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Original Article

Internal Medicine Section

Hemophagocytic Lymphohistiocytosis in Adults and Adolescents - Experience from a Tertiary Care Centre in South India

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ABSTRACT

Introduction: Hemophagocytic lymphohistiocytosis (HLH) is a rare syndrome of immune dysregulation. It is characterized by hypercytokinemia and macrophage activation resulting in fever, cytopenia, splenomegaly and hyperferritinemia leading to fatal outcomes if untreated.

Aim: To study clinical profile, diagnostic and etiological workup, treatment and prognosis of hemophagocytic lymphohistiocytosis patients.

Materials and Methods: We report retrospective analysis of 8 cases of adult and adolescent HLH admitted over a period of 2 years at JIPMER, a tertiary care centre in South India.

Results: Mean age of patients was 27 year (range 13 to 57 years) and 3 were adolescents. Median duration of symptoms was 10 days (5-60 days). Common presenting symptoms were fever, jaundice, abdominal pain, rash and seizures. Physical findings included pallor, icterus, splenomegaly, hepatomegaly and lymphadenopathy. Laboratory findings were variable cytopenia with pancytopenia in 65% cases, hyperferritinemia (100%), hypertriglyceridimia (75%) and elevated serum bilirubin (62.5%) and liver enzymes (87.5%). Underlying cause could be detected in only 6 patients with one each of dengue fever, lymphoma, tuberculosis, EBV infection, scrub typhus and juvenile idiopathic arthritis. Median overall survival at the end of 1.5 years was 62%.

Conclusion: Low threshold for suspicion, adequate evaluation and timely treatment can improve outcome in patients of HLH.

Keywords: Immune dysregulation, Epstein- Barr virus, NK/T cell lymphoma

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is a rare disorder due to immune dysregulation of varied origin which leads to cytokine storm, unregulated macrophage and Natural killer and T cell (NK/T cell) activity leading to progressive cytopenias and death if untreated [1]. Relapses are common and ultimate treatment is bone marrow transplantation in primary or familial HLH due to tendency of recurrence and associated high mortality. Familial HLH is usually restricted to young children with an incidence of 1.2/1,000,000 children per year [2,3]. Most of these events are triggered by infections. Secondary HLH is mainly due to underlying infections, malignancies or autoimmune disorders. Actual incidence and prognosis of secondary HLH is not well known [4,5]. It is difficult to differentiate between primary and secondary HLH without genetic molecular studies.

MATERIALS AND METHODS

We retrospectively studied clinical presentation, underlying cause, laboratory investigations, management and follow-up outcome of eight cases of HLH.

We reviewed the case record of patients diagnosed with hemophagocytic lymphohistiocytosis during a period of January 2011 to January 2013 in Department of Medicine, Division of Hematology, JIPMER, Pondicherry, a tertiary care centre in South India. Eight cases fulfilled HLH 2004 and HLH 2009 proposed diagnostic criteria and were included in the study. All the patients were either adolescents or adults. Patients below 13 years of age were not included in this analysis. We collected and analyzed details of demographic data, clinical history, underlying etiology, laboratory investigations, management and follow-up outcomes of these 8 patients. None of the patients except one adolescent were studied for underlying genetic molecular tests or flowcytometry for perforin protein due to unavailability of these tests on routine basis. Institute ethical committee approval was taken.

RESULTS

Eight patients were diagnosed as a case of hemophagocytic lymphohistiocytosis during a period of 2 years. Mean age of our patients was 27 years (median 20.5 years, range 13-57 years). Three patients were in adolescent age group. Male to female ratio was 3:5. None of our patients had family history of rheumatic disease, consanguineous marriage, malignancy or death of siblings.

Most of the patients attended hospital with prolonged fever of more than 10 days (range- 10 days to 8 week). Fever was commonly associated with rash, jaundice, bodyache, headache, abdominal pain, breathlessness and rarely with bleeding manifestation and vomiting. Most common physical findings were rash, pallor, splenomegaly, hepatomegaly, lymphadenopathy and pedal edema. [Table/Fig-1] shows the major clinical presentations and examination findings of these patients.

Patients were extensively investigated for making diagnosis and to find out the underlying cause or precipitating infection. Bone marrow samples were sent for fungal and tubercular cultures. Blood culture, malaria parasite by smear and antigen test, NS-1 antigen and IgM antibodies for dengue, Widal, Weil-Felix, Brucella serology, Leptospira serology, HIV/ELISA, HBsAg, HCV antibody , Antinuclear antibodies (ANA) tests, and EBV antigen assay were done in all patients. [Table/Fig-2] shows values of the important laboratory parameters during evaluation of patients.

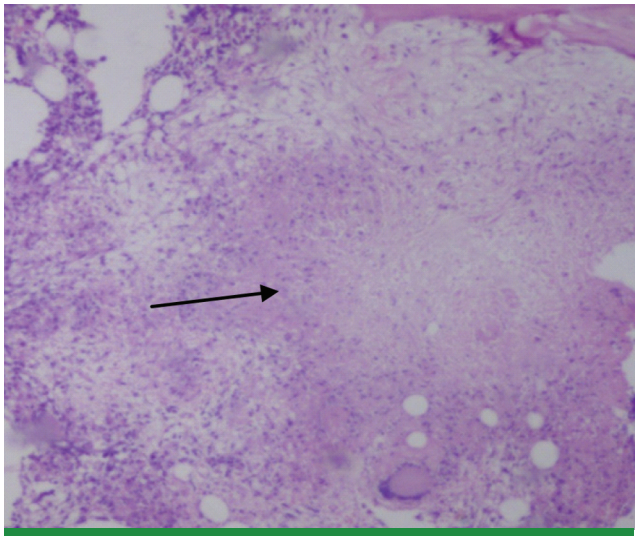
Underlying cause could be detected in 6 patients (75%). One patient had evidence of caseating granuloma in bone marrow biopsy [Table/Fig-3] and was considered to have possible tuberculosis. AFB staining and Mantoux test were negative. Patient with NK/T cell lymphoma was found to have features of

Clinical Characteristics	Case-1	Case-2	Case-3	Case-4	Case-5	Case-6	Case-7	Case-8	Total n (%)
Fever	+	+	+	+	+	+	+	+	8 (100%)
Jaundice	_	_	+	+	+	+	+	_	5 (62%)
Breathlessness	_	_	+	+	+	_	+	_	4 (50%)
Rash	_	_	_	+	+	_	+	_	3 (37%)
Body ache	+	_	+	+	_	+	_	_	4 (50%)
Abdominal pain	_	_	+	+	_	+	_	_	3 (37%)
Joint pains	_	_	+	_	_	+	_	_	2 (25%)
Headache	_	_	_	_	_	_	+	_	1 (12%)
Seizures	_	_	_	_	+	_	_	_	1 (12%)
Pallor	+	+	+	+	+	+	+	_	7 (87%)
Icterus	_	+	+	+	+	+	+	_	6 (75%)
Hepatomegaly	_	_	+	+	+	+	+	_	5 (62%)
Splenomegaly	+	+	+	+	+	+	_	_	6 (75%)
Lymphadenopathy	_	_	+	+	+	_	+	_	4 (50%)
Pedal odema	+	_	_	+	_	_	+		3 (37%)
Etiology	Not known	Not known	Tuberculosis	Lymphoma	EBV	Dengue	Scrub typhus	JIA	6 (75%)

[Table/Fig-1]: Shows patients clinical characteristics and physical findings at the time of diagnosis as HLH.

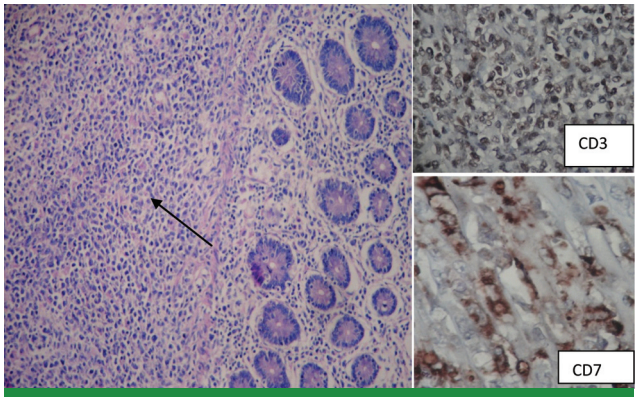
Lab Parameters	Mean Value (Range)	n (%)
Hb (gm/dl)	7.2 (3.5-11.0)	Anemia Hb < 12 = 8 (100%)
TLC (/cmm)	4377 (820-11,000)	Leucopenia < 4000 = 6 (75%)
Platelets (/cmm)	72000 (14000-200,000)	Thrombocytopenia < 100000 = 6 (75%)
Pancytopenia	-	6 (75%)
Serum ferritin (mcg/L)	13500 (820-90,000)	Ferritin>500 = 8 (100%)
Serum Triglyceride (mg/dl)	458 (251-796)	Triglyceride>265 = 6 (75%)
Serum Fibrinogen (mg/dl)	187 (60-350)	Fibrinogen<150 = 2 (50%)
Bone marrow e/o hemophagocytosis	-	6 (75%)
Serum creatinine (mg/dl)	0.83 (0.4-1.8)	Creatinine >1.5 = 1 (12.5%)
Serum Bilirubin (mg/dl)	3.8 (1-22)	S. bilirubin > 1.5mg/dl = 5 (62.5%)
SGPT	87.5 (45-463)	>ULN = 7(87.5%)
SGOT	94 (43-463)	>ULN = 6(75%)
SALP	165 (120-269)	>ULN = 1(12.5%)

[Table/Fig-2]: Shows the important laboratory parameters during evaluation of patients.



[Table/Fig-3]: H&E section of bone marrow biopsy showing evidence of caseating granuloma (10x).

perforation peritonitis with retroperitoneal lymphadenopathy. He underwent open laparotomy and multiple perforations were found in small bowel. A segment of bowel was resected. Histopathology and Immunohistochemistry was suggestive of diagnosis consistent with NK/T cell lymphoma [Table/Fig-4]. One patient had hypoechoic lesions in spleen and serology showed IgM-positivity for EBV-VCA. One case each had dengue viral infection (NS-1 Positive) and scrub typhus infection (Weil felix positive). Systemic onset Juvenile idiopathic arthritis was found in one patient as the underlying cause based on International League of Associations for Rheumatology criteria (ILAR) for diagnosis. This patient first presented with long standing (>2 weeks) fever without any localization. There was no history of joint pain, no cytopenias, no rash and no organomegaly. His serum iron was >90,000mcg/L. He was provisionally diagnosed as HLH and started on steroids and cyclosporine. He improved clinically and ferritin levels came down to normal in 2 months. After 6 months when treatment was tapered and stopped



[Table/Fig-4]: Histopathology and immunohistochemical staining of small intestinal biopsy in case of NK/T cell lymphoma showing infiltration of lymphoid cells showing positivity for CD3 and CD7.

he again developed fever associated with both knee joint pain, swelling and skin rash with a very high ferritin value. Diagnosis of underlying cause could not be established in other 2 patients. [Table/Fig-5] Shows the underlying causes, treatment and outcome of HLH in our patients.

Diagnostic Criteria[3,6]	Causes	n(%)	Treatment	Outcome (After Diagnosis)
HLH 2004, 2009	No etiology could be found	2 (25)	CSA +DEXA	Alive
HLH 2004, 2009	Tuberculosis	1 (12.5)	ATT+ Supportive	Died in 1st week
HLH 2004, 2009	Lymphoma	1(12.5)	IVIG	Died in 2nd week
HLH 2004, 2009	EBV infection	1 (12.5)	CSA+DEXA	Alive
HLH 2004, 2009	Dengue	1 (12.5)	CSA+DEXA	Alive
HLH 2004, 2009	Scrub typhus	1 (12.5)	CSA+DEXA	Died in 2nd week
Not Fulfilling criteria*	Juvenile idiopathic arthritis	1 (12.5)	CSA+DEXA	Alive

[Table/Fig-5]: Etiology, treatment and outcome of HLH in our patients.
*In view of prolonged fever, very high serum ferritin >90,000 mcg/L and evidence of hemophagocytosis in bone marrow diagnosis of HLH was entertained.

Diagnosis of HLH was also consistent with the new proposed HLH diagnostic criteria 2009 by Filipovich AH et al., [3] in all patients except one i.e. the boy with diagnosis of JIA. In this boy possibility of HLH was kept because of sky high serum ferritin levels with long standing fever and marrow evidence of hemophagocytosis.

Treatment, Follow-Up and Prognosis- Patient with NK/T cell lymphoma received IV Ig treatment but expired on day 3 of admission while on ventilator support for impending respiratory failure and sepsis. Patient with disseminated tuberculosis also expired during hospital stay due to cardiorespiratory arrest. Patient with scrub typhus had underlying sepsis and acute lung injury requiring ventilator support and expired. Rest of the patient received treatment with dexamethasone (8 mg/m^2 for 2 weeks followed by 4 mg/m^2 for 2 weeks, 2 mg/m^2 for 2 weeks and 1mg/m^2 for 2 weeks) and Cyclosporin 6 mg/kg body weight for a duration of 2 months. Cyclosporin doses were tapered later on over a period of 1 month. Patients were monitored with hemogram, renal functions, liver function and serum ferritin once in every 15 days. All patients achieved their normal ferritin levels at the end of 2 months. Median duration of follow up was 18 months (range 6-24 months). None of the patients relapsed during follow-up. One patient developed steroid induced proximal myopathy in second month of treatment which responded to lowering the doses of steroids. We observed a good response with dexamethasone with cyclosporine without adding etoposide, although HLH-2004 protocol

advocates treatment with all these 3 drugs simultaneously from the beginning.

Patient responded well to treatment and their serum ferritin gradually dropped to normal range after 2 months of treatment. Five patients were healthy and alive after median of 18 months without any relapse. One patient received only IV Ig and responded well. All patients received fluconazole and Co-trimoxazole prophylaxis. Intrathecal methotrexate was not given to any patient as none had progressive neurological symptoms. Overall mortality was 37% with a median follow-up of 18 months.

DISCUSSION

The purpose of this retrospective study was to describe the clinical features, laboratory approach and treatment of HLH in our setting. HLH is not a rare entity in India, but it is under reported especially in adolescents and adult population. Incidence and age distribution of HLH is not well known. Threshold of suspicion for this entity is high due to relative unawareness of this condition. Unavailability of diagnostic facilities is one of the common limitations in workup of HLH patients. HLH should always be considered as a differential diagnosis in any patients presenting with febrile illness and pancytopenia. Currently HLH-2004 diagnostic guidelines are used for diagnosis of HLH [6].

First major criteria for diagnosis of familial HLH gives stress on molecular diagnosis consistent with HLH. Three most common molecular defects are Perforin1 (PRF1), UNC13D, and Syntaxin 11 (STX11) gene mutations. Mode of inheritance is autosomal recessive. Presence of any defined molecular defect is enough for diagnosis. Second diagnostic criteria includes 8 criterias out of which fulfillment of at least 5 is required to diagnose HLH. Molecular tests have very limited availability. Genetic molecular tests, NK cell activity and soluble CD25 receptor assay could not be done in any of our patients. Diagnosis of most of our patients is based on clinical and other supportive laboratory features fulfilling the second criteria.

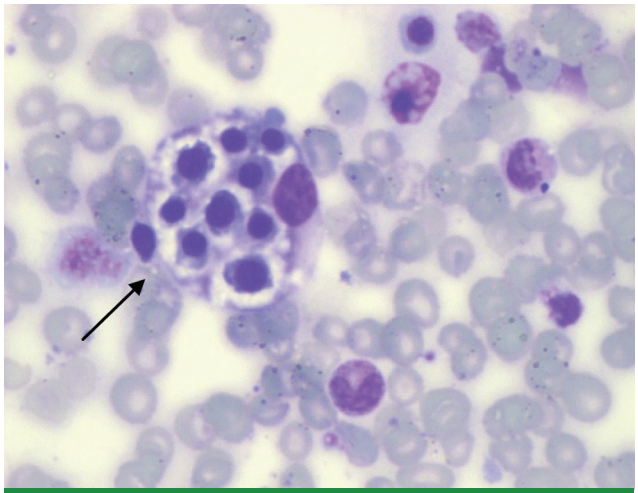
In our analysis patients age ranged from 13- 57-years and 62.5% (5 patients) were female. Most common clinical features were fever (100%) with a median duration of 3 weeks, pallor (87%), splenomegaly (75%), jaundice (62%), hepatomegaly (62%). Pedal edema (37%), abdominal pain (37%), breathlessness (50%), rash (37%) , bleeding (12.5%), and lymphadenopathy (50%), were uncommon presentations. Hyperbilirubinemia has been reported in 18% of pediatric HLH by Ramachandran et al., [7] and 25% of paediatric HLH by Janka G et al., [1]. Fever has been reported in 100% and splenomegaly in >80% of patients at the time of diagnosis [2].

Pancytopenia was present in 6 out of 8 (75%) patients, one patient had bicytopenia. Mean hemoglobin was 7.2 gm/dl and 75% patients had Hb of less than 9 gm/dl. Six patients (75%) had leucopenia and 3 of them had ANC < 1500/ mm^3.

Mean platelet count was 72,000/mm^3 with 3 patients (37%) of patients having platelet count < 20,000/mm^3. Median serum bilirubin was 3.8 mg/dl and one patient had bilirubin of 22mg/dl. All but one had elevation of hepatic enzymes ranging from 2 to 10 times upper limit of normal. Although the HLH-2004 diagnostic criteria do not include hepatic dysfunction as criteria, we found hepatic dysfunction in 87% of our patients. Recent review article in blood 2011 “How I treat HLH” also describes the commonness of hepatic dysfunction in HLH patients [8]. Recently proposed HLH diagnostic criteria (2009) by Filipovich AH et al., also includes hepatitis as one of the important criteria’s in diagnosis of HLH [3].

Median serum ferritin was 13500 mcg/L. All patients had serum ferritin > 500 mcg/L and one patient with underlying diagnosis of juvenile rheumatoid arthritis had extreme elevation of serum ferritin (90,000 mcg/L). Ferritin is a valuable and easily available marker and levels >10,000 mcg/L were found to be highly sensitive and specific for diagnosis of HLH [4]. Fever, cytopenias and elevated ferritin was found in >85% of cases in a recent study including 73 patients by Otrock ZK et al., [9]. Hypertriglyceridemia (>265mg/dl) was present in 75% of our patients and mean triglyceride levels were 458 mg/dl. Elevated triglyceride levels have been reported in upto 64%-70% of pediatric patients with HLH [2]. Serum fibrinogen was done in 4 patients and 2 had hypofibrogenemia (<150 mg/dl).

Bone marrow evidence of hemophagocytosis was present in 6 patients (75%). Two patients had only increase in number of macrophages and no evidence of hemophagocytosis. One patient had evidence of caseating granuloma along with hemophagocytosis [Table/Fig-6]. Presence of bone marrow hemophagocytosis alone is not a diagnostic marker of HLH and it can be seen in various infections without full clinical syndrome. Reported incidence of hemophagocytosis on bone marrow examination ranges from 25-100% in HLH [8].



[Table/Fig-6]: Bone marrow aspirate showing evidence of Hemophagocytosis, Leishman stain (40x).

Some patients may show hemophagocytosis later in disease course even when they are clinically improving.

There is no standard treatment protocol for secondary HLH. Etoposide, cyclosporine and dexamethasone based treatment, IVIg, and CHOP chemotherapy, all have been tried. We used Cyclosporin and dexamethasone without etoposide in most of our patients. Overall survival in our patients was 62.5% at a median follow-up of 18 months. Three patients died (37.5%) and all deaths occurred in the first few week. In a retrospective series of 162 patients with HLH, 94 patients survived (58%). Of the patient who did not survive half died within 1 month of diagnosis especially those with hematological malignancies [10].

LIMITATIONS

This is a retrospective analysis of limited number of patients with HLH. Studies including large number of patients are required for better understanding of best management strategy in secondary HLH.

CONCLUSION

Hemophagocytic lymphohistiocytosis is a rare life threatening disease. Common manifestations in our patients were fever, cytopenia, splenomegaly, hepatic dysfunction and hyperferritinemia. Etiology includes viral infections, tuberculosis and lymphoma. Early treatment with cyclosporine and steroids with or without etoposide, and treatment of underlying cause can improve survival of these patients.

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