Profile of Hemophagocytic Lymphohistiocytosis; Efficacy of Intravenous Immunoglobulin Therapy

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Abstract
Objective To study the profile of children with Hemophagocytic lymphohistiocytosis (HLH) and compare the outcome of treatment with intravenous immunoglobulin therapy and Dexamethasone vs. HLH-2004 protocol.

Methods The present retrospective cohort study was conducted in a tertiary care pediatric hospital in Chennai. Children with a diagnosis of HLH admitted to the hospital from June 2008 through June 2011 were included. Medical records of the subjects were reviewed and their clinical and demographic profile studied. Difference in outcome between treatment modalities was analysed.

Results Of the 40 children studied, all had fever of 38.5 °C for more than 7 d. Splenomegaly was noted in 25 children at admission, but eventually occurred in all the patients. All children had bicytopenia. Mean laboratory values were as follows: neutrophil count 3,400/cu.mm, hemoglobin 8.75 g/dl, platelet count 84,000/cu.mm, fasting triglycerides 358 mg/dl, ferritin 8,139 mg/dl and fibrinogen 137 mg/dl. All children had evidence of hemophagocytosis in bone marrow smear. Good outcome was seen in 19/22 children treated with IVIG therapy (Group 1) vs. 10/12 children treated with HLH-2004 protocol (Group 2), \( P=1.00 \). Good outcome was seen in 4/6 children treated with IVIG therapy followed by HLH-2004 protocol (Group 3). Serum ferritin levels of more than 3,000 mg/dl were present in 13 children. In this group, good outcome was seen in 7/8 patients treated with IVIG vs. 4/5 treated with the HLH-2004 protocol (\( P=1.00 \)).

Conclusions IVIG and HLH-2004 protocol may be equally effective in the management of HLH. IVIG may be a preferable initial regimen, to avoid the risk of secondary malignancy associated with etoposide.

Keywords Hemophagocytic lymphohistiocytosis · Intravenous immunoglobulin

Introduction
Hemophagocytic lymphohistiocytosis (HLH) is a rare, fatal disorder of childhood, affecting predominantly the mononuclear phagocytic system. Majority of patients with hematologic disorders such as leukemia, lymphoma, and aplastic anemia are treated on well-controlled protocols that allow one to evaluate the effectiveness and adverse effects of different therapeutic regimens [1–5]. A conspicuous exception can be found in virus-associated hemophagocytic syndrome or hemophagocytic lymphohistiocytosis (HLH) [6–11], for which treatment tends to, vary from patient to patient and among small cohorts. Although etoposide-containing immunotherapy has become the recommended treatment for HLH, immunomodulatory therapy with corticosteroids, IVIG, or a combination of these agents continues to be widely used. To better assess the role of IVIG therapy and HLH-2004 protocol in the management of HLH, retrospective comparative analysis of the patients with this clinical diagnosis was conducted.
The aim was to study the profile of children with HLH and to compare the outcome in children treated with immunomodulatory therapy (intravenous immunoglobulin and Dexamethasone) vs. those treated with HLH 2004 protocol (etoposide, cyclosporine, dexamethasone).

Material and Methods

This study was conducted at Kanchi Kamakoti Childs Trust hospital, a tertiary care hospital for children in Chennai, Tamil Nadu, India during the period June 2008 through June 2011. Case records of children who were admitted during the above period with the main diagnosis of HLH were reviewed and their clinical, demographic profile studied. Patients had to have at least one year of follow-up documented in medical records to be included in the study.

For the purpose of present study, a case of HLH was defined as per the criteria of the Histiocyte society [12]. A diagnosis of HLH required the presence of fever >38.5 °C persisting beyond seven days and splenomegaly with

1. Two of the following abnormalities: Anemia (<9 g/dl), Neutropenia (<1,000 cells/cu.mm), Thrombocytopenia (<100,000 cells/cu.mm).
2. One of the following abnormalities: Hypertriglyceridemia (fasting TGL >265 mg/dl), hypofibrinogenemia (<150 mg/dl), hyperferritinemia (>500 mcg/L).
3. Hemophagocytosis in Bone marrow.

Patients with suspected familial HLH were excluded. Familial HLH was identified using the following criteria-

1. Family history OR
2. Absence of history suggestive of preceding viral illness (such as Dengue or Epstein Barr virus) or febrile episode OR
3. Recurrence of HLH on follow-up examination

Where, on the basis of the above mentioned criteria, there was suspicion for familial HLH, assessment of the Perforin gene was performed. Routine gene assessment could not be performed because of cost of the test.

The variables like age, sex, symptoms, signs, laboratory findings were analyzed with group statistical and descriptive analysis along with independent sample test. Selection of IVIG vs. the HLH protocol for treatment was entirely at the discretion of the Consultant physician. Group 1 consisted of children treated with IVIG (Immunorel®) of 1 gm/kg/d for 2 d, Group 2 consisted of children treated with the HLH-2004 protocol (etoposide, cyclosporine and Dexamethasone) and Group 3 consisted of patients treated with IVIG followed by the HLH-2004 protocol.

IVIG was given at a dose of 1 g/kg/d for 2 d with Dexamethasone at 10 mg/m²/d for 7 d followed by 6 mg/m²/d till complete clinical and lab response. Supportive care was given in the form of packed red cells for severe anemia (less than 5 g/dl) and platelet concentrate if platelet counts was less than 20,000/cu.mm or in the presence of significant spontaneous bleeds.

All patients were seen in follow-up visits at least once a month. Primary outcome assessment for purposes of this study was at the end of one year follow up. Good outcome was defined by the disappearance of fever, improvement in the clinical condition and a normalization of cell counts with Event Free Survival (EFS) of 12 mo (absence of disease activity measured by ferritin, TGL and LDH). Poor outcome was defined as worsening of clinical condition and occurrence of death. The outcomes of patients within the groups were analyzed with Pearson Chi square and Fisher Exact tests as appropriate. ‘P’ value less than 0.05 was considered statistically significant.

Results

Forty children fulfilling the diagnostic criteria were studied. In three young infants where there was a suspicious family history; Perforin gene was assessed and was normal. The demographic profile of children in the study along with the duration of fever is shown in Table 1.

Gastrointestinal symptoms in the form of abdominal pain, vomiting and abdominal distension were found to be more common and were present in 18 children associated with liver derangement and coagulation abnormalities. Neurological symptoms in the form of seizures, irritability were found in seven cases. Lymphadenopathy was present in 27 % of children and rash was seen in 25 % of children. Splenomegaly was noted in 25 children at admission, but gradually evolved over the period in the rest. Figure 1 depicts the distribution of symptoms and laboratory findings in all patients. Figure 2 depicts the distribution of gastrointestinal, neurological and respiratory symptoms in patients in the three treatment groups. Six children had parental consanguinity but none of the patients had a family history of HLH.

Laboratory findings showed bicytopenia in all the children. The total neutrophil count ranged from 300 to 20,000 cells/cu.mm with a mean value of 3,400 cells/cu.mm. The hemoglobin levels ranged from 5.1 to 13.8 g/dl with mean of 8.75 g/dl. The platelet counts ranged from 10,000 to 95,000 cells/cu.mm with the mean being 84,000 cells/cu.mm. Fasting triglyceride levels ranged from 87 to 629 mg/dl with the mean of 358 mg/dl. Serum ferritin levels ranged from 104 to 107,791 mcg/L with a mean value of 8,139 mcg/dl. Out of 40 children with hyperferritinemia, 6 children had ferritin of more than 3,000 but less than 10,000 and 7 children had very
high levels of ferritin of more than 10,000. Serum fibrinogen levels were between 30 to 173 mg/dl with mean of 137 mg/dl. Bone marrow showed evidence of hemophagocytosis in all the children. Serological tests in search of diagnosis showed positive Dengue IgM ELISA in seven children, EBV VCA IgM ELISA in eight children and HSV 2 IgM in two children.

Then the baseline laboratory parameters prior to onset of treatment were compared between the three treatment groups. The findings are shown in Tables 2 and 3.

Subsequently, response to treatment and outcome was assessed in the three groups. An initial clinical response to treatment was defined as the disappearance of all the clinical signs and symptoms of the disease with normalization of laboratory findings. Worsening of previous clinical manifestations or laboratory profile was considered evidence of disease progression for which switching of therapy was considered. Special reasons for therapy switch were persisting fever and reemergence of high fever after transient response.

The group statistical analysis was done for the individual variables. The analysis showed that, all the clinical features and laboratory findings were distributed similarly across all the three groups. Of the total 22 cases treated with immunomodulation therapy (IVIG and Dexamethasone) 19 had a good outcome while ten of 12 children treated with HLH 2004 protocol (etoposide, cyclosporine and Dexamethasone) had a good outcome ($P=1.00$). In six children, where immunomodulation therapy was followed by initiation of HLH 2004 protocol, the outcome was good thereafter in four children and poor in two children. Serum ferritin levels of more than 3,000 mg/dl were present in 13 children. In this group, good outcome was seen in 7/8 patients treated with IVIG vs. 4/5 treated with the HLH-2004 protocol ($P=1.00$). There were no relapses seen in any of the groups- all patients either had a good outcome or died.

**Discussion**

Hemophagocytic lymphohistiocytosis is a disorder of the mononuclear phagocytic system where infiltrating lymphocytes and histiocytes destroy the CNS, bone marrow and other organs [1]. It is characterized by cytotoxic deficiency which leads to abnormal T-cell activation and inflammatory cytokine production, which drive disease development [2].

![Fig. 1 Distribution of symptoms and laboratory findings in all patients with HLH](image1)

![Fig. 2 Distribution of symptoms in the three treatment groups](image2)

<table>
<thead>
<tr>
<th>Table 1 Demographic profile and duration of fever of children included in the study</th>
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<tbody>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Fever duration</td>
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</table>
HLH being a disease of excessive immune activation, various forms of immunomodulatory therapy have been tried [13]. The treatment of HLH with intravenous immunoglobulin was once thought to be beneficial [14]. Yet definite evaluation of either IVIG or an IVIG plus corticosteroid combination in this disease is lacking. The exact mechanism by which IVIG exerts its immunomodulatory effects is uncertain [15] but may include alteration of the T cell activation and cytokine production [16].

The HLH study group began its first intervention treatment strategy using HLH-94 protocol. The present treatment protocol HLH 2004 has been designed for the primary, inherited disease FHL, as well as any severe form of HLH, in patients aged <18 y [18]. Steroids, cyclosporin and etoposide are the treatment agents used in the induction phase and the continuation phase for patients in this regimen [16]. Early introduction of etoposide based regimen is purported to be a critical factor in securing long-term survival in patients with HLH. However, there is increased risk of acute myeloid leukemia and myelodysplastic syndrome following the use of etoposide [18, 19]. Also the potential for neutropenia associated opportunistic infections [16] in patients treated with etoposide raises serious questions about intensive use of this agent.

The overriding question which led to this study was, whether or not etoposide is essential for successful management of hemophagocytic disease. The clinical characteristics, laboratory data and treatment variables for both the groups are reported in the Tables 2 and 3. Patients in the present study met most of the HLH diagnostic criteria proposed by Henter et al. [20] and lacked a family history of HLH. The present study also revealed that all the features were equally distributed across the three groups. It also reveals that the use of treatment regimens with immunoglobulin and etoposide have been equally effective and have a similar outcome. Though this result is in contrast to the previous studies, considering the benefits of IVIG therapy in the form of short treatment, less adverse effects and less chance of opportunistic infections [21, 22] along with response that is equal to etoposide containing regimens, intravenous immunoglobulin may be tried as the first line of management in all cases of HLH. It may be possible to begin treatment with IVIG, switching to an HLH-2004 like protocol at the first sign of failure. Support for this strategy comes again from the observation that overall survival rates in the groups were not significantly different. Although this therapy with intravenous immunoglobulin may produce positive effects against HLH, its role in first line regimen is unclear and will likely require additional data on the efficacy of IVIG with careful evaluation in future prospective trials for any firm conclusions.

Serum ferritin levels of more than 3,000 mg/dl were present in 13 children of whom 8 were treated with IVIG and 5 with HLH 2004 protocol. The outcome was good in 7 and bad in 1 in IVIG group. Likewise, good outcome was seen in 4 and bad outcome in 1 in the HLH 2004 protocol group. There was no statistical difference.

Drawbacks in the index study are, it was a retrospective study; genetic analysis was not done in all cases (though done in three cases, for which the results came as negative); NK cell activity and soluble CD25 receptor assay could not be done in any child.

### Table 2 Hematological profile (neutrophil count, hemoglobin and platelet count) of the three treatment groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Immunomodulatory group (IVIG + Steroid)</th>
<th>HLH protocol group</th>
<th>Those who received both</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>700–28,100</td>
<td>8,104.5</td>
<td>90–9,500</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>5.1–13.8</td>
<td>8.8</td>
<td>5.8–10</td>
</tr>
<tr>
<td>Platelets</td>
<td>15,000–95,000</td>
<td>67,000</td>
<td>15,000–90,000</td>
</tr>
</tbody>
</table>

### Table 3 Laboratory profile (triglycerides, ferritin and fibrinogen levels) of the three treatment groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Immunomodulatory group (IVIG + Steroid)</th>
<th>HLH protocol group</th>
<th>Those who received both</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>91–529</td>
<td>286</td>
<td>87–639</td>
</tr>
<tr>
<td>Ferritin</td>
<td>181–53,744</td>
<td>6,921</td>
<td>205–107,791</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>30–594</td>
<td>140</td>
<td>23–260</td>
</tr>
</tbody>
</table>
Conclusions

Thus, both IVIG therapy and HLH protocol 2004 were found to be equally efficient in the management of hemophagocytic lymphohistiocytosis. The use of IVIG therapy may be considered in all patients as the initial regimen as the outcome is equally good compared to HLH 2004 protocol. HLH 2004 protocol may be considered in cases with poor clinical response or when there is evidence of disease progression. This may avoid etoposide induced toxicity.

Contributions SR: Conceived the study, study design, data collection and analysis, helped writing, editing the manuscript and will act as guarantor for this paper; IA: Assisted with data collection, analysis and editing the manuscript; NM and RJ: Assisted with data collection and editing the manuscript; KG and ES: Helped conceive of the study, data collection, analysis and editing the manuscript.

Conflict of Interest None.

Source of Funding None.

References