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ORIGINAL ARTICLE

Hemophagocytic Lymphohistiocytosis in Children

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Abstract

Objective To study the profile of children with Hemophagocytic Lymphohistiocytosis (HLH) in a tertiary care hospital for children.

Methods A retrospective analysis of case records of 52 children diagnossed with HLH was performed.

Results Of the 52 children 13% ($n = 7$) had Familial HLH and 87% ($n = 45$) had secondary HLH (sHLH). Common manifestations were fever (100%), organomegaly (87%), respiratory distress (54%), neurological symptoms (31%) and skin rashes (26.2%). Anemia and thrombocytopenia were present in 51% and 73% respectively. Hyperferritinemia was present in 96% and hypofibrinogenemia in 42% and high lactate dehydrogenase (LDH) in 91%. Bone marrow examination showed hemophagocytosis in 80%. Most common etiology among infections was viral infections (67%), of which Dengue was the most common (52%). Among children with sHLH 51% received supportive care only. Thirty-seven percent ($n = 17$) received intravenous (IV) immunoglobulin and steroids. Of these 77% ($n = 35$) recovered completely. Children with familial HLH were initiated on HLH 2004 protocol but all of them expired due to disease progression.

Conclusions Identifying HLH early and managing it, poses a significant challenge. Prompt recognition and initiation of immunosuppressive therapy is extremely important for the better outcome; hence high clinical suspicion and structured work up including immunological, and genetic studies is required. It may be difficult to differentiate primary and secondary HLH in many instances unless genetic analysis is done. Identification of familial HLH is necessary for early referral to Hematopoietic Stem Cell Transplantation (HSCT). Hence screening for primary HLH needs to be considered in all children with HLH.

Keywords Familial HLH · Secondary HLH · Genetic analysis · HSCT

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening hyper inflammatory syndrome due to highly activated macrophages and lymphocytes [1]. HLH is divided into Primary or Familial and Secondary or Reactive HLH (triggered by infections, malignancy and autoimmune disorder) [2]. Both forms of HLH may be preceded by infection and it is difficult to differentiate both in many instances unless genetic analysis is done [3].

Early diagnosis and prompt initiation of immunosuppressive therapy is extremely important for survival. Identification of familial HLH is necessary for predicting relapses and early referral to Hematopoietic Stem Cell Transplantation (HSCT) [4]. But timely diagnosis is challenging due to its variable clinical presentation and complex nature of the disease. Hence high index of clinical suspicion and complete immunological and genetic workup is required.

The authors describe the etiology, varied clinical presentation, laboratory findings, and difficulties in diagnosis, management and outcome of children with HLH.

Material and Methods

A retrospective study was performed at Kanchi Kamakoti Childs Trust Hospital, a tertiary care hospital for children in Chennai during from June 2016 through May 2018.

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The criteria for diagnosis of HLH were based on the proposed 2009 HLH diagnostic criteria of Histiocyte Society [5].

Case records of children who fulfilled the criteria for HLH were reviewed and following details were collected and analyzed: age at presentation, sex, relevant family history, clinical details (including fever, organomegaly, lymphadenopathy, and CNS symptoms), laboratory data mandatory or supporting the diagnosis of HLH, etiology (familial *vs*. secondary), management (including need of intensive care, drugs used), and outcomes. Perforin protein expression and CD107a expression on NK cells were done only in proportion of children with HLH by flowcytometry at the National Institute of Immuno-Hematology, Mumbai.

Results

Fifty two children who fulfilled the criteria for HLH during the study period were analyzed. The new proposed HLH diagnostic criteria were used as it was operator-friendly and included the common manifestation of hepatitis.

Of the 52 children with HLH, 13% ($n = 7$) were confirmed to have Familial HLH (FHL) (Table 1) and 87% ($n = 45$) of them had secondary HLH (sHLH) due to underlying triggers. The ages ranged from 1 mo to 14 y with an equal male: female ratio. Infants contributed to one-third of all children (36.5%, $n = 19$). The mean age of diagnosis in sHLH was 4.4 y, which was similar to FHL, highlighting that FHL not necessarily involves only very young children. Of the seven children with FHL, six children had a family history of consanguinity and one child had history of sibling death.

The most common cause of sHLH was viral infections (67%) of which Dengue was the most common (52% $n = 27$) followed by Epstein Barr Virus (EBV) and HIV. Bacterial infections were identified in 6% (scrub typhus and mycoplasma). The other causes of sHLH were rheumatological disorders [Systemic onset juvenile idiopathic arthritis and Systemic lupus erythematosus (SLE)] (9.6%, $n = 5$) and immunodeficiency (4.4% $n = 2$). One child had *LIG4* homozygous mutation which was diagnostic of DNA ligase deficiency, a rare form of Severe Combined Immune Deficiency (SCID) and one child had *LRBA* gene mutation causative of Common Variable Immune Deficiency with autoimmunity thereby triggering sHLH and in 5 children (9.6%), etiology could not be identified (Fig. 1).

Genetic analysis was performed in 10 children based on clinical suspicion; 6 of them had a mutation consistent with FHL. One child was diagnosed with FHL type 2 based on decreased perforin protein expression (Table 1).

The most common reason for admission was fever which was present in all children (100%). Organomegaly was present in a significant number of children (87%). The other common manifestations were respiratory distress (54%), neurological symptoms (31%) and skin rashes (26.2%). Anemia and thrombocytopenia were present in 51% and 73% respectively. Hyperferritinemia was present in 96% and hypofibrinogenemia in 42% and high LDH in 91%. CSF analysis was performed in 10 children with neurological involvement. All of them had elevated CSF protein whereas CSF pleocytosis was observed in approximately two-third (60%). Bone marrow aspiration (BMA) was performed in 38 children (80%) with majority revealing moderate to severe hemophagocytosis. Clinical and laboratory features of primary and secondary HLH were highlighted in Tables 2 and 3.

Of the 27 children with Dengue HLH, median age was 3 y (4 mo – 10 y) and mean duration of fever was 8 d. Mean ferritin and serum glutamic oxaloacetic transaminase (SGOT) were 26,620 mcg/L and 3320 U/L respectively. Diagnosis of dengue was made based on NS1Ag and Dengue IgM. Fever of more than 7–8 d, persistent cytopenia, organomegaly, high ferritin and very high transaminases suggested the possibility of HLH in the children with dengue fever.

The significant proportion of children in both the groups (FHL and sHLH) presented with neurological involvement, requiring intensive care (79%), mechanical ventilation (44%), need of inotropes and dialysis (8%); this highlights the need of multidisciplinary team management for maximum outcomes.

Of the 45 children diagnosed with sHLH, 51% ($n = 23$) improved with supportive care alone. Intravenous immunoglobulin (IVIg) was used in 37% ($n = 17$); of these 4 children (9%) received only IVIg and 28% ($n = 13$) received both IVIg and steroids. Improvement in laboratory parameters was noticed within 1 wk of initiation of treatment. Chemotherapy (etoposide and cyclosporine) was initiated in 5 children with sHLH (11%) who did not respond to IVIg and steroids.

Among dengue associated HLH, 65% improved with supportive care alone and 35% of children with additional high risk features (respiratory distress, neurological involvement, hemodynamic instability) received IVIg and steroids.

Out of 45 children with sHLH, 36 children (77%) recovered completely and are doing well. Four children were lost to follow-up and 5 of them expired [4 were dengue related and one boy had Epstein-Barr virus (EBV) infection]. In the boy who died of EBV associated HLH, Duncan syndrome was strongly suspected in view of male sex and EBV trigger but this could not be proven.

All the seven children diagnosed with FHL were initiated on HLH 2004 protocol and counseled for Hematopoetic Stem Cell Transplantation (HSCT) after controlling the disease. Of these, 5 children had continued disease progression and expired, one child was lost to follow-up, one child improved and was referred for HSCT. Overall survival rate was 67%.

Table 1 Features of children diagnosed with Familial HLH (FHL)

Serial no.	Age	Sex	Consanguinity	Sibling death	Clinical features	Final diagnosis	Genetic analysis/Supportive investigations
1	1 mo	M	No	No	None	FHL3	Homozygous UNC13D mutation (AR)
2	10 mo	M	3rd degree	No	None	XLP1	SH2D1A mutation (XLR)
3	6 y	F	3rd degree	No	Partial albinism, silvery hair, recto-vesical fistula	Chediak-Higashi syndrome	Giant neutrophilic vacuoles
4	8 y	F	3rd degree	Yes, 1st male	Hypopigmented silvery gray hair, eyebrows, eyelashes	Griscelli syndrome, type 2	Homozygous RAB27A mutation (AR)
5	11 y	M	2nd degree	No	Hypopigmented hair and skin lesions since birth	Griscelli syndrome, type 2	Homozygous RAB27A mutation (AR)
6	4 mo	M	3rd degree	No	Hypopigmented hair	Griscelli syndrome, type 2	Homozygous RAB27A -Large deletion (AR)
7	4 y	F	3rd degree	No	None	FHL2	Perforin protein deficiency (6%)

Discussion

Hemophagocytic lymphohistiocytosis (HLH) is a potentially fatal multisystem hyperinflammatory disorder caused by a highly stimulated but ineffective immune response [6]. Despite increasing insights into its genetic and immunologic basis, HLH remains as a syndromic disorder diagnosed by unique pattern of clinical and laboratory findings. The estimated incidence of HLH in children <18 y old across ethnicities and races is approximately 1 in 100,000, but this is likely to be an underestimate [7] and now it's increasingly recognized.

Diagnosing HLH is the first critical step toward successful therapy but early diagnosis remains challenging. In authors' experience they have noticed that there is delay in recognition and referral. It may be due to variable presentation and complexity of diagnostic criteria. To assist in early diagnosis, the Histiocyte Society has developed diagnostic guidelines, with additional experience these diagnostic criteria have been modified (Proposed HLH 2009 criteria). It included hepatitis as a criterion, though hepatitis may be a common feature of many of the tropical infections including dengue; it allows earlier recognition of HLH which is a known mimicker of infections. The sensitivity of these criteria for early HLH is unknown as there is no gold standard test [8, 9].

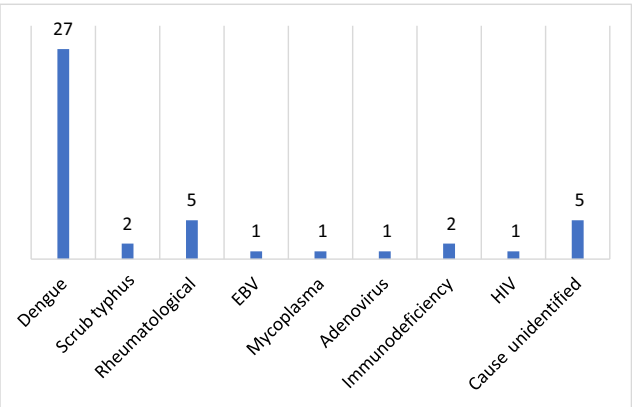


Fig. 1 Causes of secondary HLH. *EBV* Epstein-Barr virus; *HIV* Human immunodeficiency virus

This is the largest case series of HLH from India. Median age of present cohort was 4 y with an overall survival rate of 67%. Better survival and older age of presentation in present study as compared to other studies could be probably due to higher incidence of secondary HLH and early institution of therapy.

The Proposed 2009 HLH criteria allowed diagnosing HLH in 10 children who would have been otherwise excluded by the 2004 criteria. This highlights the need for us to adopt newer modifications proposed by the Histiocyte Society for further studies. Of note is the importance of diagnosing HLH even if all the necessary criteria are not met as the disease's manifestations may evolve over time. Normal bone marrow cytology was found in 20% of children in whom bone marrow was performed. Hemophagocytosis may not be clearly apparent in the bone marrow early in the disease process; therefore, absence of hemophagocytosis does not exclude the HLH [10].

FHL was identified in 13% of index children which is less than the reported incidence (24.6%) [11] and this study was limited as perforin and CD107a assay could not be performed in all children. Identifying FHL with hypopigmentation was by characteristic phenotypes, hair shaft examination (abnormal melanosome clumping) and bone marrow examination and genetic analysis was required only for confirmation. Whereas, FHL without hypopigmentation (children with FHL type 2 and 3, Duncan syndrome) was identified solely based on genetic analysis. This highlights the need for performing perforin and CD107a assay as screening tests in all children to rule out FHL.

Secondary HLH can be caused by infections (bacteria, viruses, and fungi), malignancies, autoimmune diseases and immune deficiency states [3]. In the present cohort, Dengue was the most common infectious cause. The previous studies from south India and Srilanka highlight the higher incidence of

Table 2 Clinical features of HLH children in the present study

Clinical feature	Primary HLH (%) ($n = 7$)	Secondary HLH (%) ($n = 45$)
Fever	100	100
Rash	42.8	24.4
Organomegaly	100	86.6
Lymphadenopathy	0	8.8
Respiratory distress	42.8	53.3
CNS involvement	28.5	31.1
PICU stay	85	54.2
Ventilation	42.8	44.4
Inotrope	42.8	37.7
Dialysis	28.5	11.1
Superadded infections	42.8	17.7

CNS Central nervous system, *PICU* Pediatric intensive care unit

sHLH due to dengue [12–14]. The results of present study too concur with the same, with dengue contributing to 78% of the total infection associated sHLH. In the West and South-East Asia, sHLH due to infections is usually EBV-triggered and it is associated with increased mortality if there is delay in diagnosis and initiation of etoposide [15].

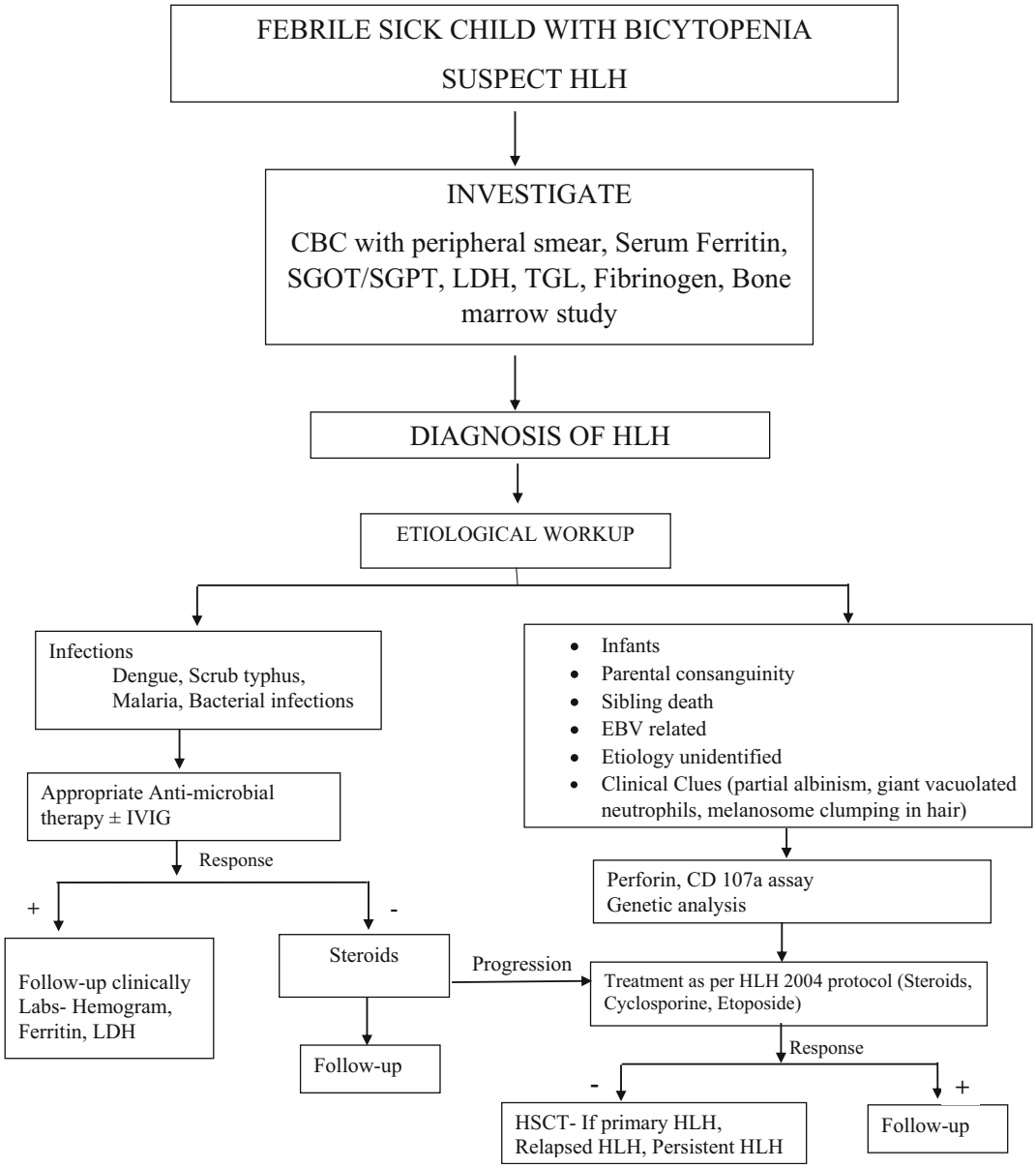
In the present study, majority of children with sHLH improved with supportive care alone. IVIG was used in one-third of all children which is comparable to the reported data in children [11]. Four children improved with IVIG only obviating the need of steroids, thereby avoiding immunosuppression and the risk of secondary infections. Steroids were used in 44%; with reduced duration of therapy adopted for children with sHLH who responded well to steroids. Etoposide and/or cyclosporine were used only in 14% of children who had persistent disease [11].

The use of the toxic HLH-2004 protocol using upfront CSA might not be warranted in sHLH who respond well to IVIG only or to steroids. Moreover, early introduction of CSA did not improve HLH outcome in patients treated with the etoposide-dexamethasone backbone ($P = 0.06$) [16]. Hematopoetic stem cell transplantation was indicated in patients with FHL, relapsing, or severe persisting disease [17].

Going by the outcomes of present study and a previous flowchart of management for HLH [18], authors suggest an algorithm for guiding evaluation and management of children with HLH in Indian subcontinent, especially in resource limited settings (Algorithm 1). Adopting such protocols balances the search for tropical triggers and early initiation of HLH- 2004 (including etoposide) in children with FHL and sHLH not responding to steroids alone [19].

Table 3 Laboratory data of HLH children in the present study

Investigations	Primary HLH		Secondary HLH	
	Mean value	Minimum–maximum values	Mean value	Minimum–maximum values
Hemoglobin (g/dl)	7.15	6–8.9	9.94	2.9–17.4
Absolute neutrophil counts (cells/mm^3)	2843	550–12,848	4901	342–27,040
Platelets (cells/mm^3)	94, 143	52000–1,56,000	1,09,089	10000–3,70,000
Ferritin (mcg/L)	46, 290	277–3,00,000	30,703	549–2,54,625
Triglycerides (mg/dl)	314.6	208–425	264.481	67–1017
Fibrinogen	276	148–453	214	28–917
Total bilirubin (mg/dl)	3	0.6–8.2	2	0.2–13
Serum glutamic oxaloacetic transaminase (SGOT)	1085	44–6485	2049	20–16,378
Serum glutamic pyruvic transaminase (SGPT)	291	17–1580	615	12–4800
Lactate dehydrogenase (IU/L)	5773	755–28,720	8337	672–25,800



Of note is that FHL can present with identified triggers and can present at any age. If a patient is successfully treated and weaned off therapy, they should be monitored closely even if it is due to trigger, because the distinction between primary and secondary HLH is difficult to make [19]. Regarding outcomes, 69% of index children recovered completely. The mortality rate was seven times higher in the FHL group compared to children with sHLH (71%% *vs*. 11%). Overall HLH-related mortality (23%) was less compared to the previous data [12]. Diagnosis of FHL warrants aggressive HLH directed therapy and early referral to HSCT. Despite this, all FHL children in present study had a fulminant course of HLH which was further complicated by super-added infections.

Multi-centric prospective nationwide web-based registry on HLH in India (Indian Hemophagocytic Lymphohistiocytosis Registry: http://indianpidregistry.com/) has been initiated and details for participating are available on request. Such registries and data analysis will clarify the etiology, management and outcomes of HLH in the sub-continent and whether the treatment protocols in the sub-continent merit reconsideration.

Conclusions

HLH is a multi-system inflammatory disorder caused by cytokine storm due to multitude of causes including underlying genetic diseases, or particular triggers in children with no known inherited disorder. Initial presentation may be vague and misleading. Therefore, a very high clinical suspicion and structured work up including immunological, and genetic

studies is required. Screening for primary HLH is desired in all children with HLH though rarely performed. Tropical infections are the major causes of HLH in India. Prompt initiation of appropriate antimicrobial therapy as indicated; supportive care along with HLH directed therapy is lifesaving. Genetic diagnosis in children with strong suspicion of FHL warrants aggressive management with chemotherapy and early referral to transplant physician. Enroling in national HLH registries allow data analysis contributing to understanding disease pathology, identifying new biomarkers and formulating tailored therapy in the long run.

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Authors' Contribution SS and DM managed the cases, analyzed the data and stand as guaranteers for the manuscript. DN, AL and MS collected and analyzed the data and prepared the manuscript.

Compliance with Ethical Standards

Conflict of Interest None.

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