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ORIGINAL RESEARCH ARTICLE

Clinicomorphological spectrum of hemophagocytic syndrome in a tertiary care hospital

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Abstract

Introduction: The HLH-2004 trial established the diagnostic criteria for hemophagocytic lymphohistiocytosis (HLH), a severe hyperinflammatory condition. It typically develops due to inappropriate macrophage activation.

Our objective was to assess the spectrum of hemophagocytic syndrome presentations by identifying hemophagocytic activity in the bone marrow, and to unravel the etiopathogenesis of this condition.

Material and methods: A retrospective study was carried out in the Department of Pathology in a tertiary care hospital reporting the clinical and laboratory findings of patients who had been previously diagnozed with hemophagocytosis in the bone marrow. The parameters in the diagnostic criteria of HLH of the same patients were documented and analyzed.

Results: The characteristics of the 32 patients who presented with hemophagocytosis in the bone marrow were documented. Persistent fever was the most frequent presentation. Mild to moderate anemia (69%), severe leucopenia (59%), and mild to moderate thrombocytopenia (63%) were other frequent findings. The incidence of primary HLH was found to be only 3%; 87% had hyperferritinemia, 78% had bicytopenia, 59% had hypertriglyceridemia, and 53% had splenomegaly. Infections followed by malignancies were shown to be the most frequent cause of secondary HLH, while the prognosis for malignancy-associated HLH appeared to be poor.

Conclusions: Based on the findings of this study, conclusions about the clinical symptoms and etiologies of HLH may be drawn, which will assist in early identification. Hence, all subjects with a clinical suspicion of HLH should be thoroughly investigated for a possible etiology.

Key words: bone marrow, hemophagocytic lymphohistiocytosis (HLH), hyperferritinemia, primary HLH, secondary HLH

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Introduction

Hemophagocytosis is a pathological disease in which activated macrophages phagocytoze bone marrow cellular components (erythrocytes, leukocytes, platelets, and their progenitors) [1, 2]. Hemophagocytic syndrome (HPS), also known as hemophagocytic lymphocytic lymphohistiocytosis (HLH) is a rare, potentially fatal condition of immune dysregulation due to failure in making a timely diagnosis and appropriate treatment [3–6]. It describes a spectrum of enhanced macrophage activity as a result of a cytokine storm and multi-organ failure, as measured by certain laboratory markers (i.e. elevated ferritin, triglycerides, soluble CD25, transaminases, lactate dehydrogenase, and fibrinogen) [3, 5, 7].

Although HLH has been observed in people of all ages, it is most frequent in children and young adults [8]. It is usually split into two categories: primary/familial HLH (fHLH) and acquired/secondary HLH. Primary HLH is a condition that affects children and is caused by a hereditary defect

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in natural killer and T-cell cytotoxic activity [9]. Secondary HLH is most common in adults, with average age at diagnosis being 48 to 50 years. Infection, cancer, immunological insufficiency, and rheumatological diseases are the most common causes of secondary HLH [3–5, 7].

Hyperpyrexia, spleen enlargement, and pancytopenia are clinical features of HLH, whether primary or secondary [4, 6]. Rashes, liver dysfunction, hyperferritinemia, hypertriglyceridemia, coagulation problems, and renal insufficiency are some other clinical manifestations. The pathognomonic hallmark of HLH is the presence of hemophagocytosis in the bone marrow, lymph nodes, spleen, or liver [10]. The liver and spleen are the most commonly affected organs. The lungs, intestines, kidneys, and skin are also organs that are regularly implicated. The prognosis for certain individuals with neurological disorders is exceedingly dismal [11]. In 1994, the Histiocyte Society published therapeutic recommendations for the treatment of HLH, and in 2004 a revised set of diagnostic criteria was released. Diagnostic criteria with their clinical and laboratory features have been previously established in the literature [4].

The objective of this study was to determine the range of hemophagocytic syndrome presentations by identifying hemophagocytic activity in the bone marrow and correlating it with clinical and biochemical parameters found in HLH. Since the patient's overall prognosis is determined by prompt diagnosis and treatment, the current study also aimed to demonstrate the etiopathogenesis of this disorder, which can play a key role in determining the patient's prognosis.

Materials and methods

This retrospective study was conducted in the Department of Pathology in a tertiary care hospital which reports the clinical observations and laboratory findings of individuals who have presented with hemophagocytosis in the bone marrow. Thirty-two individuals who had visited the department in the past, irrespective of age, sex, or health status, were incorporated into the present study.

Patient requisition forms, the hospital information system, and discharge summaries were used to collect clinical and laboratory data for each patient. The patient's age, fever, splenomegaly, peripheral blood counts, triglycerides levels, fibrinogen levels, ferritin levels, bone marrow findings, and examinations pertinent to the underlying pathology of hemophagocytosis were all recorded.

Bone marrow aspiration and trephine biopsy were performed under aseptic conditions and local anesthesia. A smear was prepared using the squash technique and stained with Giemsa stain. The trephine samples of bone marrow were sent to a histopathology facility in 10% neutral buffered formalin. These biopsies were decalcified with pH 7.6 aqueous ethylene diaminetetraacetic acid (EDTA). The blocks were processed, and sections were taken for hematoxylin and eosin (H&E) staining and immunohistochemical marker (IHC) studies. All tests for viral markers [including Epstein-Bárr virus (EBV), and cytomegalovirus [CMV]) were conducted pertaining to the clinical presentation of the patients.

With the Institutional Human Ethics Committee, PSG Institute of Medical Sciences & Research (IMS&R) approval (Project No. 15/394), the present study was conducted and carried out as per the ICH-GCP/ICMR/Schedule Y guidelines. Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) software. Results were presented as mean, frequency, and percentages.

Results

The characteristics of 32 subjects who presented with hemophagocytosis in the bone marrow were documented. These included age, fever, splenomegaly, and laboratory parameters such as blood counts, ferritin, triglyceride, and fibrinogen levels.

Out of the total number of patients incorporated in our study, 59% were males and 41% were females; 62% were ≥18 years and 38% were <18 years. The mean age was 30.7 years.

The clinical and laboratory observations of the subjects according to the HLH-2004 criteria were extracted from the medical records and were analyzed (Figure 1). At diagnosis, all patients (100%) had a fever, 87% had hyperferritinemia, 78% had bicytopenia, 59% had hypertriglyceridemia, 53% had splenomegaly, and 31% had hypofibrinogenemia.

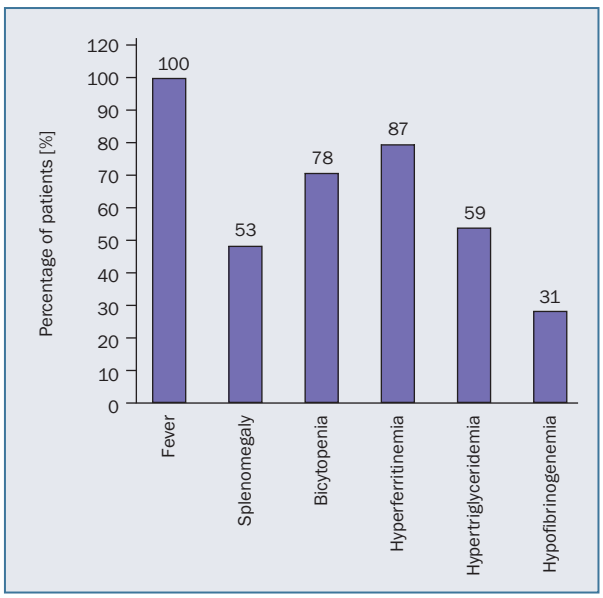

Figure 1. Clinical and laboratory observations of patients according to Hemophagocytic Lymphohistiocytosis-2004 criteria

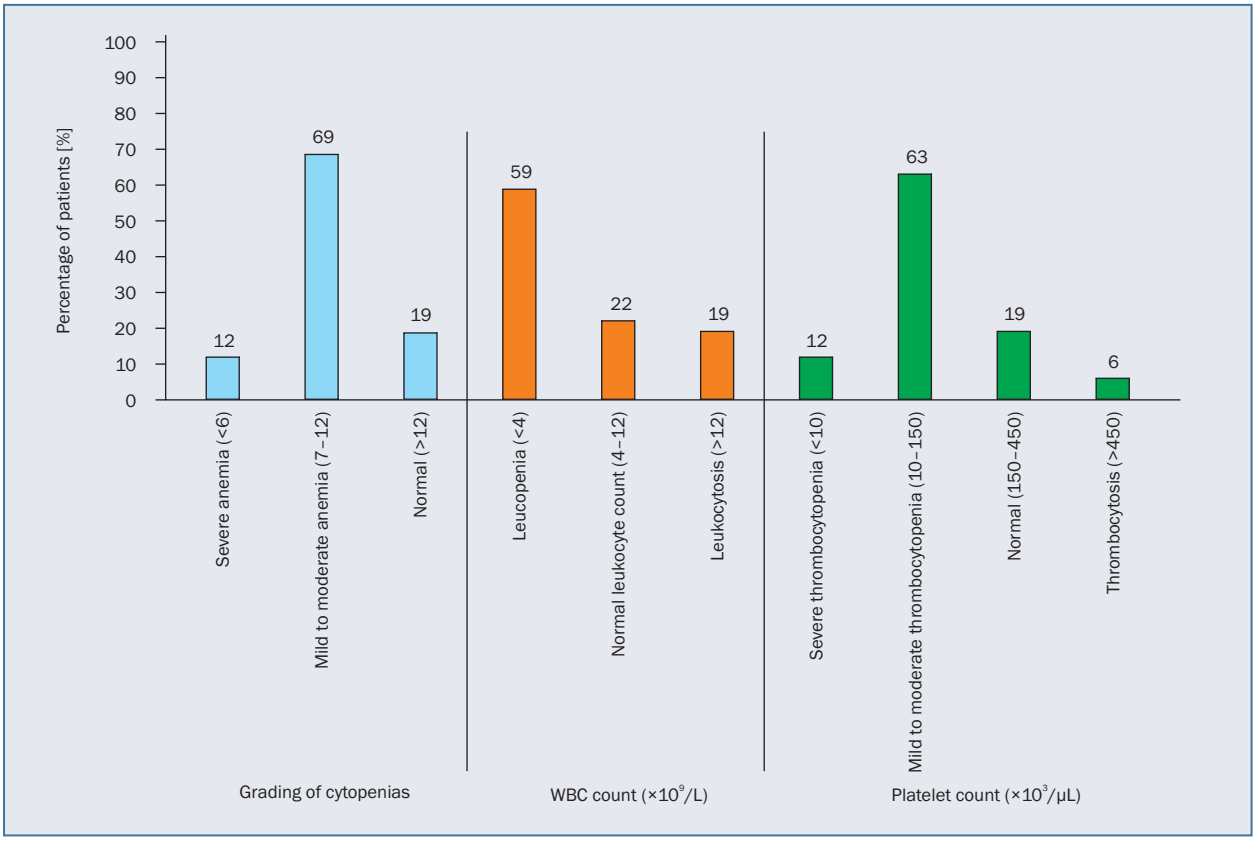

Figure 2. Grading of cytopenias and their percentage of presentation in subjects with hemophagocytic lymphohistiocytosis; WBC — white blood cells

Grading of cytopenias

Figure 1 shows that 78% of subjects presented with bicytopenia. The hematological parameters were again graded according to their severity [12].

Observations of the subjects according to the grade of severity of the hematological parameters are set out in Figure 2.

Bone marrow trephine

About 19% of the participants had hemophagocytosis in both the bone marrow aspirate and the trephine, whereas the remaining 81% had hemophagocytosis solely in the bone marrow aspirate. No patient tested positive for trephine but negative for bone marrow aspirate. IHC marker CD68 was used to indicate hemophagocytic activity in the bone marrow trephine (Figures 3A, B).

Types of HLH

In the present study, 3% of subjects (one case) had primary HLH (associated with Cheidiak Higashi syndrome) and the remaining 97% had secondary HLH. Abnormal granules in leucocytes and hemophagocytosis in the same subject is represented in Figures 4A, B.

Etiology

Infection was the most prevalent cause of HLH in this study, accounting for almost 70% of the participants. Bacterial infections accounted for 55%, viral infections for 28%, parasitic infestations for 9%, and fungal infections for 4%. The causal bacterium could not be isolated in the remaining 4% of cases. Tuberculosis was the most common infection, accounting for 23% of those infected. Disseminated sepsis was the second most prevalent cause, accounting for 19% of cases. Typhoid fever accounted for 15%, dengue fever, also known as scrub typhus, accounted for 9%, and human immunodeficiency virus (HIV) accounted for another 9%. Hepatitis A, nocardiosis, viral pneumonia, and pyrexia of unknown origin (PUO) each accounted for 4% of the total.

Neoplasms associated with HLH

After infections, neoplasm-associated HLH was the most common cause of hemophagocytosis, accounting for 16% of all patients. Lymphomas accounted for 40% of the HLH linked with neoplasms. (Figures 5A, B).

Non-lymphoma Hodgkin patients made up roughly 6% of the total. The remaining 3% of patients had myelofibrosis,

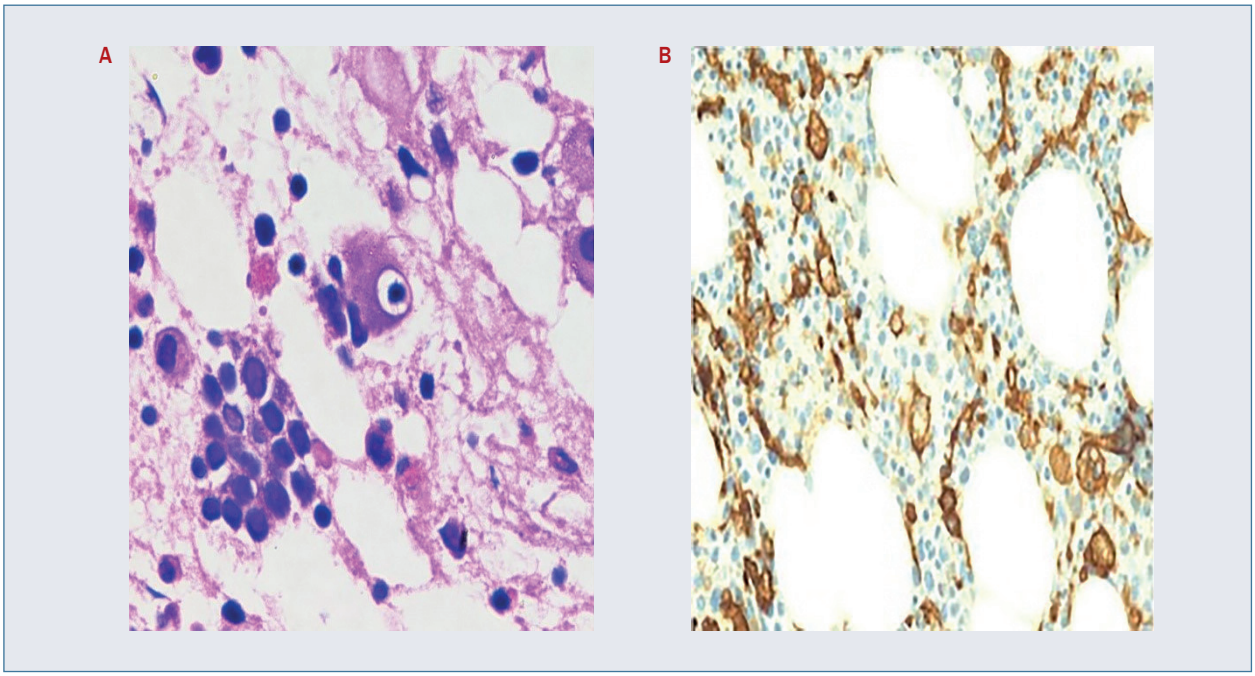

Figure 3. Hemophagocytosis in trephine (**A**) highlighted using immunohistochemical marker CD68 (**B**)

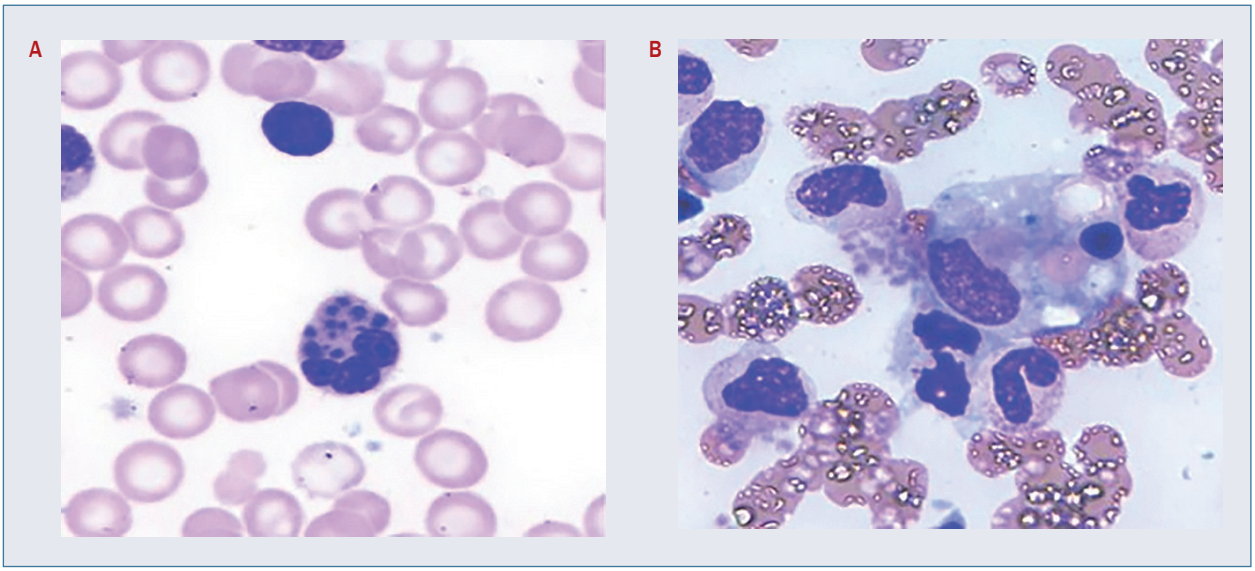

Figure 4. Abnormal granules in cytoplasm of white blood cellss in peripheral smear of individual with Chediak Higashi syndrome: **A.** Within neutrophil; **B.** Hemophagocytosis in one marrow of same individual, in Giemsa 100×

multiple myeloma, myelodysplastic syndrome, aplastic anaemia, Kikuchi Fujimoto Disease, Chediak Higashi Disease, or systemic lupus erythematosus.

Twenty per cent of subjects had a diagnosis of multiple myeloma. Myelodysplastic syndrome and myelofibrosis (Figure 6) each accounted for 20%.

Deaths associated with HLH

Nineteen per cent of the total participants in this study had an extremely unstable clinical course, which culminated in their deaths. Sepsis was responsible for 32% of the fatalities related to HLH. Scrub typhus, hepatitis A, diffuse large B-cell lymphoma (DLBCL), and multiple myeloma (MM) each accounted for 17%.

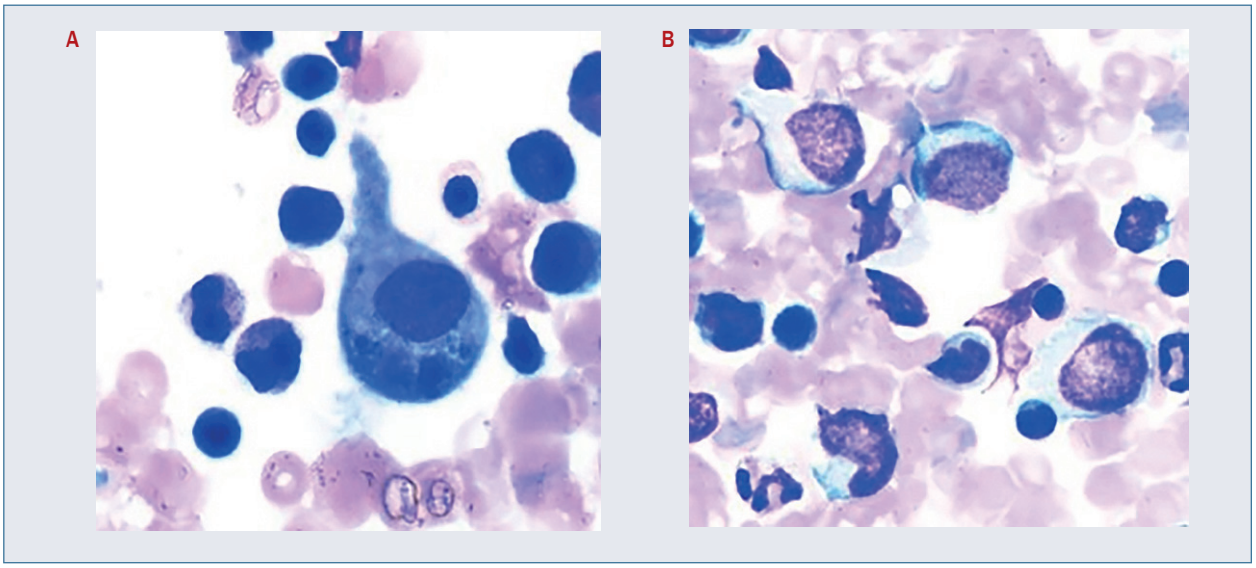

Figure 5. Hemophagocytosis (**A**) in subject with atypical lymphoid cells (**B**) in bone marrow diagnosed to be diffuse large B-cell lymphoma, in Giemsa 100×

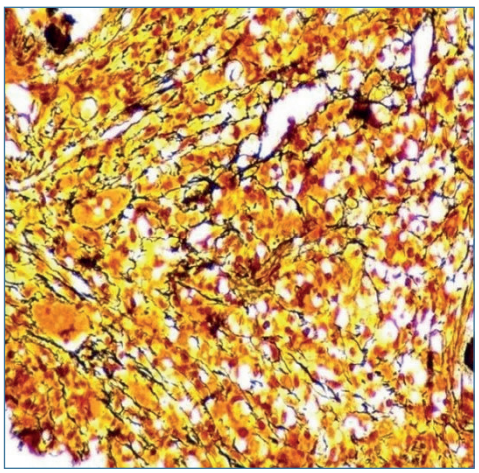

Figure 6. Bone marrow trephine of patient with myelofibrosis in reticulin stain 40×

Discussion

HLH is a syndrome, not a specific disease, that can manifest itself in a variety of circumstances. The incidence of HLH is estimated to be 1 in 100,000 live births [3].

The purpose of this study was to determine the range of hemophagocytic syndrome presentations and to elucidate the varied etiopathogenesis of HLH. Awareness of the various etiologies of this important condition will go a long way toward ensuring prompt diagnosis and treatment.

59% of the total individuals in the study were males, while 41% were females; 62% were adults (18+ years), while 38% were in the paediatric age group (0-17 years). The average age of the participants was 30.7 years. These findings are almost identical to those of Iqbal et al. [12], who found that the average age of HLH presentation was 30.8 years. In our investigation, there was no significant difference between the presentations of males and females, and this again was similar to the findings of Iqbal et al. [12].

In the diagnostic criteria, fever was the common clinical presentation in our study. Fever was the most prevalent clinical manifestation, according to the diagnostic criteria. This was in accordance with George et al. [13] and Otrock et al., [10] who observed that almost 100% of HLH subjects presented with fever. Iqbal et al. [12] stated that 65.2% of patients presented with fever, which was less than in our study. It may be inferred that the most prevalent symptom of HLH is a prolonged and persistent fever that is refractory to treatment.

In this study, splenomegaly was seen in 53% of subjects. This result approximately correlates with Fardet et al. [14], where splenomegaly was seen in 65% of patients, whereas Iqbal et al. [12] observed splenomegaly in 37.2%. In contrast to this, George et al. [13] stated that splenomegaly was present in 100% of individuals with primary HLH and in 80–90% with secondary HLH. Iqbal et al. [12] graded the cytopenias and gave percentages accordingly.

George et al. [13] observed cytopenias in 80%; in line with this, 78% of subjects in our study presented with bicytopenia.

The level of ferritin in macrophages is a good predictor of their phagocytic activity [15]. Hyperferritinemia was discovered in 87% of the individuals in our study, which matches the findings of George et al. [13], who reported hyperferritinemia in 70–90% of patients, whereas Chandra et al. [2] found it in only 40%.

In this study, hypertriglyceridemia was detected in 59% of HLH cases, whereas George et al. [13] found

hypertriglyceridemia in 40% of patients and Chandra et al. [2] found abnormal lipid levels in 45% of cases. In the studies by George et al. [13] and Chandra et al. [2], 40% of participants presented with hypofibrinogenemia. In contrast with this, in our study, fewer (31%) cases presented with low fibrinogen levels.

IHC marker CD68 was utilized by Caleb Ho et al. to identify hemophagocytosis in bone marrow samples. This improved sensitivity allowed the assessment of hemophagocytic activity in trephine biopsies [16]. In contrast to this, using the marker CD68 on trephine biopsies did not improve the sensitivity of identification of aberrant phagocytic activity in our study.

HLH is divided into two groups: primary HLH and secondary HLH. Primary HLH is mostly seen in children of less than one year of age. Primary HLH accounts for 25% of the HLH presenting in the pediatric age range, according to Zhang et al. [17]. HLH was observed in 38% of the pediatric age group in our study. In contrast to this, the percentage of individuals with primary HLH was just 3%. This is due to a lack of equipment for diagnosing genetic alterations, as well as the study population's financial restrictions.

In this study, infection was the common cause of secondary HLH, accounting for 70% of the total cases. George et al. [13] reported a 50% infection-related HLH, while Chandra et al. [2] reported a 13% infection-related HLH. Though the percentage of cases varied, infection-related HLH was the most prevalent cause of secondary HLH in all three studies.

Typhoid, according to Non et al. [18], is an extremely uncommon cause of HLH. In this study, typhoid was found in 15% of the infection-associated HLH patients. In our investigation, dengue and HIV-associated viral infections were found to be more prevalent, accounting for 9% of all instances of hemophagocytosis. Malaria was shown to be the most frequent parasite illness linked with HLH by Chandra et al. [2]. Scrub typhus, on the other hand, was the most frequent parasite infection in the current research (9%).

The second most prevalent etiological factor for secondary HLH, according to George et al. [13], Zhang et al. [17], and Hust et al. [19], is neoplasm-associated HLH (both hematological and non-hematological). Of the malignancies, lymphoma was common in all the studies. In the present study, 16% of cases were due to malignancies (both hematological and non-hematological).

There are a few limitations to our present study. Firstly, this was a retrospective study based on case selection criteria and diagnosis coding in medical records. Since many patients were lost to follow-up, information on therapy response and prognosis of the subjects was not accessible. The procedures for assessing NK cell activity and CD25 levels were not accessible at the research institution, and hence those parameters were not examined. There were also no resources for molecular diagnosis of mutations associated with primary hemophagocytic lymphohistiocytosis, which may have contributed to the lower incidence of primary HLH (3%) in the current research when compared to other investigations [20].

Conclusions

A confirmed diagnosis of HLH is based on a comprehensive examination of patients in a clinical setting. Conclusions on the clinical symptoms and etiologies of HLH may be drawn from this study, which will assist in early diagnosis. As a result, all individuals with a clinical suspicion of HLH should be thoroughly investigated for a probable etiology. For early diagnosis and therapy, a complete and detailed examination of the bone marrow of individuals suspected of having HLH is required. As a result, further research is needed to increase awareness of this condition, and enhance the efficacy of the current treatment regimen.

Authors' contributions

The manuscript has been read and approved by all the authors, that the requirements for authorship as stated earlier in this document have been met, and that each author believes that the manuscript represents honest work.

Conflict of interest

The authors declare no conflict of interest.

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This study did not receive any form of funding from public, government, or not-for-profit sectors.

Ethics

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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