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Secondary hemophagocytic lymphohistiocytosis – a common ramification of different diseases

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ABSTRACT

Hemophagocytic lymphohistiocytosis is a rare and potentially fatal disorder caused by immune dysregulation. It can occur as a primary genetic disease or secondarily due to various causes including infections, malignancies or autoimmune diseases. In this case report, we present two cases of Hemophagocytic lymphohistiocytosis which were secondary to typhoid and dengue fever. While primary disease occurs predominantly in infants, secondary hemophagocytic lymphohistiocytosis can occur in any age group. Both primary and secondary hemophagocytic lymphohistiocytosis are characterised by fever, hepatosplenomegaly, pancytopenia and multiorgan dysfunction. But unusual persistence of fever and other organ involvement should need further workup for hemophagocytic lymphohistiocytosis. Secondary hemophagocytic lymphohistiocytosis may resolve on treating the underlying disorder. But severe cases need treatment with immunosuppressive/immunomodulation therapy to

prevent morbidity. Early clinical suspicion, prompt diagnosis and treatment of hemophagocytic lymphohistiocytosis are essential to prevent deleterious effects to health.

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INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH), also identified as hemophagocytic syndrome, is an aggressive, life-threatening hematological syndrome characterised by unregulated immune activation resulting in malignant inflammation and multi-organ failure (1). The reported annual incidence of HLH is approximately 1.2 per million individuals. The mortality rate is very high, 95% if left untreated. Based on the underlying etiology, HLH is classified into primary (genetic) and secondary (acquired), both of which clinically manifest as acute or subacute febrile illness with hepatosplenomegaly, bi- or trilineage cytopenia, hypertriglyceridemia and hypofibrinogenemia. Inherited genetic mutations play a major role in primary HLH, whereas secondary HLH is commonly associated with infections, autoimmune disorders or malignancies (2). We report here the cases of two children who presented with acquired HLH secondary to enteric fever and dengue.

CLINICAL CASE DESCRIPTION

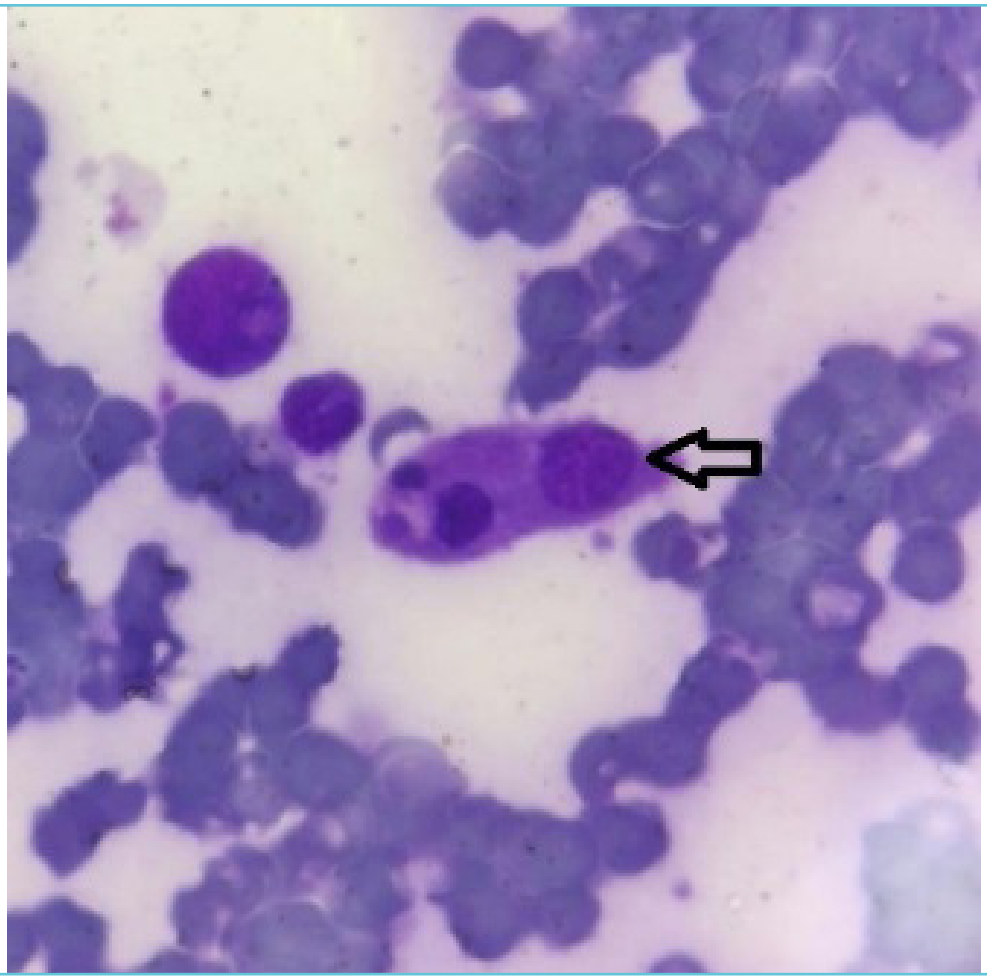
Case 1

A 5-year-old girl presented with high grade fever, abdominal pain and loose stools for 5 days, associated with reduced activity and poor oral intake for 2 days. There was history of fast breathing and reduced frequency of urine for a day. No history of vomiting, rash, joint pain, jaundice or edema. On examination, the child had tachypnea (respiratory rate - 87/min), tachycardia (heart rate - 160/min), cool peripheries, peripheral pulses were feeble and blood pressure was 100/70 mmHg. Her liver was enlarged to 4 cm below right costal margin and spleen was 3 cm below left costal margin. Both were soft in consistency and non-tender. There were no added sounds on auscultation. Provisional diagnosis of sepsis with compensated shock was made. Differential diagnosis was pneumonia, typhoid fever, scrub typhus and dengue fever.

Management and outcome

Baseline investigations revealed pancytopenia (total WBC count-3820 cells/mm^3, hemoglobin - 8.4 g/dl, platelets – 39×10^9/L), elevated liver enzymes (SGOT - 368 U/L, SGPT - 167 U/L), C reactive protein 86 mg/dL and normal renal parameters. Thorax ultrasound showed right-sided pleural effusion with bilateral B lines. Treatment was commenced with high flow oxygen by nasal cannula with FiO_2 of 30% and flow of 24L/min and was resuscitated with fluid bolus 20 ml/kg, normal saline. Intravenous ceftriaxone was started after drawing blood for culture. In view of high-grade fever which did not touch the baseline, increasing hepatosplenomegaly and worsening pancytopenia on day 8 of illness, HLH work-up was initiated. Serum ferritin was elevated (11167 ng/mL) with hypofibrinogenemia (152.3 mg/dL) and hypertriglyceridemia (279mg/dL). Brain natriuretic peptide was very high (6622 pg/mL) and echocardiogram (ECHO) revealed left ventricular dysfunction (ejection fraction-45%, normal being >65%) suggesting myocarditis. Hence, she was started on dobutamine and milrinone infusion. Bone marrow aspiration showed hemophagocytes (Figure.1). Blood culture grew Salmonella typhi, which was sensitive to ceftriaxone. In view of worsening clinical condition and elevated inflammatory markers, child was given intravenous immunoglobulin 2 g/kg over 48 hrs. Child gradually improved, fever spikes started decreasing, tachypnea settled and she was weaned off from HFNC and inotropic support. She was continued on

Figure 1 Leishman Stain 100X magnification. Arrow pointing to a hemophagocyte



enalapril, aspirin, furosemide and spironolactone. On follow up at 4 weeks, ECHO was normal and anti-failure measures were stopped.

Case 2

A 4-year-old girl presented with fever and skin rash over the lower limbs that commenced 5 and 3 days ago, respectively. She also had vomiting and reduced urine output for a day. On examination, she was conscious and oriented. Her heart rate was 170/min, respiratory rate was 40/min, blood pressure was 70/50 mmHg with cool peripheries, feeble peripheral pulses and capillary refill time > 3 seconds. She had macular erythematous blanching rash over both lower limbs and forearms. Her liver was enlarged to 4 cm, soft in consistency, with mild tenderness. Other examinations were normal.

Management and outcome

She was provisionally diagnosed to have dengue fever with hypotensive shock and resuscitated with 30 ml/kg normal saline fluid bolus. She had severe thrombocytopenia (30 $\times 10^9$/L) with hemoconcentration (Hb- 14.5 g/dL; PCV 48). Her liver enzymes were elevated (SGOT: 524 U/L and SGPT: 124 U/L). Dengue IgM was positive. On day 3 of admission, she had persistent fever and developed pleural effusion, requiring oxygen support. In view of persistent fever for 8 days, HLH workup was done. Child had hyperferritinemia (7500 ng/mL), hypofibrinogenemia

(132.7 mg/dL) and hypertriglyceridemia (251 mg/dL). ECHO was normal. Intravenous immunoglobulin was given at 2 g/kg over 48 hours. During the next 48 hours, her fever spikes settled and platelet count started increasing and she was discharged.

Laboratory parameters of both cases have been listed in Table 1.

DISCUSSION

Hemophagocytic lymphohistiocytosis is an immune-mediated life-threatening hyperinflammatory syndrome, which was initially described by pediatricians Robb-Smith and Scott in 1939. It is characterized by uninterrupted hyperinflammatory response associated with abnormal activation of macrophages and lymphocytes, resulting in hypercytokinemia (3)(4). Primary HLH occurs due to a variety of genetic abnormalities and frequently presents during infancy and early childhood, while secondary HLH is less age-restricted and it is more common in older children and adults. HLH can be triggered by a variety of events that disrupt immune homeostasis, with infection being a common trigger in both genetic and sporadic cases. Viruses are most commonly associated with secondary HLH, particularly Epstein-Barr virus, but tuberculosis, malaria, dengue, leishmaniasis and typhoid are important infections that trigger HLH, especially in tropical countries (5). In the present report, the triggers implicated were dengue and typhoid fever.

The pathogenetic mechanisms of HLH is impaired activation of innate immune system, precisely natural killer (NK) cells and CD 8+ cytotoxic T-lymphocytes (CTL), resulting in release of abundant inflammatory cytokines that promote cytokine network formation and macrophage infiltration. The liver, spleen and lungs are the commonly affected organs, but HLH can affect all organs of the body. In a healthy immune system, CD 8+ cytotoxic T-lymphocytes and NK cells secrete two cytolytic enzymes: granzyme and

Table 1 Laboratory values of the cases

Parameter	Case 1	Case 2	Normal values
Total Count (cells/mm^3)	3820	5700	5000 - 17000
Hb (g/dL)	8.4	10.8	11-14
Platelets (×10^9/L)	36	30	150-400
SGOT (U/L)	368	524	<35
SGPT (U/L)	167	124	13-45
Ferritin (ng/mL)	11167	7500	13 - 150
Triglyceride (mg/dL)	279	251	<150
Fibrinogen (mg/dL)	152.3	132.7	170 - 405

perforin. Perforin creates destabilizing pores in the membrane of the target cell which results in target cell destruction.

In patients with HLH, dysfunction of these cytosolic proteins causes reduced function of CD 8+ cytotoxic T-lymphocytes and NK-cell. The impaired cells loose its ability to eliminate virus-infected cells and instead uninterruptedly secrete inflammatory cytokines. In addition, these cytokines activate antigen-presenting cells (histiocytes and macrophages) to produce more cytokines. This results in a vicious cycle that amplifies the cytokine secretion of CD 8+ T-lymphocytes, NK cells, and macrophages, thus generating a cytokine storm. Hyperproduction of cytokines, including tumour necrosis factor α, interleukin 6 and interferon γ by virus-infected T-lymphocytes may play a role in the pathogenesis of dengue-associated HLH. Recently, enhanced antigen presentation and repetitive interferon γ-dependent stimulation of Toll-like receptors (TLRs) have also been postulated as causal mechanisms of reactive HLH. Direct activation of TLRs by intracellular pathogens that persist in histiocytes may explain the pathogenesis of typhoid-associated HLH.

The clinical features of HLH can be explained by 3 cellular pathways: 1) uncontrolled activation of macrophages and CD8+ T-lymphocytes; 2) hyperproliferation and infiltration of these cells into various organs; and 3) hypercytokinemia, resulting in progressive multi-organ dysfunction. Early symptoms of HLH are non-specific and can mimic common infections. The cardinal symptoms of the disease include continuous fever (>38·5°C), enlarged lymphohemopoietic organs and bi- or tri-lineage cytopenia. Neurological abnormalities are observed in nearly one-third of patients with HLH. The disease can also affect other vital organ systems, including the respiratory system, heart, kidneys and skin. Both the children presented here had lung involvement and one child had myocarditis in addition to respiratory distress.

Diagnosis of secondary HLH is challenging because of nonspecific clinical features. Hemophagocytic lymphohistiocytosis should be clinically suspected in all patients presenting with undiagnosed, continuous high-grade fever and evidence of multi-organ involvement. The diagnostic criteria proposed by the HLH-2004 study, requires 5 out of 8 parameters for the diagnosis of HLH (Table 2)(6). An elevated serum ferritin level is very common in children with HLH and has high sensitivity and specificity. Most patients with HLH will have hepatitis, characterised by elevated liver enzymes, lactate dehydrogenase and bilirubin. Hypertriglyceridemia and abnormal coagulation parameters are also frequently seen. The detection of hemophagocytosis, a hallmark of activated macrophages in the bone marrow, is supportive for diagnosis, however bone marrow biopsies are neither specific nor sensitive for conclusive diagnosis. Recently, flow cytometry has been implicated as a screening tool for identifying patients with genetic predisposition to HLH. Transient defect in NK cells and CTL cells function may be noted in secondary HLH while the changes will be persistent in primary HLH (7). Similar manifestation of continuous fever with multi-organ involvement can happen in multisystem inflammatory syndrome in children (MIS-C) following COVID-19 infection. But both the patients were negative for COVID-19 serology.

The initial goal of therapy in patients with HLH is to suppress the unregulated severe hyperinflammation, since both genetic and acquired forms of HLH can initially be managed by the same treatment protocol. Following this, the goal of therapy shifts towards identification of underlying triggering agents, as patients with primary HLH may require hematopoietic stem cell transplantation. Most cases of secondary

HLH resolve once the underlying disease is treated like in our children.

Regarding treatment of dengue-associated HLH, other similar cases reported in the literature showed that few cases have recovered with supportive therapy only. Intravenous immunoglobulin G has been used in few cases either alone or with steroids (8)(9). The treatment of dengue-associated HLH using intravenous immunoglobulin G seems to be associated with favourable outcome, as in this case report. On the other hand, typhoid is one of the bacterial infections, that can be a potential trigger for HLH. Treatment of the inciting bacterial pathogen is often inadequate and in most of the cases it is essential to start lifesaving therapy with immunosuppressants and immunomodulators as early as possible to prevent mortality(10).

Given the poor outcomes of HLH and the rapidity with which the disease progresses to cause fatal multi-organ failure, it is essential that signs and symptoms of hypercytokinemia are not overlooked and that the hyperinflammatory state is suppressed as soon as possible. In addition, it is imperative to detect the triggering event in secondary HLH and initiate prompt treatment to achieve successful outcome.

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Compliance with ethical standards

- **Conflict of interests**: The authors have declared that no Conflict of interest exists.
- **Ethical approval**: It was not obtained as this is a case report.
- **Informed consent**: Informed consent was obtained from the participants parents.
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Table 2 Diagnostic criteria for hemophagocytic lymphohistiocytosis based on HLH-2004

Diagnostic criteria for hemophagocytic lymphohistiocytosis (HLH)
1. Persistent fever - >/=38.5°C
2. Splenomegaly
3. Bicytopenia or pancytopenia (Hb<9 g%, Platelets<1 lakh/µL, Neutophils<1000/µL)
4. Hypertriglyceridemia and/or hypofibrinogenemia
5. Low or absent NK cell activity
6. Hyperferritenemia (>500 ng/mL)
7. High sCD25 (sIL-2R>2,400 U/ml)
8. Hemophagocytosis in bone marrow, spleen, lymph nodes or liver

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LEARNING POINTS

- Secondary hemophagocytic lymphohistiocytosis should be suspected in all children presenting with continuous high-grade fever beyond the natural course of illness and evidence of multi-organ involvement.
- Prompt treatment of the underlying triggering event of secondary HLH is essential
- It is prudent to start lifesaving therapy with immunosuppressants and immunomodulators in case of hypercytokinemia leading to multi organ involvement.

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