ORIGINAL ARTICLE

Infections and HLH - Experience from a Tertiary Care Centre

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Abstract

Introduction: HLH is a rare but potentially fatal condition that may be triggered by connective tissue disorders, malignancies and in a significant number of cases by infections. HLH is often difficult to diagnose because of its similarity with a host of other infective and inflammatory conditions. Prompt identification of the underlying cause is important as it guides treatment decisions. Early initiation of appropriate therapy reduces morbidity and mortality.

Aims: To study patients with infection associated secondary HLH in a tertiary care facility.

Settings and Design: We conducted a prospective study to identify patients with secondary HLH triggered by infections (IA HLH), the type of infections triggering HLH and the course and outcome of the patients. Patients' data were collected from September 2015 to October 2016.

Methods and Material: Between September 2015 and October 2016, consecutive patients meeting the diagnostic criteria for IA-HLH, based on the HLH2004 protocol of the Histiocyte Society, were included in the study.

Results: In the course of over a year we diagnosed 20 patients with infection associated secondary HLH. Twelve cases were secondary to dengue, four were triggered by typhoid fever and two cases each were precipitated by tuberculosis and Epstein Barr virus infection. Of the 20 patients, three patients with dengue induced HLH died of hemorrhagic complications. Rest recovered without any sequel.

Introduction

Haemophagocytic lymphohistiocytosis (HLH) is characterized by dysregulated activation of CD 8+ T lymphocytes and macrophages that engulf erythrocytes, leucocytes, platelets and their precursor cells. HLH is a distinct clinical entity characterized by fever, pancytopenia, splenomegaly, and haemophagocytosis in bone marrow, liver or lymph nodes and is associated with considerable mortality and morbidity. Hemophagocytic lymphohistiocytosis (HLH) is a devastating and rare disorder with an estimated incidence of around 1.2 cases per million patients per year and a high mortality rate of around 47%.[1] HLH may be familial or secondary to a variety of infections, collagen vascular diseases or malignancies. Infections, particularly infections with intracellular microbes constitute about 40% of all secondary or reactive HLH. Unlike other forms of HLH, infection associated HLH carries a relatively better prognosis provided the inciting infection is diagnosed early and treated promptly.

Subjects and Methods

From September 2015 to October 2016 patients diagnosed with infection associated HLH (IA-HLH) were included in the study.

The diagnosis of HLH was based upon the criteria, which were used in the HLH-2004 trial.[2]

Five of the 8 criteria are required to be fulfilled for diagnosis of HLH to be made.

The last two tests soluble CD25 level and NK cell activity are not done in our hospital and patients who satisfied at least 5 out of the remaining six criteria were included in the study.

H score of the patients were also calculated. The "H score" is a scoring system that has been developed to generate a diagnostic score that estimates the probability of HLH; this combines scores for immunosuppression; fever; organomegaly; levels of triglycerides, ferritin, alanine aminotransferase, and fibrinogen; degree of cytopenias; and presence of hemophagocytosis in the bone marrow aspirate.[3] An Hscore of ≥250 confers a 99 percent probability of HLH, whereas a score of ≤90 confers a <1 percent probability of HLH.

Results

Between September 2015 and October 2016, 20 patients with infection associated secondary HLH were diagnosed. The patients fulfilled at least five criteria required to establish HLH as per the HLH diagnostic criteria, 2004. The mean age at diagnosis was 28.4 years {median 23 (range 4-67)}. All patients had fever at presentation and at least a bi or trilineage cytopenia, elevated liver enzymes and hyperferritinemia. The mean ferritin level was 30893.545 ng/ml (Reference range: paediatric: 7-140 ng/ml; adult: 10-250 ng/ml). The average platelet count was 54000 mm^{-3}; (reference value [RV]: 4000 - 10000 mm^{-3}). Twelve cases were secondary to dengue virus infection, four were triggered by typhoid fever and two cases each were precipitated by tuberculosis and Epstein Barr virus infection. Out of the 12 patients in whom bone marrow study was undertaken, nine of them had evidence of haemophagocytosis in bone marrow biopsy (Figure 1). The H scores of all the patients were calculated. Three patients had H score less than 190. In these patients the probability of HLH varied from 8.8% to 70.9%. The remaining patients had H score over

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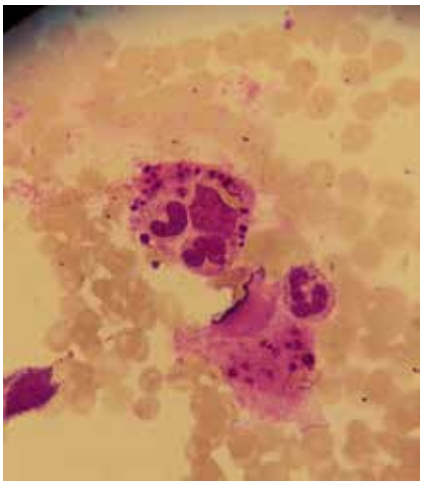

Fig. 1: Bone marrow aspirate showing macrophage with marked hemophagocytic activity

190 which is associated with more than 85% probability of developing HLH (Table 1).

Of the 12 cases of dengue associated IA-HLH, six were primary dengue cases and five cases were those of secondary dengue infection.

The patients of tuberculosis initially presented as pyrexia of unknown origin (PUO). Extensive investigations revealed disseminated tuberculosis. There were no other underlying disorders. In both patients, laboratory investigations revealed deranged LFT, moderate anaemia and decreased blood platelet counts besides high serum ferritin and triglyceride levels. The bone marrow studies showed presence of caseating epithelioid granulomas suggestive of tuberculosis along with evidence of haemophagocytosis. Radiological investigations were corroborative of disseminated tuberculosis. Imaging studies showed bilateral pulmonary miliary nodules in both patients. The patients also had hepatosplenomegaly with minimal ascites and in addition one of the patients had periportal, peripancreatic and retroperitoneal lymphadenopathy. Following establishment of diagnosis, the patients were treated with anti-tubercular drugs and dexamethasone.

The patients diagnosed as typhoid induced HLH initially presented with high fever. The diagnosis of enteric fever was established following isolation of *Salmonella typhi* from blood cultures. Progressive bicytopenia despite appropriate antibiotics prompted investigations for HLH. All four patients were treated with intravenous ceftriaxone to which the strains were susceptible. Of the four patients one required dexamethasone as per HLH 2004 protocol for resolution of HLH.

Table 1: Depicting the clinical and laboratory features of the 20 patients with IA-HLH Y: Yes ; N: No; TG: Triglyceride; ND : Not done

Sl.	Age	Sex	Infection	TG	Fibrinogen	Ferritin	Bone marrow	Treatment for HLH	Outcome	H-score
1	56	M	Dengue	219	240.9	609.5	Present	Steroids	Discharge	199
2	12	M	Dengue	293	ND	39400	Present	Steroids	Discharge	244
3	12	F	Dengue	201	201	35863	Present	N	Discharge	227
4	4	M	EBV	193	409	13269	ND	N	Discharge	224
5	24	F	Typhoid	ND	103	15720	Present	N	Discharge	245
6	15	M	Typhoid	284	ND	7285	Absent	Steroids	Discharge	224
7	14	M	Typhoid	283	ND	1385	ND	N	Discharge	130
8	67	M	Dengue	ND	ND	64337	Present	IVIG	Discharge	196
9	26	M	Dengue	275	ND	42468	Absent	N	Discharge	224
10	16	F	Tuberculo	414	297	3268.4	Present	N	Discharge	226
11	30	F	Dengue	155	99.8	93273	ND	N	Death	216
12	24	F	Typhoid	215	100	12000	ND	N	Discharge	264
13	21	F	Dengue	196	117.9	31479	ND	N	Death	216
14	5	F	Dengue	271	170.4	69340	ND	Steroids	Death	172
15	46	M	Tuberculo	586	<70	33908	Present	Steroids	Discharge	309
16	32	M	EBV	396	250.4	26902	Present	Etoposide IVIg	Discharge	302
17	50	M	Dengue	190	91.9	11611	Absent	IVIG	Discharge	215
18	38	M	Dengue	111	185	16830	Present	N	Discharge	217
19	46	M	Dengue	357	344	63972	ND	N	Discharge	224
20	31	F	Dengue	550	148.2	34951	ND	N	Discharge	182

All patient had fever & Pan/Bicytopenia. Except Patient No.3 all had Splenomegaly

Two patients with reactive HLH had evidence of Epstein Barr virus infection. Serology for viral capsid antibody IgM was reactive in one patient and the second patient had significant number of EBV viral copies (10^4EBVviral copies/ml) in the blood. The second patient was subsequently diagnosed with pre B cell ALL.

Discussion

HLH is a potentially fatal hyperinflammatory condition that can be secondary to autoimmune diseases, infections or malignancies. Infections are major triggers of HLH and around 50% of secondary HLH are triggered by infections, the majority being viral infections (29%).[4]

Despite advances in the diagnostic work up of febrile illness, the IAHLH remains undiagnosed due to lack of awareness amongst clinicians about this entity. Various tropical infections such as dengue, typhoid, kala azar, leishmania, tuberculosis, leptospirosis, malaria are important triggers of HLH. IAHLH has been frequently associated with intracellular pathogens that stimulate the TH 1 response.

Dengue associated HLH

Out of the 20 cases of IAHLH diagnosed over a period of one year, 12 cases were triggered by dengue. In 2016, there was a major outbreak of dengue and consequently the increased number of dengue related HLH cases.

A mild disease in majority of cases, less than 2% of dengue patients present with severe manifestations, dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS), both conditions resulting in considerable mortality and morbidity. A total of 74 dengue-associated pediatric and adult HLH cases have been described in the published literature since 1966, with a cumulative case-fatality rate (CFR) of 9.5%.[5] It is possible that the diagnosis of dengue induced HLH is missed as clinicians tend to focus on the underlying infection rather than the overwhelming cytokine production. HLH is a potentially life threatening disorder and several cases reported in the literature show dengue induced HLH can be fatal.[6-8] The pathophysiology of severe dengue (DHF, DSS) is not always well understood and it is now being increasingly believed that macrophage activation may play a role in some cases of severe dengue. It is fairly well established that cross protection among the four dengue serotypes is limited and secondary infection by a different serotype may predispose to DHF and DSS. Many studies found that primary dengue infections could be associated with severe DHF and dengue-associated hemophagocytic

syndrome.[9-11] The increasing reports of haemophagocytosis associated with dengue infection in both endemic and non- endemic areas have raised concern of the potential threat of this emerging syndrome in causing severe and fatal complications. So it is important to look for associated HLH in cases of severe dengue. Amongst our patients, three patients with dengue induced HLH succumbed to hemorrhagic complications. Two of them had H score of 216 and one patient had score of 172.The patient with the H score 172 had the stormier onset and succumbed a day after admission in our hospital. The fibrinogen and triglyceride levels were normal in the patient probably as a result of therapeutic interventions in an outside facility and hence the low H score.

TB associated HLH

TB-HLH was first published in the 1980s[12] and there has been increasing number of reports of TB HLH in the recent years. Padhi *et al* , reviewed a total of 55 articles describing nearly 70 cases of TB-HLH published in the world literature till March 2014.[13] Since then around 16 more cases have been reported in the literature including a series of 8 cases by Zhang Yun *et al*.[14-22] One aspect that is almost certain from these case reports and reviews that that HLH associated with TB carries a high mortality (of approximately 50%) for patients not receiving appropriate treatment or due to a delay in commencing treatment.[22] Treatment with anti-TB drgus and chemo-immunotherapy improves outcome.

Both the patients of disseminated tuberculosis improved after institution of anti- tubercular therapy and dexamethasone. TB HLH patients generally present with PUO and establishing the etiological diagnosis without much delay can be challenging. In our patients, radiological findings along with histopathological evidence in the bone marrow helped in the diagnosis of tuberculosis. The bone marrow study proved to be particularly helpful as it provided evidence of tuberculosis infection and suggestion of haemophagocytosis.

Typhoid induced HLH

Enteric fever, transmitted via the faeco-oral route, is endemic in the developing countries of Asia, Africa, Latin America, the Carribean, and Ocenia with an estimated 13.5 million episodes in 2010.[23] There are few cases of enteric fever induced HLH reported in the literature and it is possible that secondary HLH due to typhoid fever go unrecognized possibly due to lack of awareness about this entity.[24-25] As early as the mid-1800s, haemophagocytic macrophages, which are macrophages that have consumed red and white blood cells, were observed in the tissues and blood of recently deceased typhoid fever patients.[26]

Leucopenia and splenomegaly, two of the diagnostic criteria of HLH are also pathognomonic features of typhoid fever and as a result secondary HLH triggered by enteric fever may be difficult to identify. In enteric fever patients with progressive bicytopenia despite appropriate antibiotics, the diagnosis of HLH should be taken into consideration and investigated accordingly.

EBV induced HLH

EBV-HLH is a major subtype of secondary HLH that is induced by a primary EBV infection. Epstein–Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH) is the most frequent subtype of secondary HLH triggered by infections. EBV-HLH is a clinicopathological syndrome comprising of a dysregulated immune response and cytokine storm, manifested clinically by fever, splenomegaly, and cytopenia. Without early and effective therapy, EBV-HLH has a high mortality rate, frequently due to multiorgan failure. Recently established diagnostic and therapeutic guidelines, particularly the introduction of the chemotherapeutic drug etoposide have contributed to improvements in survival rates. Etoposide appears to interfere with EBV – induced lymphocyte transformation and suppresses formation of EBV nuclear antigen.[4] The incidence of EBV-HLH is relatively high in Asian countries, indicating the underlying genetic background in the pathogenesis of EBV-HLH. In Japan EBV associated HLH is seen in around 40% of all secondary HLH patients.[27]

In children and adolescents EBV HLH has been observed in primary EBV infections whereas in adults it may be possible that acquired immune dysfunction is required to trigger the onset of EBV-HLH in EBV-immune adults.[27,28] Among our cases, patient 1 (4 years old male boy) had no history to suggest immune dysfunction and the second patient (31 years adult male) subsequently received a diagnosis of pre B cell ALL. The first patient recovered spontaneously but in the second case IVIg followed by etoposide was administered for resolution of the HLH.

Diagnostic challenges

The main challenge in recognizing IA-HLH is due to its wide spectrum of manifestations but lack of specificity in the clinical findings. Persistent cytopenias, rising liver enzymes, unresponsiveness to appropriate antibiotics in the setting of an infectious disease are some of the diagnostic pointers for this disease. In case of infections, a simple blood investigation that shows elevated levels of serum ferritin should raise the suspicion of a coexisting HLH. A single value of ferritin more than 10,000 ng/ml in the absence of iron overload conditions like hemochromatosis and thalassemia syndromes can act as a surrogate marker for HLH with a sensitivity of 90% and specificity of 96%.[29] Fever, hepatosplenomegaly, cytopenias, hepatitis, hypofibrinogenemia, hyperferritinemia, hypertriglyceridemia, hyponatremia with evidence of haemophagocytosis in the bone marrow are amongst the clinical features that support the diagnosis of HLH. All our patients except three had ferritin levels over 10000ng/ml. In our study the mean ferritin level was found to be as high as 30893.545ng/ml. Although haemophagocytosis is the hallmark of the disease, it is seldom found at presentation in case of secondary HLH and may not be evident until late in the course of disease progression. Bone marrow biopsies performed in the initial stage of disease may be normal or demonstrate very non-specific changes.[4]

Tests for molecular markers such as the soluble CD 25, natural killer cell activity, are not regularly performed in most hospitals and if available the reports are obtained after some time and therefore may not be a timely preference for diagnosing HLH. Consequently, IA-HLH may be under diagnosed with the HLH 2004 or proposed HLH 2009 Diagnostic Criteria as these protocols include molecular biomarkers. So a modification of the diagnostic criteria has been proposed .In this approach, diagnosis requires three of four clinical findings (fever,

splenomegaly, cytopenias, hepatitis) plus one of four immune markers (hemophagocytosis, increased ferritin, hypofibrinogenemia, absent or very decreased NK cell function).[3] Along with these criteria the H Score may be a useful tool which can complement these diagnostic criteria and help in establishing IA-HLH.

The H Score was recently designed and validated to assess individual risks of HLH. Fardet *et al* in their study reported that a cut off H score of 169 ensured rather high degrees of sensitivity, specificity, and classification accuracy.[3] The H Score is now preferred by many to the previous HLH-2004 criteria because the latter were suffering from substantial limitations. First, these criteria were established in a pediatric population to diagnose the hereditary primary form of HLH and suffer from substantial limitations in diagnosing the adult or the reactive form of the disease. Second, the weight of each criterion was unknown and the cut-off values were merely empirical. Third, some of the investigations (eg, NK cell activity, soluble interleukin-2 receptor level) are high end tests performed by reference laboratories and are not available in many of the centers.

This present study also corroborated the usefulness of the H score for establishing the diagnosis of HLH. All our patients expect one had H Score more than 169. In fact 17 out of the 20 patients included in the study had H score over 200 with a probability of more than 90% of developing HLH

The majority of the laboratory work-up necessary to establish the diagnosis of HLH include tests done routinely with a fast turn-around time (e.g. CBC, triglycerides, fibrinogen, and ferritin). These findings, combined with a good clinical examination should be enough to raise suspicion of HLH in an appropriate clinical setting, prompting exhaustive investigations to establish HLH and detect the underlying pathology that triggered the cytokine storm.

Treatment

The mortality of HLH is very high without HLH directed therapy. Early recognition and initiation of therapy is therefore of utmost importance Regarding EBV-associated HLH, early immunochemotherapy with etoposide results in high cure rates. For infection associated HLH, the treatment of the underlying infection and supportive care is sufficient in 60-70% of cases. Patients with reactive HLH associated with an infectious organism except leishmaniasis, may need specific HLH therapy since pathogen specific therapy cannot stabilize the disease activity by itself in around 30-40 % of cases. In this study, patients diagnosed with tuberculosis and typhoid fever received specific antibiotics for the underlying infections and the rest were managed symptomatically. Nine of the patients were administered HLH specific therapy in the form of dexamethasone, IVIg or etoposide. Of the three patients who died, two of them received HLH specific therapy.

Conclusion

Hemophagocytic lymphohistiocytosis (HLH), a life-threatening clinicopathological condition caused by excessive immune activation is being increasingly recognized in clinical practice. The high mortality rate associated with HLH is due at least in part to delays in diagnosis that result from the similarity between the initial clinical features and a wide range of infective and inflammatory conditions. Acquired HLH is often caused by infections, malignancies or collagen vascular diseases. Infections are responsible for precipitating HLH in around 40% of cases. Infection associated HLH carry a slightly better prognosis if diagnosed and treated sufficiently early. Dengue induced HLH seems to be the cause of hemorrhagic complications in certain patients. The recently developed H Score is a useful tool and can be used to estimate an individual's risk of having reactive hemophagocytic syndrome.

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