Original Article

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Secondary Hemophagocytic Lymphohistiocytosis

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ABSTRACT

Introduction: Hemophagocytic lymphohistiocytosis (HLH) is a rare hematological disorder which may be fatal due to uncontrolled activation of immune system. It is characterized by hemophagocytosis in bone marrow, liver or lymph nodes along with presence of fever, pancytopenia, splenomegaly. It has been associated with bacterial, viral, fungal and parasitic infection.

The study aims to explore the utility of bone marrow aspiration in patients with pancytopenia and bicytopenia which supports the diagnosis in a scenario where there is clinical suspicion of HLH.

Material and Methods: prospective study carried out in department of pathology from August 2018 to July 2019. We studied 88 cases of pancytopenia and bicytopenia. All relevant clinical history and investigations were collected. Bone marrow aspiration done and smears were studied in all the cases.

Result: Out of 88 cases studied 28 patients were having bicytopenia and 60 were having pancytopenia with male to female ratio 13 :9.Out of these 88 patients we reported 3 patients as having HLH who fulfill the criteria according to Revised Diagnostic Guidelines for hemophagocytic lymphohistiocytosis (HLH).

Conclusion: An early diagnosis and treatment is the key to decrease the mortality caused by this dreadful disease and if left undiagnosed and undertreated, Hemophagocytic lymphohistiocytosis can be rapidly progressive and potentially fatal.

Keywords: *Hemophagocytic Lymphohistiocytosis; Activated Macrophage, Immune Activation.*

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a life threatening hematological disorder characterized by over activation of macrophages and T lymphocytes leading to uncontrolled over production of inflammatory cytokines in the body which results in multi organ inflammation. HLH can be familial or acquired, with a spectrum of etiologies including autoimmune disorders, malignancies as well as infections. Varied skin manifestations of this disease including generalized purpuric macule and papules , erythroderma, and morbilliform eruption can assist in the initial diagnosis of this disease with better outcome and potentially signify recurrence.

The study aims to explore the utility of bone marrow aspiration in patients with pancytopenia and bicytopenia which supports the diagnosis in a scenario where there is clinical suspicion of Hemophagocytic lymphohistiocytosis.

Material and Methods

This study was conducted at Mahatma Gandhi Medical College Indore (M.P) between June 2016 to July 2019 in department of pathology from August 2018 to July 2019 in which a total of 88 patients with pancytopenia and bicytopenia were included in the study. Clinical details of the patients were collected from the case sheet from respective departments. Bone marrow aspiration was done under all aseptic precautions using Jamshidi's needle after written consent . The bone marrow slides were stained using Romanowsky stain. Simultaneously blood sample in EDTA was also obtained from brachial vein and complete blood count and peripheral smear was examined along with bone marrow reporting.

Exclusion criteria :- All samples showing diluted marrow in the smears were excluded from this study.

Result

Out of total 88 cases , 60 cases were of pancytopenia and 28 cases were of bicytopenia (Graph 1). Age of patients ranged from 5 years to 70 years. Maximum cases fall between age group of 5 to 10 years. There were 52 males and 36 females. Male to female ratio was 13:9. (Graph 2). Out of 88 cases we diagnosed 3 cases having HLH which was secondary to viral infection and autoimmune disorder. Out of 3 cases of HLH, two were male and one female. The complete presentation of three cases has been discussed below-

Case Study 1**:** A 12 year old male presented with two month history of progressive malaise, body aches, fever for

one month associated with cough and not taking feeds for 5-6 days along with multiple pustules and papule on dorsal surface of hands and forearm.(Figure 1). All relevant clinical history and investigations were collected from patient's case sheet. On general examination patient was febrile with fever 102 °F and respiratory rate 24/min along with multiple pustules and papule on dorsal surface of hands and forearm along with generalized lymphadenopathy. On per abdomen examination-hepatosplenomegaly was present. Complete Haemogram revealed pancytopenia with Hemoglobin 5.6 gm/dl, Platelet count 22000/mm3 and Total leukocyte count 900/mm3 .Peripheral blood examination revealed Normocytic anaemia with thrombocytopenia. (Figure 2) Raised ESR (52mm at end of 1 hr) .Biochemical profile levels revealed raised SGPT (70u/L), Ferritin Raised (694ng/ml), Triglyceride Raised(275 mg/dl) and LDH raised (876U/L) Besides that peripheral smear does not show any presence of abnormal or blast cells. In view of pancytopenia, his bone marrow aspiration was done. Bone marrow aspiration smears revealed lymphocytosis, increased histiocytes showing hemophagocytosis along with foamy macrophages with cell debris.(Figure 3 and 4)

In our case 6 out of 8 criteria were fulfilled; fever ,hepatosplenomegaly, pancytopenia, raised triglyceride, raised ferritin and hemophagocytosis in bone marrow examination. We correlates all the clinical and laboratory findings and a final diagnosis of Hemophagocytic lymphohistiocytosis secondary to viral infection was made. The patient was supplemented with intravenous immunoglobulin along with antibiotics, steroids and pack cells and PRP transfusion. Finally the patient recovered very fast and was discharged with fruitful outcome.

Case Study 2 : 20 year old female presented with history of fever for one month associated with arthralgia and one episode of GTCS 1 day back. All relevant clinical history and investigations were collected from patient's case sheet. On general examination patient was febrile with fever of 101.2 °F and respiratory rate 16/min along with oral ulcers , pallor and icterus. Systemic examination had generalized maculopapular rashes, nasolabial fold spare , oral ulcers, splenomegaly. Complete Haemogram revealed pancytopenia with Hemoglobin 5.7 gm/dl, Platelet count 50000/mm3 and Total leukocyte count 2000/mm3 .Peripheral blood examination revealed Normocytic anaemia with thrombocytopenia. Biochemical profile levels revealed raised SGPT (877U/L), Ferritin Raised (1650ng/ml), Triglyceride raised (528 mg/dl) and direct bilirubin raised (6.8mg/dl). ANA by IFA revealed +4 homogenous pattern. Besides that peripheral smear does not show any presence of abnormal or blast cells. Anti SMA and Anti LKM both are negative. In view of pancytopenia, her bone marrow aspiration was done. Bone marrow aspiration smears revealed lymphocytosis, increased histiocytes showing hemophagocytosis along with foamy macrophages. In this case 6 out of 8 criteria were fulfilled; fever , hepatosplenomegaly, pancytopenia, raised triglyceride, raised ferritin and hemophagocytosis in bone marrow examination. So a final diagnosis of Hemophagocytic lymphohistiocytosis secondary to SLE was made. Patient was given IV pulse therapy with methylprednisolone 1gm for 5 days, meropenam 1 gm , levetiracetam 500 mg , fluconazole and pack cells and PRP transfusion.

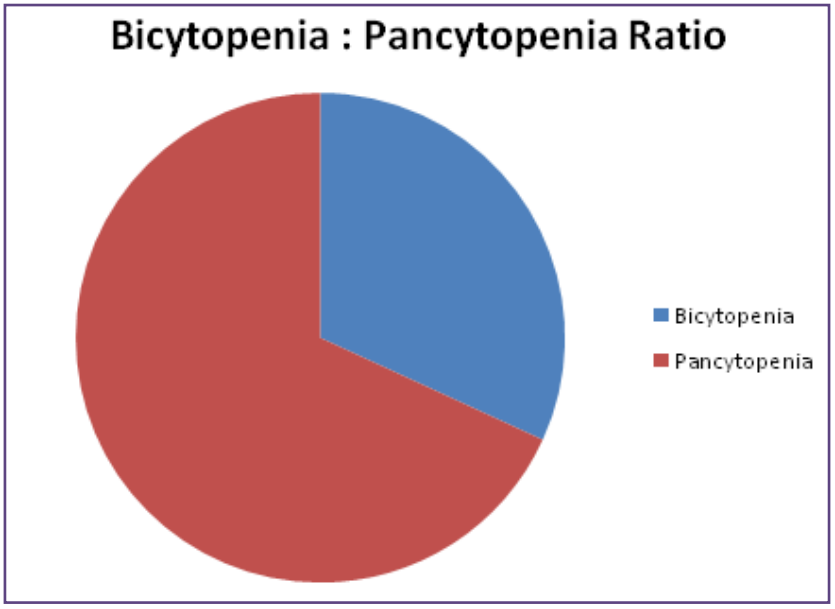
Case Study 3 : 40 year old male presented with history of fever , generalized weakness and yellowish discoloration of sclera since 2 weeks. All relevant clinical history and investigations were collected from patient's case sheet. On general examination patient was febrile with fever of 103.0 °F, pallor and scleral icterus. Systemic examination shows hepatosplenomegaly only as positive finding. Complete Haemogram revealed bicytopenia with Hemoglobin 9.0 gm/dl, Platelet count 16000/mm3 and Total leukocyte count 8000/mm3 with 7 % atypical cells. Peripheral blood examination revealed blast cells with high N:C ratio, irregular nuclear borders, prominent nucleoli, mitosis and azurophilic cytoplasmic granules along with cells showing multilobated nuclei resembling clover leaves. Biochemical profile levels revealed raised SGPT (987u/L), Ferritin Raised (5578ng/ml), direct bilirubin raised (7.2mg/dl), LDH raised (6374U/L), decreased serum fibrinogen (0.9 g/L) and elevated serum EBV DNA level 55842 copies/ml as detected by RT-PCR. His bone marrow aspiration was done. Bone marrow aspiration smears revealed 66% atypical cells along with lymphocytosis, increased histiocytes showing hemophagocytosis. Immunophenotyping showed atypical cells positive for CD2, CD16, CD56, CD7, CD45 suggestive of Natural killer cells. In this case 6 out of 8 criteria were fulfilled; fever, hepatosplenomegaly, bicytopenia, raised ferritin , decreased fibrinogen and hemophagocytosis in bone marrow examination. So a final diagnosis of Natural Killer Cell Leukemia With Hemophagocytic Lymphohistiocytosis was made. Complete observation of all the findings of three cases has been shown in Table 1.

Discussion

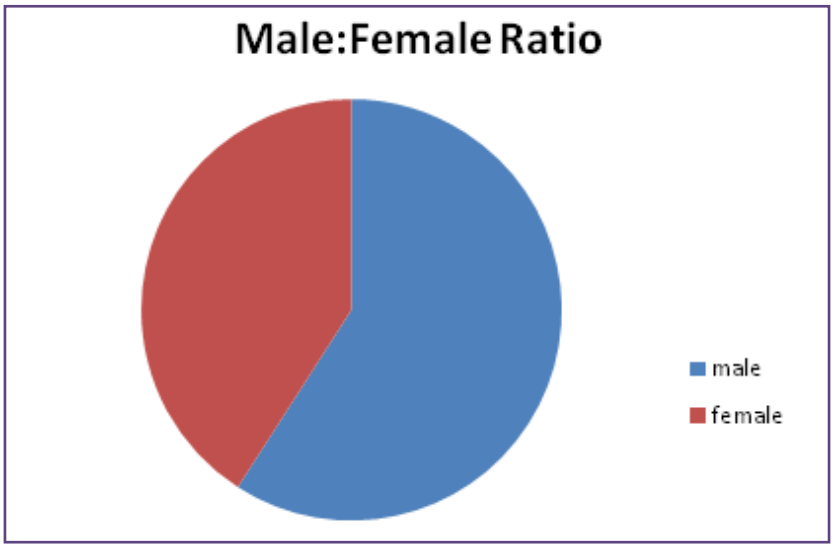
Hemophagocytic lymphohistiocytosis (HLH) is a clinical entity caused by a sustained activation of the mononuclear phagocytic system that may result in an extreme hyper inflammatory response(2) . HLH can be diagnosed by the criteria proposed by the Histiocyte Society(4) that include clinical, laboratory, and histopathologic features. The diagnosis of HLH can be established if either 1 or 2 below

Table 1: Complete observation of all the findings of three cases.

Case	Clinical features	Temp-erature	Hemoglobin (gm/dl)	TLC(/ cumm0	Platelet (/cumm)	Serum ferritin (ng/dl)	Serum triglyceride (mg/dl)	BMA	Fibrinogen (ng/l)	Associate illness
12Yr/M	Fever, hepatosplenomegaly, papules on hand	102°F	5.6	900	22000	694	275	hemophagocytosis	-	Viral infection
20 Yr/F	Fever, hepatosplenomegaly, arthralgia	101.2°F	5.7	2000	50000	1650	528	hemophagocytosis	-	SLE
40Yr/M	Fever, hepatosplenomegaly ,jaundice	103°F	9.0	8000	16000	5578	Not done	hemophagocytosis	0.9	NK cell leukemia



Graph 1 :- Bicytopenia : Pancytopenia ratio.



Graph 1 :- Bicytopenia : Pancytopenia ratio.

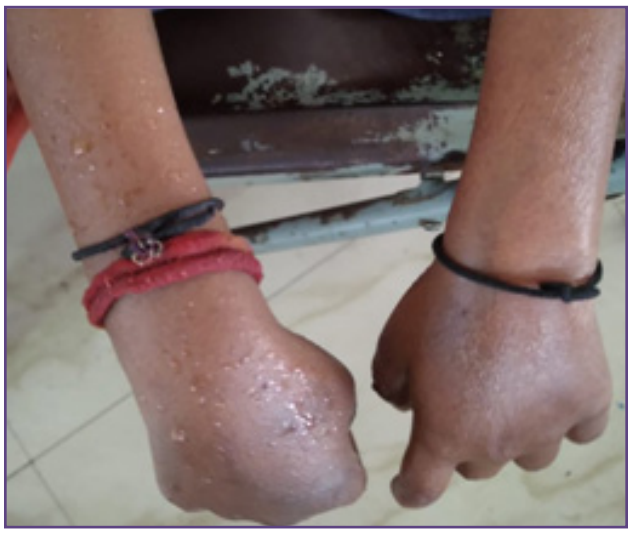


Fig. 1: Multiple pustules and papule on dorsal surface of hands and forearm.

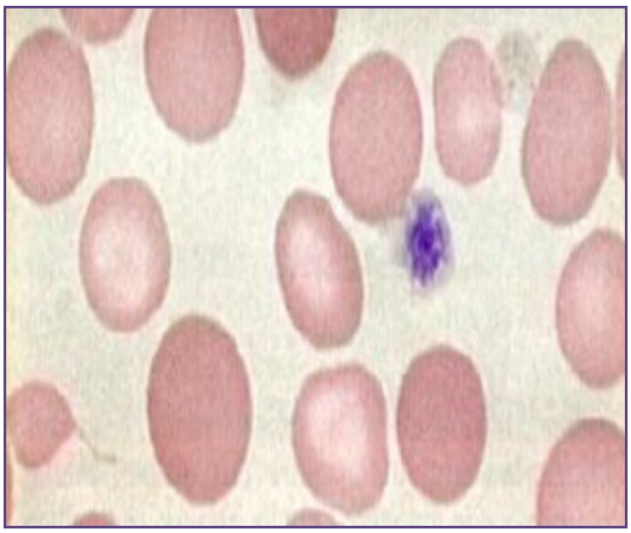


Fig. 2: Peripheral blood examination showing Normocytic anaemia with thrombocytopenia.

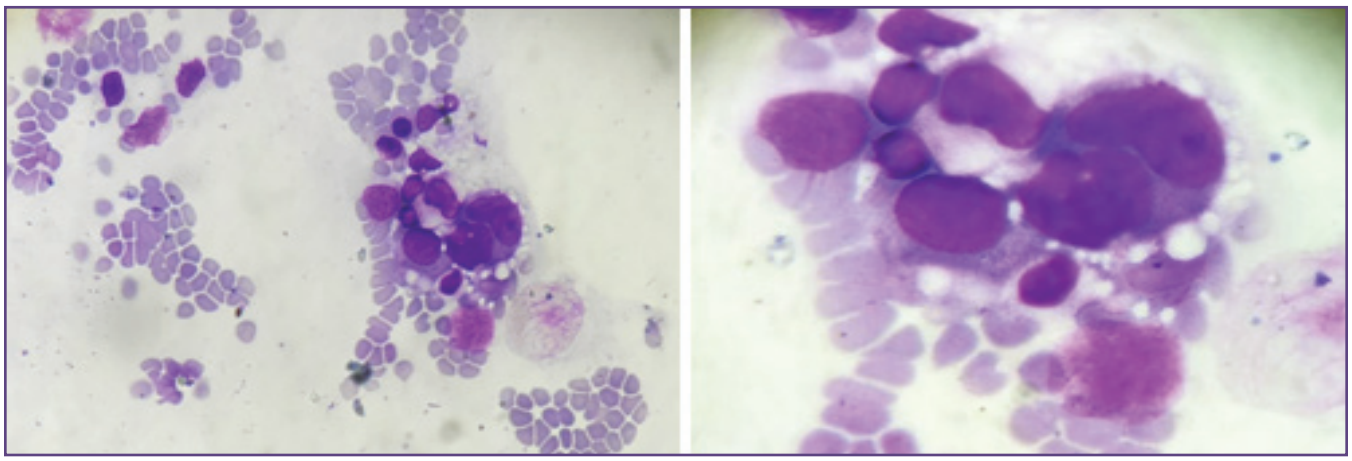


Fig. 3 & 4: Bone marrow aspiration smears showing increased histiocytes with hemophagocytosis along with foamy macrophages with cell debris.

are fulfilled according to Revised Diagnostic Guidelines for hemophagocytic lymphohistiocytosis (HLH) ie; Molecular diagnosis consistent with HLH or diagnostic criteria for HLH fulfilled (5 out of 8 criteria below) that are - Fever, splenomegaly, cytopenias affecting = 2 of 3 lineages: Hemoglobin < 9 g/dl , Platelets < 100 x10^9 /L; Neutrophils < 1.0 x 10^9 /L, hypertriglyceridemia and/ or hypofibrinogenemia: fasting triglycerides = 3.0mmol/L (= 265mg/dl), OR fibrinogen = 1.5 g/L, hemophagocytosis in spleen, bone marrow or lymph nodes, low or absent Natural Killer -cell activity Ferritin = 500 ug/L and soluble CD25 (i.e., soluble IL-2 receptor) = 2,400 U/m.

The disease presents as fever, hepatosplenomegaly, lymphadenopathy, jaundice, and skin rashes. HLH can occur in two forms: primary and secondary. Primary HLH is also called as familial form (familial hemophagocytic lymphohistiocytosis [FHLH]) is rapidly fatal autosomal recessive disorder and is associated with immune deficiencies such as Chédiak Higashi syndrome(CHS). Secondary HLH commonly presents in adulthood, is also known as acquired HLH. It is usually associated with an underlying infection, malignancy or an autoimmune disease which can lead to a hyperactive immune response(3) and may be due to presence of perforin gene in some patients . Familial HLH triggered by an infection may be difficult to distinguish from HLH associated with infection. The distinction is important; as allogeneic bone marrow transplantation is the therapy of choice in patients with familial HLH (5) whereas secondary HLH respond well when the underlying cause is treated. HLH triggered by bacterial infection has a high recovery rate. Histopathologically hemophagocytosis is seen in bone

marrow, lymph nodes, spleen, and occasionally the skin and central nervous system. Erythrocytes, leukocytes platelets, their precursors, and cellular fragments are engulfed by activated macrophages. Appropriate broad spectrum antibiotics, steroids, blood transfusion and supportive therapy are given. Early recognition and treatment with chemotherapeutic agents or bone marrow transplant may reduce mortality and morbidity caused by the disease. The prognosis is guarded with an overall mortality of 56 %. Poor prognostic factors included HLH associated with malignancy, with half the patients dying by 1.4 months compared to 22.8 months for non-tumour associated HLH patients(9).

Secondary HLH in some individuals may be self-limited because patients are able to fully recover after having received supportive medical treatment (i.e., IV immunoglobulin only)(8) as seen in our case number 1 in which patient show quick recovery after supplementation of immunoglobulin. However, long-term remission in cases of HLH without the use of cytotoxic and immune-suppressive therapies is unlikely in the majority of adults with HLH and in those with involvement of the central nervous system (brain and/or spinal cord)(11).

Conclusion

HLH is a clinical syndrome of uncontrolled and ineffective immune response along with hyper inflammation. In patients presenting with splenomegaly, cytopenias, elevated liver markers and increased inflammatory markers, a high level of suspicion for HLH is required. If left undiagnosed and undertreated, HLH can be rapidly progressive and potentially fatal. Early diagnosis and treatment may decrease the morbidity and the fatal outcome of the disease.

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