RESEARCH PAPER

# Profile of Hemophagocytic Lymphohistiocytosis in Children in a Tertiary Care Hospital in India

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**Objective:** To describe the epidemiology, clinical features, laboratory findings, outcome and the difficulties in diagnosis and management of children with Hemophagocytic Lymphohistiocytosis (HLH) in a tertiary children's hospital in India.

**Study Design:** Retrospective analysis of case records of all the children with a diagnosis of HLH from December 2006 to December 2008.

**Setting:** Tertiary care children's teaching hospital in Chennai, India.

**Results:** 43 children had a diagnosis of hemophagocytosis, of who only 33 (19 male, mean age 46 months, range 50 days-14 years) met the inclusion criteria based on the HLH-2004 protocol of the Histiocyte Society. The predominant presenting features included prolonged fever and hepatosplenomegaly. CNS symptoms were present in 36%. Anemia (Hb <9gm/dL), and thrombocytopenia (platelets <1,00,000/mm$^3$) were present in 97% and 72%, respectively. Among the biochemical markers, hyperferritinemia was present in 97%, and hypofibrinogenemia and high LDH in 92%. Bone marrow examination showed hemophagocytosis in 84%. Infectious agents were identified in 42% children, with viruses accounting for 2/3 of them (5 Dengue virus, 3 EBV, 1 CMV, 1 TB and 5 bacterial agents). The mean duration between the onset of symptoms and the diagnosis was 16 days. Corticosteroids were the most commonly used immunomodulatory agents (67%), followed by IVIg (64%). Cyclosporine was used in 33% and Etoposide in 15%. Improvement of laboratory parameters was noticed within 5-7 days of starting treatment. Overall survival rate was 76%.

**Conclusion:** HLH should be considered in the differential diagnosis of children with prolonged fever, hepatosplenomegaly and cytopenia. Prompt recognition and appropriate therapy may result in good outcome, particularly in Infection associated HLH.

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Hemophagocytic Lymphohistiocytosis (HLH) is a rare macrophage related hyper inflammatory disorder that presents as prolonged fever and a sepsis like syndrome [1,2]. There are 2 types - Primary (familial) and Secondary or Reactive HLH (associated with viral, bacterial, fungal or parasitic infections, as well as with autoimmune disorders or malignancy). Familial HLH (FHL) is a rapidly fatal autosomal recessive disorder typically affecting young children. Both forms of HLH may be preceded by infection and can be differentiated only by molecular genetic techniques [2,3].

We outline the epidemiology, clinical features, laboratory findings, outcome and the difficulties in diagnosis and management of these children, from a tertiary children's teaching hospital in southern India.

## METHODS

This was a retrospective study of case-records of all the children who were diagnosed to have HLH during the period December 2006 to December 2008 at the Kanchi Kamakoti CHILDS Trust Hospital. Only those children who met the diagnostic criteria of the HLH-2004 protocol of the Histiocyte Society

(***Table* I**) were included. Genetic analysis, NK cell activity and soluble CD25 receptor assay could not be done in any child.

The case records of the children who fulfilled the criteria for HLH were analyzed and the following details collected: age at presentation, sex, relevant family history, clinical and laboratory data, course, treatment for HLH, and outcome (survival or death). For peripheral blood counts and biochemistry, nadir or peak values were recorded. In some children perforin expression was determined in cytotoxic cells using flow cytometry at the Institute of Immunohaematology, KEM Hospital, Mumbai, and compared with age matched controls.

## RESULTS

Forty three children were diagnosed with HLH over a 25-month period. Of these, only 33 met the inclusion criteria and were analyzed. The remaining 10 children presented with prolonged fever and had hemophagocytosis, either in the bone marrow or lymph nodes but were excluded since they did not meet the minimum diagnostic criteria.

Of the 33 children analyzed, 19 (58%) were male. The ages ranged from 50 days to 14 years (mean 46 months, median 33 months). Six children had a family history of consanguinity and three had a history of sibling death (anemia requiring blood transfusion and hospitalization elsewhere).

The most common reason for hospital admission was fever with or without additional symptoms or signs such as rash, hepatosplenomegaly, lymphadenopathy, respiratory symptoms [cough, tachypnea, dyspnea] or CNS involvement (irritability, altered behavior, meningeal signs or seizures). The interval between the onset of symptoms and diagnosis of HLH varied from 7 to 53 days (mean 16 days). The clinical and laboratory features are highlighted in ***Tables* II** and **III**. Eighteen (55%) children required intensive care for either respiratory distress or sepsis and 7 (22%) were ventilated.

**TABLE I** Diagnostic Guidelines for HLH-2004

The diagnosis of HLH can be established if one of the either 1 or 2 below is fulfilled.

1. A molecular diagnosis consistent with HLH.
2. Diagnostic criteria for HLH fulfilled (5 out of 8 criteria below).

A. Initial criteria (to be evaluated in all patients with HLH)

*Clinical criteria*

- Fever
- Splenomegaly

*Laboratory criteria*

- Cytopenias (affecting ≥2 lineages in peripheral blood): anemia (hemoglobin <9gm/dL), thrombocytopenia (<100,000/ $mm^3$) and neutropenia (ANC <1000/$mm^3$)
- Hypertriglyceridemia (≥265mg/dL) and/or Hypofibrinogenemia (<150mg/dL)

*Histopathologic criteria*

- Haemophagocytosis (in bone marrow, spleen or lymph nodes).
- No evidence of malignancy.

B. New diagnostic criteria

- Low / absent NK cell activity
- Hyperferritinemia (>500mcg/L)
- Increased soluble CD 25 ≥2400 units /mL.

**TABLE II** CLINICAL AND LABORATORY FEATURES

| | *n* (%) |
|---|---|
| Rash | 58 |
| Lymphadenopathy | 39 |
| Respiratory symptoms | 30 |
| CNS symptoms | 36 |
| Hepatomegaly | 100 |
| Splenomegaly | 88 |
| Anemia (Hb <9g/dL) | 94 |
| Thrombocytopenia (platelet<1lakh/ $mm^3$ ) | 79 |
| Neutropenia (ANC<1000/ $mm^3$ ) | 24 |
| Coagulopathy (prolonged PT and APTT) | 33 |
| Elevated liver transaminases (>60 IU/L) | 67 |
| Hyperbilirubinemia (>2mg/dL) | 18 |
| High creatinine (>1.4mg/dL) | 9 |
| Hyperferritinemia (>500mcg/L) | 97 |
| Hypertriglyceridemia (>265mg/dL) | 64 |
| Hypofibrinogenemia (<150mg/dL) | 92 |
| High LDH (>450 IU/L) | 92 |

**TABLE III** LABORATORY VALUES

| | Median (range) |
|---|---|
| WBC, per $mm^3$ | 5900 (700-54500) |
| ANC, per $mm^3$ | 2288 (80-28215) |
| Platelet, per $mm^3$ | 60000 (5000-330000) |
| Hemoglobin, gm/dL | 7.35 (5.7-9.6) |
| Ferritin, mcg/L | 3171 (188-49968) |
| Fibrinogen, mg/dL | 72 (38-326) |
| Triglycerides, mg/dL | 296 (70-520) |
| LDH, IU/L | 1103 (352-7098) |
| AST, IU/L | 103 (17-3790) |
| ALT, IU/L | 57 (11-1250) |

*ANC, absolute neutrophil count; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase WBC, total leucocytes.*

Nineteen of the 21 children in whom ferritin was repeated showed a decreasing trend, and they survived. CSF analysis was carried out in all children with neurological involvement ($n$ = 12) and was abnormal [cells >15 and/or elevated CSF protein (>50mg/dL)] in 4 of them. Bone marrow aspiration was performed in 31 children and showed hemophagocytosis (macrophages engulfing blood cells and their precursors) in 26 children (84%). There was no evidence of malignancy in any patient.

Infectious etiology was identified in 14 (42%) children, with viruses accounting for the majority (EBV-3, Dengue-5, CMV-1). Bacteria were isolated in 5 children – *Mycobacterium tuberculosis* (1), Klebsiella (1), Leptospira (1), Pneumococcus (1) and Sphingomonas (1). The child with tuberculosis has been reported earlier [4].

All 33 children received supportive treatment including antibiotics and blood products when necessary. Immunomodulatory treatment was initiated within 1-10 days of admission, at the treating pediatrician's discretion. Corticosteroids (IV methylprednisolone and/or dexamethasone) were used in 22 children, followed by Intravenous immunoglobulin (IVIg) in 21. Chemotherapy was initiated in 11 children who either did not respond to IVIg/steroids or were considered very sick. Cyclosporine was used in all 11 children and etoposide added in 5 children who had an ANC >500/$mm^3$ [1]. Improvement in laboratory parameters (especially increasing platelet counts) was noticed within 5-7 days of starting immunomodulatory treatment. Out of 33 children, 25 (76%) survived and were discharged home.

Two children required full 40 weeks treatment as per the HLH-2004 protocol and are doing well. In three children, chemotherapy was stopped after the initial 8-week course and they are doing well now. Perforin expression in cytotoxic cells was very low in 3 out of 10 children in whom the test was performed. Of these, two survived and are doing well.

## DISCUSSION

HLH is a rare disorder that is being increasingly recognized in children now. This is the largest case series of HLH from India. The median age at diagnosis of HLH in our series was 33 months, similar to other Asian studies [5,6]. The overall mortality (24%) in our series was lower than in series reported earlier [5-10]. Better survival rate in this series might be due to a high incidence of secondary HLH, early diagnosis and early institution of immunomodulatory treatment.

Mutations in the perforin gene have been reported to be present in 20-40% of FHL [1,11]. Perforin staining in cytotoxic cells by flow cytometry has been documented as a screening test to identify children with FHL, who can then be subjected to genetic analysis [12,13]. A very low perforin expression was detected in 30% children in whom the test was done but we could not perform genetic analysis in them. The most common presentation at admission was fever and hepatomegaly (100%). Hyperferritinemia, hypofibrinogenemia and high LDH levels were observed in the majority of our patients, suggesting that these tests could serve as important diagnostic clues to HLH. Similar findings were reported in an earlier study [14]. We had normal bone marrow histopathology in 16% of the patients in whom bone marrow was performed. Hemophagocytosis in bone marrow may not be present during the early phase of disease, and therefore the absence of hemophagocytosis does not exclude a diagnosis of HLH [15].

**WHAT IS ALREADY KNOWN?**

- HLH is a rare hematological disorder which can be hereditary or associated with other diseases.

**WHAT THIS STUDY ADDS?**

- Bone marrow examination may not always show hemophagocytosis in early phase of this disorder. Prompt recognition and appropriate therapy may result in good outcome, especially in Infection associated HLH.

None of the clinical and laboratory parameters were significantly associated with mortality in our series. In survivors, recovery of laboratory parameters (increasing platelet count), decrease in ferritin levels, and clinical improvement (subsidence of fever and regression of organomegaly) was noted about 5-7 days after starting immunomodulatory treatment.

Dengue virus was the most common infectious agent identified in our series. Work up for HLH was done in these children as fever, thrombocytopenia and anemia, persisted for more than 2 weeks, without any proven secondary infection. Very few cases of dengue associated HLH have been reported in the literature [10]. EBV infection has been reported to be the commonest cause of infection associated HLH and has been observed to be a bad prognostic factor in earlier studies [16,17]. EBV accounted for only 20% of the cases in our series, which is much lower than in other reports [9,18]. All patients with EBV infection survived without cytotoxic drugs.

Stem cell transplant is the standard treatment for FHL, once remission is achieved on immuno-modulatory therapy [1]. However, genetic diagnosis is not available and stem cell therapy difficult in India. In our series, steroids with or without IVIg were used commonly in the early phase of the disease. A favorable response with IVIg has been observed in some studies [19,20], but a lack of efficacy was also reported [16]. Etoposide containing regimens have been reported to have better outcomes [16,17,21].

HLH should be considered in the differential diagnosis of children with sepsis or presumed sepsis that do not respond to the conventional treatment. Patients may not fulfill all the criteria during the early phase of the disease. Bone marrow examination may not show hemophagocytosis initially. Cytotoxic agents should be considered early in patients who do not respond to other therapies. Presence of an infection during illness does not exclude FHL. Prompt recognition and appropriate therapy may result in a good outcome.

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*Contributors*: BR and KGR managed the cases, analyzed data and stand as guaranteers for the manuscript. SBS contributed to patient management and writing of the manuscript. AVR initiated the design of the study and helped in editing the manuscript. AN collected and analyzed the data, outcome assessment and prepared the manuscript.

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*Competing interests:* None stated.

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