

HEMOPHAGOCYtic LYMPHOHISTIOCYTOSIS: CASE SERIES IN INFANTS AND CHILDREN

KHAN F.S.,¹ AHMAD A.,² ANWAR S.,³ RATHORE A. H.⁴ AND BILAL S.⁵

¹Departments of Heamatology and Transfusion Medicine, ^{2,3}Heamatology Oncology, ⁴Pediatrics and ⁵Radiology
The Children Hospital and The Institute of Child Health, Ferozepur Road, Lahore – Pakistan

ABSTRACT

Background and Objective: Hemophagocytic Lympho Histiocytosis (HLH) is a rare disorder. It can be primary or secondary, despite the existence of diagnostic guidelines it often remains unrecognized. Familial Hemophagocytic Lymphohistiocytosis occurs typically in children under 1 year of age, secondary HLH typically occurs after 6 years of age. The objective of the study was to highlight the clinical problem and increase awareness of the disease in children in order to facilitate diagnosis and treatment.

Methods: This retrospective study was conducted over 17 months period from 1.1.2014 to 30.5.2015 in the hematology department of children's hospital and included all pediatric patients diagnosed with HLH fulfilling 5 out of 8 revised diagnostic criteria guideline of the HLH – 2004 protocol. None of the patients had genetic testing, sCD25 levels or NK cell activity testing.

Results: The diagnosis of HLH was made by fulfilling 5 out of 8 clinical and laboratory diagnostic criteria for HLH in 12 patients. Their ages ranged from 3 months to 10 years, male:female ratio was 2:1, fever was present in 100% cases, splenomegaly in 100%, cytopenias in two out of three cell lines in 100%, hypertriglyceridemia in 50%, hypofibrinogenemia in 50%, elevated ferritin in 66.6%, hemophagocytosis in bone marrow in 83%, HLH was diagnosed in one patient with Chedik – Higashi syndrome, in one patient with malaria, in one patient with typhoid fever and in one case with systemic juvenile idiopathic arthritis (sJIA).

Conclusion: HLH presents with a varied clinical manifestations, often under – recognized, thereby contributing to its high morbidity and mortality. Early diagnosis of HLH and underlying condition and appropriate therapy is crucial to start lifesaving management, this being possible only by keeping a high index of suspicion and using diagnostic guidelines.

Key words: Hemophagocytic Lympho Histiocytosis (HLH). Familial Hemophagocytic Lymphohistiocytosis (FHL). Systemic juvenile idiopathic arthritis (sJIA). Cytotoxic T lymphocytes (CTLs). Natural killer cells (NK Cells).

INTRODUCTION

Hemophagocytic Lympho Histiocytosis (HLH) is primarily a pediatric syndrome, with highest incidence in those < 3 months.¹ HLH is a syndrome of excessive inflammation and tissue destruction.²

HLH can be divided into primary (familial) hemophagocytic lymphohistiocytosis (FHL) and secondary HLH. The incidence of FHL is 1:50,000 births with an equal gender distribution.³ Primary HLH is caused by gene mutation, either at one of the FHL loci or in a gene responsible for one of several immunodeficiency syndromes.⁴ HLH is often associated with infections especially viral, less commonly bacterial, fungal or parasitic³ malignancies, most commonly lymphoid cancers.^{5,6} HLH may develop during the course of a rheumatologic disorder, autoimmune diseases like dermatomyositis, systemic sclerosis, mixed connective tissue

disease, antiphospholipid syndrome, Sjögren's syndrome, ankylosing spondylitis, vasculitis, and sarcoidosis are associated with HLH.⁷

Primary mediator of tissue damage is excessive cytokine production by NK cells, macrophages and CTLs.² Cytokines found at extremely high levels in the plasma of patients with HLH include interferon gamma (Ifn- γ); tumor necrosis factor alpha (TNF- α); IL-6, IL-10, and IL-12; and the soluble IL-2 receptor (CD25).⁸⁻¹⁰

There is coexistence of immune dysregulation with unchecked inflammation.¹¹ Mutations of perforin gene, are seen in 20 to 30% of FLH patients.¹²

HLH has varied clinical manifestations, poor prognosis and high mortality. The median survival without treatment is estimated at less than 2 months,¹³ therefore it is crucial to make early diagnosis by perform-

ing relevant investigations and start treatment without delay.

PATIENTS AND METHODS

This retrospective study was conducted over 17 months period from 1.1.2014 to 30.5.2015 in the hematology department of children's hospital and included all pediatric patients diagnosed with HLH fulfilling 5 out of 8 revised diagnostic criteria guideline of the HLH – 2004 protocol. None of the patients had genetic testing, sCD25 levels or NK cell activity testing.

RESULTS

The diagnosis of HLH was made by fulfilling 5 out of 8 clinical and laboratory diagnostic criteria for HLH in 13 patients. Ages of the patients ranged between 3 months to 11 years, with mean age of 2 years and 8 months with six patients being under one year of age. There were eight males and five females and male:female 2.2:1.

Fever and cytopenias in two out of three cell lines were present in 100% cases, hemoglobin ranged from 5 to 10g/dl (mean 7.3 g/dL), platelets ranged between $11 - 73 \times 10^3/l$ (mean $40 \times 10^3/ul$), absolute neutrophil count $< 1 \times 10^3/ul$ was present in 30.7% cases. Hypertriglyceridemia in 53% and hypofibrinogenemia in 46%, elevated ferritin in 10 out of 13 cases (76%), Hemophagocytosis in bone marrow was found in 11 out of 13 (84%). Parental consanguinity was present in 30.7% of cases. HLH was diagnosed in one patient each with Chedik–Higashi syndrome, Malaria, typhoid fever and systemic juvenile idiopathic arthritis.

DISCUSSION

HLH is a syndrome of excessive inflammation and tissue destruction, caused by a lack of normal down regulation of activated macrophages and lymphocytes.² Primary mediator of tissue damage being excessive cytokine production by macrophages, NK cells, and CTLs.²

Genetic defects play a major role in childhood HLH.¹⁴ Most of the implicated genes encode for perforin – dependent cytotoxicity.¹⁵ Cytotoxic cells exert their effect by releasing cytotoxic granules, containing perforin and granzyme toward the immune synapse, connecting granules with the cell membrane, releasing the contents of granules, leading to death of the target cell through apoptosis.^{16,17} Unlike FHLH, most patients with sHLH do not present detectable abnormalities in the mechanisms of cytotoxicity. Studies in animal models suggest at least two different mechanisms of sHLH: enhanced antigen presentation and excessive signaling of Toll-like receptors.¹⁸ TLRs are non-antigen-specific receptors on the surface of NK cells that are activated by components of bacteria, fungi, viruses, or mycoplasma.¹⁹

The official diagnosis of HLH, is based on fulfilling

one or both of the following criteria established by the Histiocyte Society.¹¹

Molecular identification of an HLH – associated gene mutation (ie, *PRF1*, *UNC13D*, *STX11*, *STXBP2*, *Rab27A*, *SH2D1A*, or *BIRC4*). Children require documentation of homozygosity or compound heterozygosity for HLH – associated gene mutations for adults heterozygosity may be sufficient if they have HLH associated clinical findings.

OR

Five of the following eight findings:

- Fever $\geq 38.5^\circ\text{C}$.
- Splenomegaly;
- Peripheral blood cytopenia, with at least two of the following: hemoglobin < 9 g/dL (for infants < 4 weeks hemoglobin < 10 g/dL), platelets $< 100,000/microL$, absolute neutrophil count $< 1000/microL$.
- Hypertriglyceridemia (fasting triglycerides > 265 mg/dL) and/or hypofibrinogenemia (fibrinogen < 150 mg/dL).
- Hemophagocytosis in bone marrow, spleen, lymph node, or liver.
- Low or absent NK cell activity.
- Ferritin > 500 ng/mL.
- Elevated soluble CD25 (soluble IL-2 receptor alpha) two standard deviations above age – adjusted laboratory – specific norms.

Fever along with anemia and thrombocytopenia, are seen in majority of patients with HLH, They were present in all cases in this study with similar findings by Rajajee et al²⁰ while Liu et al²¹ have reported these in 100% and 94.8% cases.

Bone marrow cellularity can be variable in HLH.¹¹ Incidence of hemophagocytosis on bone marrow examination varies from 25 to 100 percent,²² in our study it was observed in 84% cases. Pancytopenia and higher grade of hemophagocytosis on bone marrow examination may be helpful in differentiating HLH from non HLH cases.²³ Despite the fact that hemophagocytosis is prominently featured in the name of this disease, it is rarely found at presentation in secondary cases and may not be visible until late in disease progression, Bone marrow biopsies performed early in the course of secondary disease may be normal or demonstrate very nonspecific findings, but FHL, may demonstrate prominent hemophagocytosis from the start. An immunohistochemical stain for CD163 may be useful, as up-regulation of this receptor facilitates hemophagocytosis.²⁴

Hyperferritinemia was found in 76% cases in this study with highest level of 91114 ng/l in a patient with sOJIA. in contrast to the 93.2% and 98% reported in other studies.^{21,25} Higher cutoff value of ferritin level may have improved utility in the diagnosis of secondary HLH in the critical care setting.²⁶ Ferritin accu-

mulates during the anti-inflammatory process of macrophage scavenging of heme via the CD163 receptor.²⁷ Growth differentiation factor 15, a protein responsible for modulation of iron homeostasis, is upregulated in patients with HLH.²⁸

Hypertriglyceridemia may be due to severe liver involvement, and triglycerides may not be elevated until the liver has been affected for some time. In a review of patients with HLH, 68% and 55% had elevated triglycerides,^{29,21} in our study it was present in 53% cases.

Coagulopathy is a prominent feature of HLH, as low fibrinogen is found in the majority of patients, Hypofibrinogenemia was present in 46% in this study while Liu et al²¹ reported it in 61.8% cases. Coagulation studies have demonstrated normal factor V and VIII levels and an absence of fibrin split products. These findings provide evidence against disseminated intravascular coagulation, a diagnosis that may overlap with HLH due to the shared findings of thrombocytopenia and hypofibrinogenemia.³⁰ Cutaneous eruptions as a consequence of HLH are variable in presentation³¹ and take the form of generalized rashes, erythroderma, edema, petechiae, and purpura,⁵ generalized rashes were present in 30.7% in our study.

A high percentage (up to 24%) of FHL cases are associated with parental consanguinity.³² In the present study it was present in 30.7% cases.

While many of the laboratory tests are readily available, evaluation of IL-2 receptor and NK-cell activity may require sending specimens out to specialized laboratories and may not be a timely option for clinical diagnosis.³³ Flow cytometry for perforin staining in cytotoxic lymphocytes, including NK-cells, CD8+ T-cells, and CD56+ T-cells, is a marker for perforin gene mutations seen in HLH and proven to be a rapid and useful modality to reliably diagnose HLH and shorten the time to treatment.³⁴ Additionally, measuring plasma levels of CD163, a receptor for hemoglobin – haptoglobin complexes, may also be helpful in distinguishing HLH from other purely infectious diseases.³⁵

It is therefore **concluded** that HLH poses real serious clinical problem, remains underrecognized, as the initial symptoms and laboratory findings may be nonspecific and thus not helpful in diagnosis. There is significant overlap with other illnesses resulting in diagnostic delay, poor prognosis and high mortality. A very high index of suspicion in patients with prolonged fever, cytopenias, and hyperferritinemia accompanied by relevant immunological, and genetic workup and bone marrow aspirate and trephine biopsy to determine the cause of cytopenias and document hemophagocytosis enables timely diagnosis to initiate life – saving treatment.

Conflict of Interest

The authors have no conflict of interest to declare.

Author's Contributions

All the authors contributed equally in the study.

ACKNOWLEDGEMENT

The authors wish to thank medical director and staff of hematology, pathology oncology departments of the hospital who helped and facilitated this study.

REFERENCES

- Henter JI, Elinder G, Söder O, Ost A. Incidence in Sweden and clinical features of familial hemophagocytic lymphohistiocytosis. *Acta Paediatr Scand.* 1991; 80: 428.
- Filipovich A, McClain K, Grom A. Histiocytic disorders: recent insights into pathophysiology and practical guidelines. *Biol Blood Marrow Transplant.* 2010; 16: S82.
- Henter JI, Arico M, Elinder G, Imashuku S, Janka G. Familial hemophagocytic lymphohistiocytosis. Primary hemophagocytic lymphohistiocytosis. *Hematol Oncol Clin North Am.* 1998; 12: 417–433.
- Larroche C. Hemophagocytic lymphohistiocytosis in adults: diagnosis and treatment. *Joint Bone Spine.* 2012; 79: 356.
- Ramos – Casals M, Brito – Zerón P, López – Guillermo A, et al. Adult haemophagocytic syndrome. *Lancet.* 2014; 383: 1503.
- Otrock ZK, Eby CS. Clinical characteristics, prognostic factors, and outcomes of adult patients with hemophagocytic lymphohistiocytosis. *Am J Hematol.* 2015; 90: 220.
- Fukaya S, Yasuda S, Hashimoto T, et al. Clinical features of haemophagocytic syndrome in patients with systemic autoimmune diseases: analysis of 30 cases. *Rheumatology (Oxford)*, 2008; 47: 1686.
- Aricò M, Danesino C, Pende D, Moretta L. Pathogenesis of haemophagocytic lymphohistiocytosis. *Br J Haematol* 2001; 114: 761.
- Komp DM, McNamara J, Buckley P. Elevated soluble interleukin-2 receptor in childhood hemophagocytic histiocytic syndromes. *Blood*, 1989; 73: 2128.
- Tang Y, Xu X, Song H, et al. Early diagnostic and prognostic significance of a specific Th1/Th2 cytokine pattern in children with haemophagocytic syndrome. *Br J Haematol.* 2008; 143: 84.
- Jordan MB, Allen CE, Weitzman S, et al. How I treat hemophagocytic lymphohistiocytosis. *Blood*, 2011; 118: 4041.
- Janka GE, Schneider EM. Modern management of children with haemophagocytic lymphohistiocytosis. *Br J Haematol.* 2004; 124: 4–14.
- Janka GE. Familial hemophagocytic lymphohistiocytosis. *Eur J Pediatr.* 1983; 140 (3): 221–230.
- Stepp SE, Dufourcq – Lagelouse R, Le Deist F, et al. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. *Science*, 1999; 286: 1957.
- Freeman HR, Ramanan AV. Review of haemophagocytic lymphohistiocytosis. *Arch Dis Child.* 2011; 96: 688.
- Gupta S, Weitzman S. Primary and secondary hemophagocytic lymphohistiocytosis: clinical features, pathogenesis and therapy. *Expert Rev Clin Immunol.* 2010; 6: 137–154.
- Janka G. Hemophagocytic lymphohistiocytosis: when

- the immune system runs amok. *Klin Padiatr.* 2009; 221: 278–285.
18. Canna SW, Behrens EM. Not all hemophagocytes are created equally: appreciating the heterogeneity of the hemophagocytic syndromes. *Curr Opin Rheumatol.* 2012; 24: 113–118.
 19. Fall N, Barnes M, Thornton S, et al. Gene expression profiling of peripheral blood from patients with untreated new-onset systemic juvenile idiopathic arthritis reveals molecular heterogeneity that may predict macrophage activation syndrome. *Arthritis Rheum.* 2007; 56: 3793.
 20. Rajajee S, Ashok I, Manwani N, Rajkumar J, Gowrishankar K, Subbiah E. Profile of hemophagocytic lymphohistiocytosis; efficacy of intravenous immunoglobulin therapy. *Indian J Pediatr.* 2014 Dec; 81 (12): 13.
 21. Liu Y, Zhou S, Zhang L, Xu L, Jin Y, Liu A, Zhang X, Yao H, Jia Y, Zhang X, Su Y, Li Z. Analyses of 77 patients with hemophagocytic syndrome. *Zhonghua Yi Xue Za Zhi.* 2015 Mar 10; 95 (9): 681.
 22. Rivière S, Galicier L, Coppo P, et al. Reactive hemophagocytic syndrome in adults: a retrospective analysis of 162 patients. *Am J Med.* 2014; 127: 1118.
 23. Chandra H, Chandra S, Kaushik R, Bhat N, Shrivastava V. Hemophagocytosis on bone marrow aspirate cytology: single center experience in north himalayan region of India. *Ann Med Health Sci Res.* 2014 Sep; 4 (5): 692–6.
 24. Filipovich AH. Hemophagocytic lymphohistiocytosis and related disorders. *Curr Opin Allergy Clin Immunol.* 2006; 6 (6): 410–415.
 25. Xiao L, Guan X, Meng Y, Su Y, Xian Y, Xiao J, Cui Y, Yu J. Analysis of clinical and laboratory features of 217 pediatric hemophagocytic lymphohistiocytosis. *Zhonghua Xue Ye Xue Za Zhi.* 2014 Jul; 35 (7): 628–32.
 26. Saeed H, Woods RR, Lester J, Herzog R, Monohan G. Evaluating the optimal serum ferritin level to identify hemophagocytic lymphohistiocytosis in the critical care setting. *Int J Hematol.* 2015 May 13.
 27. Janka GE. Familial and acquired hemophagocytic lymphohistiocytosis. *Eur J Pediatr.* 2007; 166 (2): 95–109.
 28. Wu JR, Yuan LX, Ma ZG, et al. GDF15 – mediated up-regulation of ferroportin plays a key role in the development of hyperferritinemia in children with hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer.* 2013; 60: 940.
 29. Okamoto M, Yamaguchi H, Isobe Y, et al. Analysis of triglyceride value in the diagnosis and treatment response of secondary hemophagocytic syndrome. *Intern Med.* 2009; 48: 775.
 30. McClure PD, Strachan P, Saunders EF. Hypofibrinogenemia and thrombocytopenia in familial hemophagocytic reticulosis. *J Pediatr.* 1974; 85 (1): 67–70.
 31. Zerah ML, DeWitt CA. Cutaneous findings in hemophagocytic lymphohistiocytosis. *Dermatology.* 2015; 230 (3): 234–43.
 32. Arico M, Janka G, Fischer A, et al. Hemophagocytic lymphohistiocytosis. Report of 122 children from the International Registry. FHL Study Group of the Histiocyte Society. *Leukemia.* 1996; 10 (2): 197–203.
 33. Xu XJ, Tang YM, Song H, et al. Diagnostic accuracy of a specific cytokine pattern in hemophagocytic lymphohistiocytosis in children. *J Pediatr.* 2012; 160 (6): 984–990.
 34. Kogawa K, Lee SM, Villanueva J, Marmer D, Sumegi J, Filipovich AH. Perforin expression in cytotoxic lymphocytes from patients with hemophagocytic lymphohistiocytosis and their family members. *Blood.* 2002; 99 (1): 61–66.
 35. Filipovich AH. Hemophagocytic lymphohistiocytosis (HLH) and related disorders. *Hematology Am Soc Hematol Educ Program.* 2009: 127–131.