Acta Scientific Cancer Biology (ISSN: 2582-4473)

Volume 7 Issue 3 May 2022

Research Article

A Case Series of Hemophagocytic Lymphohistiocytosis from a Tertiary Care Centre: A Rare and Underdiagnosed Entity

Gurpreet Kaur[1], Ankur Ahuja[1]*, Vikram Singh[1], Paresh Singhal[1], Arijit Sen[2], Metaanksha[3] and Gaurav Gahlot[4]

[1]*Associate Professor, Department of Pathology, Armed Forces Medical College, Pune, India*

[2]*Professor and HOD, Armed Forces Medical College, Pune, India*

[3]*Second Year MMBS Student, Himalayan Institute of Medical Sciences, Jolly Grans, Dehradun, India*

[4]*Professor, Department of Pathology, Armed Forces Medical College, Pune, India*

***Corresponding Author:** Ankur Ahuja, Associate Professor, Department of Pathology, Armed Forces Medical College, Pune, India.

DOI: 10.31080/ASCB.2023.07.0419

Received: April 17, 2023

Published: April 29, 2022

Abstract

Objective: Hemophagocytic lymphohistiocytosis (HLH) is an aggressive and life-threatening syndrome of excessive inflammation due lack of normal downregulation of activated macrophages and lymphocytes. Initial signs and symptoms of HLH can mimic common infections, fever of unknown origin, hepatitis, or encephalitis. As the presenting features are non specific therefore HLH diagnosis remains a challenge and needs a high degree of suspicion. A detailed history clinical examination and the patients laboratory findings help arrive at a conclusive diagnosis. In this study we attempt to present clinical and laboratory features of a series of HLH cases.

Material and Methods: This was a retrospective study wherein all the bone marrow aspirate smears with a diagnosis of HLH between January 2018 to March 2023 were studied. The morphological clinical and biochemical parameter were correlated.

Results: A total of seventeen cases were diagnosed as HLH from January 2018 to March 2023. Twelve patients fulfilled 5 out of 8 clinical and lab diagnostic criteria of HLH (2004). Three cases of familial HLH were also diagnosed after testing for molecular alterations and these were upfront diagnosed as HLH while for the rest the diagnosis was suggested after correlating clinical and laboratory criteria with bone marrow findings.

Conclusion: We present a series of seventeen cases of Hemophagocytic Lymphohistiocytosis from a tertiary care hospital. A high degree of suspicion aided by relevant clinical and biochemical criteria is essential for diagnosis for institution of therapy and to prevent morbidity and mortality.

Keywords: Hemophagocytic Lymphohistiocytosis; Hyperferritinemia; Pancytopenia

Introduction

Hemophagocytic lymphohistiocytosis (HLH) was first reported in 1952 by Farquhar and Claireaux, and at that time it was called familial hemophagocyticreticulosis [1]. Hemophagocytic lymphohistiocytosis (HLH) is a disorder of fulminant immune activation with resembling systemic inflammatory response syndrome (SIRS). It was originally described in pediatric patients, HLH has been increasingly recognized also in adults. The hallmarks of HLH include fever, pancytopenia, splenomegaly, and liver-dysfunction. HLH in adults is usually secondary or aquired to acquired associated with infections, autoimmune disease or malignancies. HLH results from a heightened immune response due to uncontrolled activation of macrophages and cytotoxic T-lymphocytes (CTL).

The aetiology of HLH may be classified as primary or secondary based on whether it has a genetic cause or is a result of an underlying secondary disorder. Familial or primary HLH usually follows an autosomal recessive inheritance. The genetic cause of type 1 is currently unknown. Types 2-5 are caused by genetic changes in the PRF1 gene, the UNC13D gene, the STX11 gene and the STXBP2 gene, respectively. When HLH results from an inappropriate immune response to Epstein-Barr virus or another viral illness, it may be due to a separate genetic condition called X-linked lymphoproliferative disease (XLP) caused by a genetic change in the SH2D1A or XIAP gene and is in the name this is inherited in an X linked fashion [2,3]. Secondary HLH is usually acquired by viral infections, immunodeficiency states and autoimmune disorders. The commonly associated immunodeficiency syndromes associated with HLH are Chediak Higashi syndrome, Hermansky Pudlak syndrome, Griscelli syndrome, and X linked lymphoproliferative syndrome [4]. Macrophage activation syndrome (MAS) is another overlapping syndrome associated with rheumatologic diseases like systemic-onset juvenile idiopathic arthritis (SOJIA), adult-onset Still disease, and systemic lupus erythematosus [5,6].

The initial diagnostic criteria for HLH were proposed in 1991 and these were updated in 2004 [7]. The 2004 criteria are as follows. The diagnosis requires the presence of either criterion A or 5 out of 8 of criterion B [8-10].

Diagnostic criteria for HLH (2004)

A. Familial disease/known genetic defect

OR

B. Clinical/laboratory criteria

- Fever,
- Splenomegaly,
- Cytopenia (at least 2 cell lines) (HGB < 9 gram/dL, PLT< 100,000/microL ANC< 1000/microL)
- Hypertryglyceridemia and/or hypofibrinogenemia, Fasting triglyceride> 265 mg/dL, Fibrinogen < 150 mg/L,
- Hyperferritinemia, Ferritin>500ug/l
- Hemophagocytosis in bone marrow, CSF, or lymph nodes,
- Decreased/absent NK cell activity
- Soluble CD25 > 2400 U/ml

Methods

The requisition forms from January 2018 to March 2023 of our centre were reviewed to get all relevant clinical and laboratory details. We then correlated bone marrow smear findings with clinical and laboratory findings.

Findings

From January 2018 to March 2023 a total of seventeen cases of HLH were diagnosed. The clinical findings and laboratory reports of these patients are summarized in tables 1. All bone marrow smears, except one, showed increased number of histiocytes, however one did not show Hemophagocytosis raising the possibility of an underlying storage disorder.

A total of seventeen cases were diagnosed as HLH from January 2018 to March. 2023.

Table 1 highlights the clinical and laboratory profile of these patients. All bone marrow samples showed features of HLH except one. These are depicted in Figure 1.

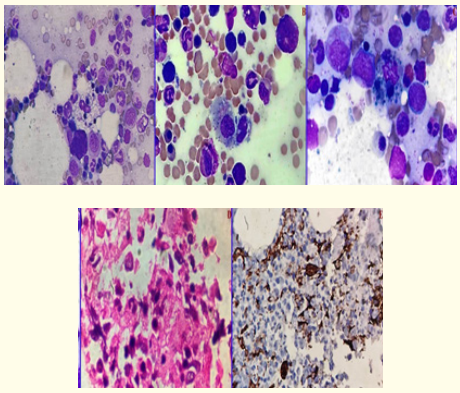

Figure 1: Bone marrow aspirate smears showing evidence of haemophagocytosis in the form of histiocytes showing prominent phagocytosis of hematopoietic elements (red cells, normoblasts, platelets) are increased. These histiocytes possess bland looking nuclei and lack distinct nucleoli. (Leishman Giemsa stain). H and E stained bone marrow biopsy also shows evidence of Haemophagocytosis. Immunohistochemistry with CD163 highlights these histiocytes.

Age/gender	Cytopenia	Triglyceride mg/dL	LDH IU/L	S. Ferritin ng/ml	Fibrinogen ng/ml	HPC	Clinical features	Aetiology
8 mnths/M	Pancytopenia	350	800	1320	230	-	Fever, rash, jaundice, HSM	PRF1 mutation
16/M	Pancytopenia	297	2169	1132	254	+	Fever, arthralgia jaundice, SM	Typhidot +
34/M	Bicytopenia	479	562	632	NA	+	Fever, SM	-
28/F	Pancytopenia	800	2190	1210	100	+	Fever, SM	-
65/M	Bicytopenia	510	600	567	210	+	Fever, pleural effusion jaundice	EBV serology +
76/M	Bicytopenia	NA	340	2000	256	+	Fever, jaundice, HSM	HIV+, CMV+
28/F	Pancytopenia	1224	465	4320	NA	+	Fever, SM	-
38/M	Bicytopenia	NA	NA	781	160	+	Fever, HSM, shock, delirium	-
61/M	Pancytopenia	786	345	910	110	+	Fever, SM	-
63/M	Pancytopenia	NA	781	670	250	+	Fever, rash, jaundice, HSM	EBV+
71/M	Bicytopenia	NA	3611	2300	NA	+	Fever, SM	HIV+, Spur cell anaemia
45/F	Bicytopenia	891	653	541	145	+	Fever, HSM	Tuberculosis
5/F	Bicytopenia	417	879	630	150	+	Fever, HSM	
6/F	Pancytopenia	464	932	1363	NA	+	Fever, SM	STX11 gene muataion
14/M	Pancytopenia	NA	NA	1500	442	+	Fever	STXBP2 mutation
45/M	Pancytopenia	621	1181	721	NA	+	Fever	-
31/M	Pancytopenia	541	932	1200	NA	+	Fever HSM	EBV+

Table 1: The table depicts the clinical and laboratory profile of the patients of HLH. Pancytopenia is defined as Hemoglobin levels < 9gm%, Platelets < 100 x 10^9/L Neutrophils < 1.0 x 10^9/L.

Abbreviations - HSM, Hepatosplenomegaly, SM, Splenomegaly, EBV, Epstein -Barr Virus, HIV, Human immunodeficiency virus.

The age of the patients ranged from 08 months to 76 years with males outnumbering the females. The aetiology spectrum included infections most commonly and three cases out of the 17 had a primary HLH. Twelve patients fulfilled 5 out of 8 clinical and lab diagnostic criteria of HLH (2004) and three patients fullfilled the molecular criteria. The two patients who did not fulfill five criteria were diagnosed as HLH because of strong clinical suspicion. After correlating clinical and laboratory criteria along with bone marrow findings, the diagnosis of HLH was suggested.

Discussion

There is scant data from the Indian subcontinent regarding HLH and is limited to a few case series [11-17]. The age range in our series varied from 3 months to 65 years with a mean age of 30 years. The male to female ratio in the present series was 1.7:1. Kumar., *et al*. also found a male preponderance with a (M:F 2:1), Reddy., *et al*. similarly found a male preponderance (M:F 4:1) whereas Joshi., *et al*. found a female preponderance (M:F 1:4) [11,12,14]. The most frequent presenting symptom in our study was fever with cytopenia and hepatosplenomegaly. Similarly in the series by Joshi., *et al.* and Ramachandran., *et al*. fever and hepatosplenomegaly were the most common findings. The study by Joshi., *et al.* had a predomiantly paediatric population and their case series had three cases of familial HLH much like ours. Ramachandran., *et al.* had 33 cases of HLH these also being paediatric patients and one third of their cases had CNS symptoms. Only one patient presented acute-

ly with neurologic symptoms. We found CNS involvement in only one patient (5%) however Joshi., *et al*. found CNS involvement in 30% while Ramachandaran., *et al*. (36%), and Reddy., *et al*. (80%) [12,14,15]. Its important to note that these were predominantly paediatric populations.

Ferritin levels were available in 16/17 patients, serum triglycerides in 12/17 and serum fibrinogen in 11/17 patients. Hyperferritenemia, raised raised triglycerides (according to the HLH 2004 criteria) were found in all these patients. In all proinflammatory condition- Ferritin increases as an acute phase reactant. In HLH serum Ferritin is increased because of marked macrophage activation and proliferation as this is primary site of iron storage in the marrow. Levels of >10,000 µg/L are >90% sensitive and ~98% specific in cases of HLH in children as per one paediatric study [18]. In adults high ferritin is neither sensitive or specific. In one large study >50,000 µg/L ferritin was mostly found in renal failure, hepatocellular injury, malignancies, infection. Only 19% of cases had HLH [19].

Fibrinogen was low in 05 patients out of the 11 in which it was available. Most of our cases were attributive to infectious aetiology 07/17 where EBV was a common pathogen. There was one case of Tuberculosis while three patients had EBV infection and two patients had HIV infection. One patient had a typhidot positivity and CMV serology positive each. In all our cases but for one 08 month old paediatric patient no evidence of hemophagocytosis was found. Hemophagocytosis though the hallmark of macrophage activation is only supportive in diagnosis and is not obligatory. It may be absent in early phase of HLH and is neither sensitive or specific. Its prevalence varied in different studies from 25% to 100%. And there is varying consensus amongst morphologists on the criteria for Hemophagocytosis. A IHC with CD163 increases sensitivity of picking of hemophagocytosis by highlighting macrophages.

Among the newer markers sCD25/serum IL2 levels can be done as a part of the 2009 criteria. This is a marker for cytokine production and is the gold standard marker for T-cell activation. There may be age related variations where infants have a higher mean sCD25 level in comparison to adults and therefore a local reference range should be established. The advantage on the other hand is low cost, commercially availability and that it can be done on frozen specimens by ELISA test. However our laboratory did not have this test available. sCD25 levels may be an be increased in ALPS, Lymphoma etc and this may lead to erroneous results. Levels of >2400 U/ml is ~100%are sensitive for HLH but specificity is low [3]. Reduced NK cell activity- reflects underlying immune defect can be used for diagnsosis using a Typical Radiolabelled chromium release assay however it is not commonly available [20].

We had three cases of familial HLH with PRFI1, STX11, STXBP2 mutation. 25% cases of HLH are FHLH (mostly <1yr age) Ethnic variation in mutations are known. All familial HLH have a AR transmission except XLPs. PERF1 null mutation present at <1yr age, while missense mutations have variable age presentation. The PERF1 mutation has higher risk of early disease onset compared to STX-11 mutation and adult patients with hypomorphic mutations in PERF1, UNC13D have indolent course in comparison to younger age group. The FHL3 has higher CNS involvement compared to FHL2. STXBP2 mutation is associated with defective erythropoiesis and hemolysis [21,22].

Conclusion

To conclude hemophagocytic lymphohistiocytosis is a rare, life-threatening disorder with a varied with a clinical features its needs a high degree of suspicion and accurate clinical, immunological, and genetic diagnostic work-up.

Bibliography

1. Farquhar JW and Claireaux AE. "Familial haemophagocyticreticulosis". *Archives of Disease in Childhood* 27 (1952): 519-525.
2. George MR. "Hemophagocytic lymphohistiocytosis: review of etiologies and management". *Journal of Blood Medicine* 5 (2014): 69-86.
3. Griffin G., *et al*. "Hemophagocytic lymphohistiocytosis: An update on pathogenesis, diagnosis, and therapy". *Best Practice and Research: Clinical Rheumatology* 34.4 (2020): 101515.
4. Rosado FG and Kim AS. "Hemophagocytic lymphohistiocytosis: An update on diagnosis and pathogenesis". *American Journal of Clinical Pathology* 139 (2013): 713-727.
5. Ravelli A., *et al*. "Macrophage Activation Syndrome". *Hematology/Oncology Clinics of North America* 29.5 (2015): 927-941.
6. Deane S., *et al*. "Macrophage activation syndrome in autoimmune disease". *International Archives of Allergy and Immunology* 153 (2010): 109-120.
7. Henter JI., *et al*. "Incidence in Sweden and clinical features of familial hemophagocytic lymphohistiocytosis". *Acta Paediatrica Scandinavica* 80 (1991): 428-435.
8. Henter JI., *et al*. "HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis". *Pediatric Blood Cancer* 48 (2007): 124-131.
9. Jordan MB., *et al*. "Challenges in the diagnosis of hemophagocytic lymphohistiocytosis: Recommendations from The North American Consortium for histiocytosis (NACHO)". *Pediatric Blood Cancer* 66.11 (2019): e27929.

10. Wang H., *et al*. "A systematic review of malignancy-associated hemophagocytic lymphohistiocytosis that needs more attentions". *Oncotarget* 8.35 (2017): 59977e85.

11. Kumar Vijay., *et al*. "Case Series of Hemophagocytic Lymphohistiocytosis from a Tertiary Care Centre: An Underdiagnosed Entity". *Turkish Journal of Pathology* 35 (2019).

12. Reddy S., *et al*. "Haemophagocytic lymphohistiocytosis (HLH): Case series in tertiary referral hospital over three years". *Journal of the Association of Physicians of India* 61 (2013): 850-852.

13. Rajagopala S., *et al*. "Severe hemophagocytic lymphohistiocytosis in adults-experience from an intensive care unit from North India". *Indian Journal of Critical Care Medicine* 16 (2012): 198-203.

14. Joshi R., *et al*. "Haemophagocytic lymphohistiocytosis: A case series from Mumbai". *Annals of Tropical Paediatrics* 31 (2011): 135-140.

15. Ramachandran B., *et al*. "Profile of hemophagocytic lymphohistiocytosis in children in a tertiary care hospital in India". *Indian Pediatrics* 48 (2011): 31-35.

16. Gopal GK., *et al*. "Scrub typhus associated macrophage activation syndrome". *Tropical Doctor* 40 (2010): 249-250.

17. Rajagopala S., *et al*. "Visceral leishmaniasis associated hemophagocytic lymphohistiocytosis-case report and systematic review". *Journal of Infection* 56 (2008): 381-388.

18. Allen CE., *et al*. "Highly elevated ferritin levels and the diagnosis of hemophagocytic lymphohistiocytosis". *Pediatric Blood Cancer* 50.6 (2008): 1227-1235.

19. Paul La Rosée., *et al*. "Recommendations for the management of hemophagocytic lymphohistiocytosis in adults". *Blood* 133.23 (2019): 2465-2477.

20. Abdalgani M., *et al*. "Accuracy of flow cytometric perforin screening for detecting patients with FHL due to PRF1 mutations". *Blood* 126 (2015): 1858-1860.

21. Canna SW and Marsh RA. "Pediatric hemophagocytic lymphohistiocytosis". *Blood* 135 (2020): 1332-1343.

22. Gadoury-Levesque V., *et al*. "Frequency and spectrum of disease-causing variants in 1892 patients with suspected genetic HLH disorders'. *Blood Advances* 4 (2020): 2578-2594.