≥ 2 of 3 lineages:
      - Hemoglobin < 90 g/L (in infants under four weeks, hemoglobin < 100 g/L; Platelets < 100 x10⁹/L; Neutrophils < 1.0 x10⁹/L)
   4. Hypertriglyceridemia and/or hypofibrinogenemia: fasting triglycerides ≥ 3.0 mmol/L (≥ 265 mg/dl), OR fibrinogen ≤ 1.5 g/L
   5. Hemophagocytosis in bone marrow, spleen or lymph nodes
   6. Low or absent NK-cell activity (using local laboratory reference ranges)
   7. Ferritin ≥ 500 ug/L
   8. Soluble CD25 (i.e., soluble IL-2 receptor) ≥ 2,400 U/ml

### Notes:

1. If hemophagocytic activity is not found at presentation, further search for hemophagocytic activity should be done. If the bone marrow is inconclusive, biopsies of other organs or serial bone marrow aspirates are helpful.
2. The following findings provide supportive evidence for HLH:
   1. Spinal fluid pleocytosis (mononuclear cells) and/or elevated CSF protein
   2. Liver histology resembling chronic persistent hepatitis
3. Other findings consistent with HLH are:
   1. Cerebromeningeal symptoms
   2. Lymphadenopathy
   3. Jaundice
   4. Edema
   5. Rash
   6. Raised liver enzymes
   7. Hyperproteinemia
   8. Hyponatremia
   9. Raised VLDL
   10. Lowered HDL
hypergammaglobulinaemia and the patients with HLH have haemophagocytes in the marrow with or without coagulopathies.

Based on this a final diagnosis of G2P1L1 with 24 weeks of gestation with oligohydramnios with vitamin B12 deficiency with visceral leishmaniasis with secondary HLH was made. Suppression of cell-mediated immune mechanisms during pregnancy favors infections sustained by intracellular agents, however, review of literature does not indicate an increase in the risk of VL in pregnant women. Higher susceptibility to parasitic infections has been reported in primi-parous women than in multiparous women because of decreased natural killer cell cytotoxicity. This may explain the higher prevalence of parasitic infections in women in their first pregnancy as observed in our cases.

Fetal transmission is reported in areas where *L. donovani* is endemic. To evaluate if vertical transmission had occurred, a placental study is recommended in order to observe the presence of the parasite just after delivery.

In our case, intravenous amphotericin B deoxycholate was continued and later on switched to liposomal preparation in order to minimize nephrotoxicity. Total duration of therapy was 3 weeks with cumulative dose of 1300 mg (21 mg/kg). During the course of amphotericin B, renal functions and electrolytes were closely monitored. Dexamethasone was switched to oral based preparation and gradually tapered with total duration of treatment being 3 weeks. Other antibiotics were de-escalated. She continued to remain afebrile with blood picture showing improving trend. She was discharged with advice to follow-up in the outpatient department under the department of Obstetrics and Gynecology. She had full term normal vaginal delivery on 28/10/2013 at 38 weeks period of gestation. Baby (birth weight 2.16 kg with APGAR score- 8/8) was kept in the Intensive Care Unit for tachypnea at birth that settled down. She was discharged after one month of hospitalization and was further followed-up.

In conclusion, this case is unique as the combination of visceral leishmaniasis (VL), pregnancy and HLH together has not been reported in the literature. This patient was not a resident of area that is endemic for VL like Bihar, Jharkhand or West Bengal. She also had no history of travel to these areas. Several case reports of VL as well as cutaneous leishmaniasis have been described from Himachal Pradesh and in most of these patients the diagnosis of VL was missed during initial evaluation. Thus, there is a need for increased surveillance and education of primary care physicians regarding endemic nature of VL in this particular area.

Also it is important to note that VL can lead to secondary HLH and early treatment with immunosuppressive treatment have good outcome. Both VL and HLH have overlapping presentation and high degree of clinical suspicion is needed for accurate diagnosis.

References