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ETIOLOGY OF HAEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS (HLH) IN A TERTIARY CARE CENTRE

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ABSTRACT

Introduction- Haemophagocytic lymphohistiocytosis (HLH) is an aggressive and life threatening syndrome of highly stimulated but ineffective immune process. It can be triggered by a variety of events that disrupt immune homeostasis. Infection is a common trigger both in those with a genetic predisposition and in sporadic cases. Acquired HLH, with or without genetic disorders, may be due to infectious like bacterial, fungal, parasitic and viral or non-infectious etiologies such as malignancies, autoimmune disorders, and drugs. Although an early diagnosis is crucial to decrease mortality, it is often challenging due to lack of specificity of the clinical and laboratory findings and less availability of genetic tests in developing country.

Materials and Method- In this retrospective study, total 857 patients referred to our department for bone marrow aspiration from other departments of Calcutta National Medical College and Hospital from February'16 to October '18 and out of these 857 patients, 18 patients who have haemophagocytes in their bone marrow findings, were evaluated for etiology.

Result- Among 857 patients, 18 patients (2.1%) had bone marrow haemophagocytic lymphohistiocytosis, of whom 11patients (61.11%) were male and 7 patients (38.89%) were female. The patients' age ranged from 1 year to 74 years. Among them 4 cases (22.22%) associated with infective etiology, 2 cases (11.11%) with megaloblastic anemia, 2 cases (11.11%) with acute leukemia, 2 cases (11.11%) with myelodysplastic syndrome, 2 cases (11.11%) associated with plasma cell dyscrasia and 6 cases (33.33%) are of unknown etiology.

Conclusion- Haemophagocytic lymphohistiocytosis has a wide spectrum of causes which can be diagnosed by detailed history, peripheral smear examination supported by bone marrow examination, biochemical tests, specific antibody detection and other relevant investigations. It's an expected situation for haemophagocytic lymphohistiocytosis reasons that the high rate of infection is one of the major causes in tertiary care centre.

INTRODUCTION

Haemophagocytic lymphohistiocytosis (HLH) is an often fatal syndrome of exaggerated but ineffective inflammatory responses, characterized by excessive macrophage and T-cell activation as well as impairment of the ability of natural killer (NK) and cytotoxic T cells to kill target cells. [1-4]HLH is a group of disorders that include familial and acquired forms of the syndrome and macrophage activation syndrome that is associated with certain autoimmune diseases. [1-3, 5] The acquired form of HLH is associated with infections, especially with Epstein-Barr virus, and malignancies, particularly peripheral T/NK- cell or anaplastic large cell lymphomas, and certain medications used for conditions such as systemic lupus erythrematosus. [1-3, 5, 6]

Histiocyte Society HLH-2004 diagnostic criteria [7, 8]-

The diagnosis HLH requires that either 1 or 2 below are fulfilled:

1. A molecular diagnosis consistent with HLH: Pathological mutations of PRF1, UNC13D, STXBP1, RAB27A, STX11, SH2D1A, or XLAP
2. OR,
3. Diagnostic criteria for HLH fulfilled (5 out of the 8 criteria below)-

Initial diagnostic criteria

- Fever 38.5^0C or more for more than 7 days.
- Splenomegaly
- Cytopenias (affecting at least 2 of 3 cell lineages in the peripheral blood):
- Hemoglobin <90g/L (in infants < 4 weeks: hemoglobin < 100g/L)

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- Platelets < 100 X 10^9/L
- Neutrophil < 1.0 X 10^9/L
- Hypertriglyceridemia and/ or hypofibrinogenemia:
- Fasting triglycerides ≥ 3.0mmol/L (i.e. ≥ 265 mg/dL)
- Fibrinogen ≤ 1.5 g/L
- Haemophagocytosis in bone marrow or spleen or lymph nodes or liver
- B) New diagnostic criteria-
- Low or absent NK-cell activity
- Ferritin ≥ 500 µg/L
- Soluble CD25 (i.e. soluble IL-2 receptor) ≥ 2400 U/ml (new data show normal variation by age. Level should be compared with age-related norms).

Bone marrow aspirations are often performed to check for evidence of hemophagocytosis when there is suspicion for HLH. Several diagnostic criteria for HLH, such as fever, cytopenias, and splenomegaly, are not very specific findings. Conventional wisdom suggests that finding evidence of hemophagocytosis can increase clinicians' confidence in making a diagnosis of HLH. Furthermore, genetic mutation analyses, NK-cell activity and sCD25 levels are usually send out tests done at specialized laboratories, which may not be as helpful in acute settings when prompt treatment decisions are crucial. In HLH, as a result of exaggerated immune activation, macrophages nonselectively phagocytize hematopoietic elements, presumably leading to the microscopic finding of hemophagocytosis. However, histologic evidence of hemophagocytosis is not specific to HLH and can be seen in other conditions as well, such as after blood transfusion, chemotherapy administration, and major operations, but the expected amount of hemophagocytic cells (HPCs) seen in these conditions has not been well defined. [9-12] At the same time, although it has been suggested that a positive finding in marrow for HPCs requires careful examination of at least three smears, each revealing at least two HPCs,[11] there is so far no accepted interpretative threshold for positive findings or standardized reporting guidelines when such findings are present.

The exact pathophysiology of HLH varies depending on the cause and trigger. [13] Based mainly on the pathophysiology of primary HLH, defective granule-mediated cytotoxicity of cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells is considered the main abnormality that causes HLH. Since CTLs and NK cells cannot insert perforin channels into the membranes of antigen presenting cells (e.g. Macrophages and histiocytes) and deliver granzymes. So, osmolysis and apoptosis of the antigen presenting cells do not occur. With persistent antigenic stimulation of CTLs and NK cells by the antigen presenting cells, an abundant release of cytokines ensues. The cytokine storm creates a systemic inflammation that can cause tissue destruction, progressive organ failure and death. Activated macrophages may engulf blood cells and create hemophagocytosis [14], one of the features of HLH.

A small case-control study found bone marrow quantitation of hemophagocytosis to be higher in patients with HLH, and hemophagocytosis had a sensitivity of 83% and a specificity of only 60% in diagnosing HLH. [9] Only bone marrow aspirates, but not biopsy specimens, were evaluated.

In this retrospective study, among 857 bone marrow aspirates, 18 cases were found with HLH. We searched for the etiology from bone marrow examination and reviewing the medical records.

Aims and Objectives

1. To study the incidence of bone marrow haemophagocytic lymphohistiocytosis.
2. To study the different causes of haemophagocytic lymphohistiocytosis.

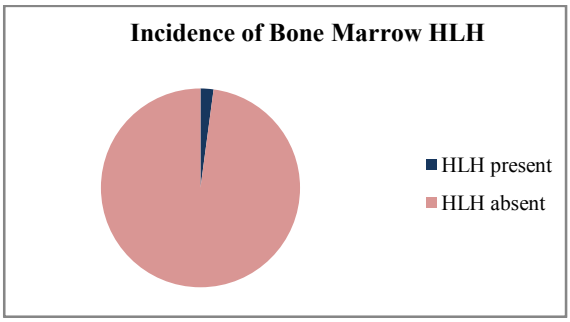
MATERIALS AND METHODS

Study design- Retrospective study.
Study duration- from February'16 to October'18
Place of study- Department of Pathology at Calcutta National Medical College and Hospital.
Period of study – 2 years 9 months.
Study population- Those patients who have haemophagocytosis in their bone marrow, included in this study.
Medical record review- Patients clinical presentations, symptoms, laboratory results, impressions and assessments of the treating clinicians, and disease courses were all taken into consideration, and data for each diagnostic criterion in the HLH-2004 guidelines and specific antibody tests were recorded.

RESULTS

Incidence of bone marrow haemophagocytic lymphohistiocytosis (HLH) -
Table 1 summarizes the incidence of bone marrow haemophagocytic lymphohistiocytosis. Among 857 bone marrow aspirates, 18 cases (2.1%) were found with HLH.

Table 1 Incidence of bone marrow haemophagocytic lymphohistiocytosis (HLH) (n=857)



Patients' characteristics

Table 2 and Table 3 summarize the patients' characteristics, including age and sex. Of the 18 patients, 11 were male (61.11%) and 7 were female (38.89%). The median age of diagnosing haemophagocytic lymphohistiocytosis was 38.0years, with an age range of 1 to 74 years.

Table 2 Age distribution (n=18)

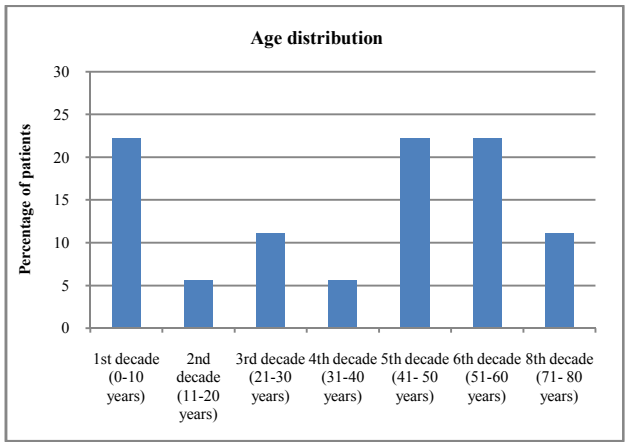
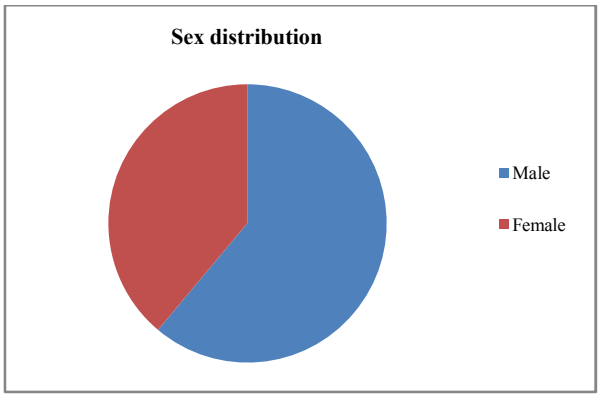


Table 3 Sex distribution (n=18)



Peripheral blood smears findings

Table 4 summarizes the peripheral blood smears findings. Of the 18 cases, 17 (94.44%) showed bicytopenia or pancytopenia and only 1 (5.56%) showed only thrombocytopenia.

Table 4 Cases showing cytopenia in peripheral blood smear (n=18)

Bi- or pancytopenia	No. of cases	% of total cases
Present	17	94.44
Absent	1	5.56

Bone marrow cellularity

At times initial stage of disease, no marrow involvement may be seen but may be seen in later in the course of disease. Varied cellular pattern of high, low or normal cells can be noted in HLH. Table 5 summarizes the bone marrow cellularity. Of the 18 cases, 16 (88.89%) showed hyper cellular marrow; whereas only 2 (11.11%) showed hypo cellular marrow.

Table 5 Cases showing bone marrow cellularity (n=18)

Cellularity	No. of cases	% of total cases
Hyper cellular	16	88.89
Hypo cellular	2	11.11

Causes of haemophagocytic lymphohistiocytosis (HLH)-
Table 6 summarizes the different causes of HLH. Of these 18 cases, 4 (22.22%) associated with infection (Kala-azar-1, Dengue-1, Enteric fever-1, HIV-1), each 2 cases (11.11%) with megaloblastic anemia, acute leukemia, myelodysplastic syndrome and plasma cell dyscrasia. But, 6 (33.33%) out of 18 cases are of unknown etiology.

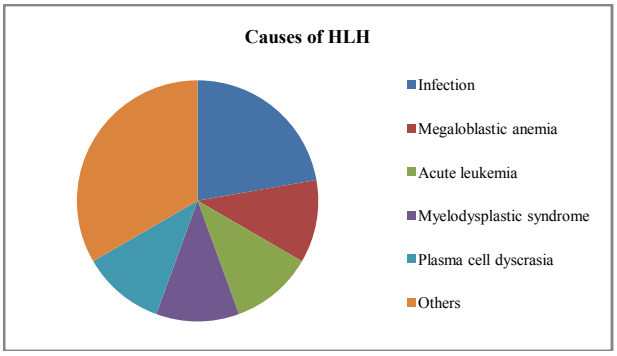


Table 6 Different causes of HLH (n=18)

DISCUSSION

HLH is a syndromic disorder that can lead to life-threatening symptoms in a short interval. Earlier reviews of the epidemiology of HLH reported incidences that were much low, likely reflecting under diagnosis of the condition. As example, the incidence of HLH in Sweden has been estimated to 1.2 children per 1 million children per year, or 1 in 50,000 live births with equal sex distribution. [15] A review of HLH cases from the largest pediatric hospital in Texas revealed an incidence of 1 in 100,000 children. [16] The true incidence of acquired HLH is unknown, and a study suggests that HLH may be significantly under recognized in many adult critical care units. [17] In this study, HLH is found in 18 cases (2.1%) out of total 857 cases.

In a study by Janka GE *et al* has showed that the disease is seen in all ages and has no predilection for race or sex. [2] Kaito *et al* described poor prognostic factor of adult HLH, including age over 30 years, presence of DIC, hyperferritinemia, increased beta 2-microglobulin, jaundice and worsening of anemia and thrombocytopenia. [18] Imashuku S *et al* has showed that HLH appears to affect all ages, although the hereditary and sporadic cases are reported more often in children. [19] Among these, 4 patients (22.22%) were under 10 years, 8 out of 18 patients (44.44%) were in 5th and 6th decade. The median age of diagnosing haemophagocytic lymphohistiocytosis was 41.5 years. Of these 18 patients, 11 were male (61.11%) and 7 were female (38.89%).

In 2011, Troltestam H *et al* have showed that cytopenias, especially anemia and thrombocytopenia, are seen in greater than 80% of patients on presentation. [16,20,21] Platelet counts range from 3000 to 2,92,000 (median 69,000)/ µL, and hemoglobin levels of 3.0 to 13.6 (median 7.2) g/dl are typical.[16] In this study, 17 patients (94.44%) with bicytopenia or pancytopenia and only 1 (5.56%) showed only thrombocytopenia.

In this study, 4 out of 18 cases (22.22%) associated with infection whereas 6 cases (33.33%) are of unknown etiology and 2 cases (11.11%) with megaloblastic anemia, 2 cases (11.11%) with acute leukemia, 2 (11.11%) with myelodysplastic syndrome and 2 (11.11%) associated with plasma cell dyscrasia. 4 cases of infective etiologies are- kala-azar, enteric fever, dengue, HIV. Dhote R *et al* have showed that a number of conditions are associated with secondary HLH. By prevalence, these include viral infections (29%), other infections (20%), malignancies (up to 27%), rheumatologic disorders (7%), and immune deficiency syndromes (6%). [22] As has been described by the others, the most common underlying malignancies were AML and MDS (21%).[23] Pancytopenia in typhoid fever may result from either

bone marrow suppression or infection associated hemophagocytic syndrome (IAHS).[24,25] Typhoid fever is rarely associated with HLH.[26] Viruses are most frequently associated with secondary HLH, particularly Epstein- Barr Virus (EBV)[27] , but tuberculosis, malaria, leishmaniasis and typhoid fever are important tropical infections that act as a trigger for IAHS.[28] Waseem Iqbal *et al* have showed that megaloblastic anemia is the most common cause (24.4%) in non malignant hematological conditions with HLH[29] which is also similar to this study.

Different studies described that perforin mutations are causative in the majority of familial haemophagocytic lymphohistiocytosis (FHL) cases, accounting for up to 58% and are considered a defining feature of FHL-2.[3,19,30] Genes involved in cytotoxic granule exocytosis have been demonstrated to bear mutations in FHL-3, FHL-4, FHL-5. FHL-3 cases, which account for 10%-32% of genetic HLH feature UNC13D mutations. [2]

CONCLUSION

HLH is a diverse condition with many causes and is likely under recognized, which contributes to its high morbidity and mortality. Though clinical findings, biochemical markers and tissue diagnostic markers fulfill diagnostic criteria, genetic analysis is warranted in all relevant cases as it has specific therapeutic and prognostic implication. Facilities for genetic study in diagnosis of haemophagocytosis lymphohistiocytosis are still less available and costly in our country. Infections are common triggers in both genetic and acquired HLH. Fair number of HLH occurs due to infection and it may be manageable. This study is too small to conclude, further study is required for better comment.

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