Liver biopsy to the aid in diagnosis of pediatric hemophagocytic lymphohistiocytosis: A case report

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Keywords: Hemophagocytic lymphohistiocytosis, liver biopsy, pancytopenia

Abstract

Hemophagocytic lymphohistiocytosis is a life-threatening, macrophage-related hyperinflammatory disorder that presents with sepsis like syndrome. The clinical presentation, laboratory findings, and histopathological features can be variable and nonspecific, and considerable overlap with other disorders is observed. Since hepatomegaly and hepatic dysfunction occur early in the disease, demonstration of hemophagocytosis in liver biopsy facilitated the diagnosis in our case, which was missed earlier on bone marrow aspiration study for pancytopenia.

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a result of uncontrolled macrophage activation with prominent phagocytosis of platelets, erythrocytes, and lymphocytes and hematopoietic precursors. Attempt should be made to establish or rule out HLH in patients presenting with fever, multisystem inflammation, and varying degrees of unexplained pancytopenia. Owing to the guarded prognosis associated with HLH, differentiation from other causes of pediatric liver disease is critical and is a diagnostic challenge.

Case Report

A 6-year-old male child born out of a consanguinous marriage presented with prolonged fever, bleeding gums, epistaxis, generalized lymphadenopathy, and hepatosplenomegaly of 1 month duration. Child had been irritable since 1 week. Melena was noticed. On examination, child had hepatosplenomegaly, mild generalized lymphadenopathy, and ecchymotic patches. Ultrasonography showed liver enlargement of 16.6 cm and spleen enlargement of 14.4 cm with few enlarged coeliac group of lymph nodes [Figure 1]. A clinical diagnosis of leukemia was followed by routine investigations that revealed anemia (hemoglobin 7.5 g/dl), pancytopenia (total leukocyte count of 3300/cm² and platelet count of 94,000/cm²) but bone marrow (BM) aspiration excluded leukemia. Megaloblastic erythropoiesis with megakaryocytic hyperplasia was seen. Coagulation profile showed prolonged bleeding time 8’30” (Duke’s method). Urine examination detected hematuria. The hepatomegaly and hepatic dysfunction with raised alkaline phosphatase 160 IU/L and serum glutamic oxaloacetic transaminase 21 IU/L and serum glutamate-pyruvate transaminase 30 IU/L warranted a liver biopsy despite the thrombocytopenia as it was necessary to establish or exclude a diagnosis of pediatric liver disease. The prominent sinusoidal Kupffer cell hyperplasia and conspicuous hemophagocytosis with extramedullary hemopoiesis led to a strong suspicion of HLH [Figure 2]. The second line of investigations revealed raised LDH levels 301 U/L (110.00-295.00 IU/L), normal ferritin levels 42.40 ng/mL (7.00-140.00 ng/mL), and hypertriglyceridemia 190 mg/dl and reduced fibrinogen levels 130 mg/dl. A careful review of BM aspiration showed hemophagocytosis of erythrocytes, platelets and lymphocytes by macrophages, more evident at the tail end in cellular trails [Figure 3].

A male sibling aged 10 years was apparently healthy, and no similar complaints were forthcoming. A gene analysis to exclude a primary (or genetic) HLH despite the parental consanguinity was not undertaken due to the nonavailability of facilities and economic constraints. HLH was diagnosed in the absence of genetic confirmation, as 5 of 5 major criteria for the disease
were identified: Fever, splenomegaly, cytopenias involving all three cell lines, hemophagocytosis in BM, and liver biopsy; hypofibrinogenemia (Table 1).

TORCH screening showed IgM and IgG antibodies against Rubella and cytomegalovirus. Serology for viral hepatitis, dengue, HIV, and tests for malaria was negative.

Discussion

The histiocytic disorders in children are a diverse group and are characterized by abnormalities in the reticuloendothelial system. A classification to better characterize these disorders that commonly have hepatic manifestations and to improve communication among physicians includes Langerhans cell histiocytosis and HLH.\(^1\)

HLH, a macrophage-related hyperinflammatory disorder is of 2 types: Primary (familial) and secondary or reactive HLH (associated with infections, autoimmune disorders, or malignancy).\(^2\) Familial HLH is an autosomal recessive disorder; seen more frequently in cases of parental consanguinity.\(^3\) It is associated with mutations in genes encoding perforin, hMunc13-4, and syntaxin 11. The mutation leads to a defect in cytotoxic granules and the impairment of their release from cytotoxic T-cells and natural killer cells.\(^4\) Disease severity and the occurrence of relapses without evidence of secondary HLH disease have been presumably defined as familial HLH.\(^5\) However, the diagnostic criteria for primary HLH are fulfilled if there is molecular confirmation of the gene mutation. It is diagnosed in the absence of genetic confirmation if 5 of the following features of the disease are identified: Fever, splenomegaly, cytopenias involving 2 or more cell lines, hypertriglyceridemia or hypofibrinogenemia, hyperferritinemia, elevated interleukin-2 receptor (sCD25), reduced or absent NK cell activity, and hemophagocytosis in BM, cerebrospinal fluid or lymph nodes. All of these criteria are not met early in the disease, and many patients do so only later in the course. The familial variant of HLH is almost universally fatal without treatment whereas secondary HLH probably has a better prognosis. Therefore, until that time the genetic test results are available, all attempts should be made to look for an infectious trigger in a case of HLH.\(^6\)

Secondary HLH is triggered by a variety of diseases such as infections, immunodeficiency syndromes, hematological neoplasias, and autoimmune diseases. It is important to note that both HLH types could be triggered by a variety of infections compounding the diagnostic dilemma.\(^7\) Alternately, whether secondary HLH is also related to a deficiency of cytotoxic molecule release is not clearly established, but the immunosuppressive conditions associated with reactive HLH could support this hypothesis.\(^8\) Infection-associated HLH has been reported in a great variety of viral, bacterial, and fungal infections. Epstein-Barr virus (EBV) infection is undoubtedly the major cause of HLH and has the worst prognosis. Occasional case reports of parasitic infections implicated include visceral leishmaniasis, toxoplasmosis, babesiosis and disseminated strongyloidiasis and plasmodium falciparum malaria.\(^9\) Apart from EBV, dengue infection, especially in epidemics, has
emerged as an important cause of HLH. Bacterial infections such as tuberculosis, enteric fever, and Ehrlichia also form a major portion of HLH, and these patients have a relatively good prognosis if timely antibiotics and supportive measures are instituted.\[10\]

Patient’s blood and bone marrow should be cultured for bacteria, fungi, and viruses. In addition, serologic tests should be obtained. Additional testing for infectious causes should be guided by epidemiologic data and the patient’s medical and travel history.\[11\]

Despite a high incidence of tropical infections in India, studies on secondary HLH are sparse and limited to case reports. The main reasons are a lack of clinical suspicion and awareness and non-availability of genetic and other molecular studies in most developing countries. Diagnosis is usually delayed, which has a negative effect on the morbidity and mortality.\[7\] Early recognition of infection-associated HLH in the tropical Asian populations should stimulate a search for common infections, with early institution of specific treatment and aggressive supportive care, which is crucial for reducing morbidity and mortality.\[6\] 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 (BM) examination may not show hemophagocytosis initially.\[2\] Although demonstration of hemophagocytosis on BM aspiration/biopsy is not mandatory for the diagnosis of HLH according to the diagnostic criteria, it is found to be a useful tool, in the absence of molecular studies.\[7\] The presence of severe HP in BM smears correlated with marked cytopenias.\[12\]

Our case had fever, splenomegaly, pancytopenia, hypofibrinogenemia, and conspicuous hemophagocytosis demonstrated both in the liver as well as bone marrow. All the five major criteria being fulfilled, our patient was diagnosed to have HLH in the absence of genetic testing. The scenario of parental consanguinity favored a familial HLH. The older age, unaffected sibling, short duration, absence of relapses and positive serology for rubella, and cytomegalovirus suggested a secondary HLH. The patient was referred to a premier child health institute in the capital and hence was not followed up. Both types of HLH can be triggered by infections, and hence it was not possible to exclude familial HLH in our case.

As these cases represent systemic diseases causing hepatic dysfunction, the pediatrician must keep them in the differential diagnosis in any young child with liver disease and refer the patient to the appropriate center for definitive evaluation and management.\[6\]

Hepatobiliary disease is a common manifestation and includes hepatomegaly, jaundice, elevated aminotransferases, and coagulopathy.\[1\] Hepatomegaly and hepatic dysfunction, typically occur early in the disease, so the liver is frequently biopsied for diagnosis in recent years. 4 histopathologic patterns of liver injury are observed (1) chronic hepatitis-like, (2) leukemia-like, (3) histiocytic storage disease-like, and (4) neonatal giant cell hepatitis-like. In addition, constellation of features including lymphocyte-mediated bile duct injury, significant endothelialitis, steatosis, cholestasis, and hemophagocytosis are also useful in making a diagnosis of HLH.\[4\]

In our case, liver biopsy was adequate and showed prominent Kupffer cell hyperplasia, erythrophagocytosis in hepatocellular sinusoids, and lobular extramedullary hematopoiesis. No macrovesicular steatosis or giant cell change or cholestasis was evident. Liver involvement characterized by Kupffer cell hyperplasia with hemophagocytosis seemed to be constant in HLH but is a rare finding otherwise, seen in few pathologic conditions and remarkably not in chronic liver disease including autoimmune liver diseases. Conversely, the specificity of this histologic lesion for the diagnosis is unknown. It has been observed in patients who did not fulfill criteria for HLH, but this fact is poorly reported. In bone marrow smears, hemophagocytosis has been observed without HLH.\[6\]

Steps to identify deficient perforin cytotoxic activity, which results in an impaired downregulation of the cellular immune response with sustained activation of macrophages and T-cells are being made by the study of familial cases. This is supported by immunosuppressive conditions being associated with secondary HLH. Further evidence has been a profound decrease of the perforin to CD3 ratio in the liver specifically associated with Kupffer cell hyperplasia with hemophagocytosis, suggesting the role of perforin cytotoxic deficiency in the development of secondary HLH.\[6\]

Table 1: Diagnostic criteria modified from study group of the Histiocyte Society 2004

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<td>Fever: Peak temperature &gt;38.5°C for ≥7 days</td>
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<td>Splenomegaly: Spleen palpated &gt;3 cm below the left costal margin</td>
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<td>Cytopenia involving two or more cell lines: Hemoglobin &lt;9.0 g/dl, or platelets &lt;100,000/ml, or absolute neutrophil count &lt;1000/ml</td>
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<td>Hypertriglyceridemia or hypofibrinogenemia: Fasting triglycerides &gt;2.0 mmol/l (177 mg/dl), or &gt;3 SD above the normal value for age, or fibrinogen &lt;1.5 g/l, or &gt;3 SD below the normal value for age</td>
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<td>Hemophagocytosis: Demonstrated in bone marrow, spleen, or lymph node. No evidence for malignancy</td>
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Alternative criteria:

- Low or absent natural killer cell activity
- Serum ferritin level >500 mg/l
- Soluble CD25 (soluble interleukin 2 receptor) >2400 U/ml

Diagnosis: The diagnosis of hemophagocytic lymphohistiocytosis (HLH) requires the presence of all five major criteria. Either criterion (A) or a combination of criteria (B) and (C) may be a substitute for one of the major criteria. If a patient meets only four criteria and the clinical suspicion for HLH is high, appropriate treatment must be started, as delays may be fatal. In the appropriate clinical setting, the diagnosis is justified by a positive family history of HLH, parental consanguinity is only suggestive of HLH.\[6\]
Conclusion

In India peculiarly, HLH is not uncommon. Awareness is the clue. HLH is an interim mechanism of disorder, an overreaction of the immune system and many diseases can trigger the same events. Early diagnosis by bone marrow or liver biopsy in the absence of genetic testing and aggressive pursuit of an infectious trigger is logical in the infection-associated subset to enable, a complete resolution of the proliferative disorder.

References
