CASE REPORT

Familial Hemophagocytic Lymphohistiocytosis due to Mutation of UNC13D Gene

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Abstract Hemophagocytic lymphohistiocytosis (HLH) are basically a heterogenous group of clinical syndromes, characterised by activation and non-malignant proliferation of benign histiocytes i.e. lymphocytes and macrophages, leading to a cytokine storm that accounts for the fever, organomegaly and multi-organ dysfunction. Two types of HLH are described, either due to known genetic defect (familial HLH/FHL) or due to some acquired cause either infection or rheumatological diseases. Here we present a case of a 3 months old baby, admitted with fever, hepatosplenomegaly and cytopenia and ultimately was diagnosed to be a case of Familial HLH type 3 due to defect in UNC13D gene as a result of compound heterozygous for two nonsense mutation resulting in the Munc13-4 protein defect.

Keywords Hemophagocytes · Familial HLH · Hepatosplenomegaly · Cytopenia

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening syndrome in which an uncontrolled and ineffective immune response, triggered in most cases by infectious agents, leads to severe hyperinflammation especially in genetically susceptible individuals [1]. Though most of the

HLH are acquired due to either secondary to infections, rheumatological diseases or malignancy, familial forms of HLH (FHL), which are increasingly being found also in adolescents and adults, are due to genetic defects leading to impaired function of natural killer cells and cytotoxic T cells [2]. These mutations occur either in the perforin gene or in genes important for the exocytosis of cytotoxic granules. These genetic defects result in a hypercytokinemia and severe hyperinflammation in the familial forms of disease in very early in life and presents with features of fever, organomegaly, cytopenia, hyperferritinemia and demonstrable hemophagocytes in the bone marrow. Our proband, a 3 months old baby boy presented with persistent fever, hepatosplenomegaly, cytopenia, and hemophagocytosis in bone marrow and found to be FHL3 due to UNC13D mutation.

Case Report

This 3 months old baby boy was born of a non-consanguineous marriage of an Indian, Hindu, Bengali couple. He had an uneventful early and post neonatal period without any significant family history and was apparently well up to 2 months of age. At the age of 2 months 4 days, he developed fever that persisted for almost 1 week and it was associated with hepatosplenomegaly. He was first admitted in a local hospital and treated with iv antibiotics but did not respond. Later on he was transferred to Kolkata city and admitted in a private hospital and developed cytopenia with progressively increasing hepatosplenomegaly and treated with broad spectrum antibiotics and multiple blood product transfusions. CSF study was done and it revealed 0 cells with normal protein and sugar and came out to be sterile. TORCH (Toxoplasma, Others, Rubella, Cytomegalovirus and Herpes) screening was also done and came out to be negative. Then he came to us

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with a h/o non resolving fever for almost 1 month with pancytopenia, hepatosplenomegaly (liver 6 cm from right costal margin and spleen 8 cm from left costal margin), and pallor. Investigations revealed a Hb of 7.8 g/dl, TLC-5400/cumm (N20, L74), platelet 18,000/cumm, CRP-124 mg/L. Bone marrow done, revealed reactive changes without any evidence of hemophagocytosis or malignancy. He was treated with broad spectrum coverage by Inj Meropenem, Vancomycin and Fluconazole with blood products transfusion and ultimately became afebrile after 7 days and then discharged in a clinically and hemodynamically stable condition. But the baby was re admitted again after 7 days with fever for 3 days, pallor, respiratory distress and increasing abdominal distension. Investigation revealed Hb of 6.4 g/dl, TLC-6800/cumm (N17L78), Platelet 26,000/cumm. With a strong clinical suspicion of Hemophagocytosis, we send a HLH Panel and reports revealed Ferritin-1,861 µg/L (Normal 20–220 µg/L), Tg-135 mg/dl (Normal <220 mg/dl), fibrinogen-1.05 g/L (Normal 1.5–3 g/L). Another bone marrow study was done and it revealed plenty of hemophagocytosis along with dyserythropoiesis and decreased megakaryocytes. Baby was treated with broad spectrum antibiotics, blood component transfusion, Intravenous immunoglobulin (2 g/kg), iv dexamethasone (10 mg/m²) and oral Cyclosporine and blood for genetic mutation analysis was sent to abroad. The baby initially responded to treatment and Hematopoietic stem cell transplantation (HSCT) was advised but the parents took the baby home due to financial constraints and the baby ultimately expired there. The mutation analysis done by gene sequencing confirmed the diagnosis of Familial Hemophagocytosis Type 3 (FHL-3 OMIM 608898) on the basis of bi-allelic mutations (c.762delC p.C255AfsX73 and c.823C>T p.Q275X) in UNC13D gene. The baby was found to be compound heterozygous for these two nonsense mutations which were also confirmed on the parents (mother heterozygous for the mutation c.762delC p.C255AfsX73 and father: heterozygous for the mutation c.823C>T p.Q275X). Functional assays for natural killer (NK) cell activity could not be performed for the long shipping time. The parents were called up again and genetic counselling was done. They were advised to go for a prenatal diagnosis by a Chorionic villous sampling and mutation analysis in the next pregnancy.

Discussion

HLH is actually not a disease, but a clinical syndrome characterized by hyperinflammation. It may have genetic causes or may be acquired under a variety of conditions. In infants and very young children, HLH is predominantly due to immune defects due to mutations in genes respon-

sible for cytotoxic function of NK cells and cytotoxic T lymphocytes (CTLs). These are called as genetic or Familial HLH [2]. In older children, adolescents, and adults, HLH is associated more often with infections, malignancies, autoinflammatory and autoimmune diseases, and acquired immune deficiency states. However, even in a number of cases of late-onset HLH, many genetic defects are increasingly being identified [3].

Cardinal symptoms of HLH whether familial or acquired are prolonged fever coupled with hepatosplenomegaly and cytopenia as our index patient who presented with all of these three features. Hemophagocytosis may be absent initially in a substantial proportion of patients and the absence of hemophagocytes in bone marrow does not exclude the disease as in our case the first marrow did not show any hemophagocytes, where the second one revealed it. HLH is diagnosed based on the revised diagnostic criteria made by Histiocytic Society [4]. Among the eight criteria (fever, splenomegaly, cytopenia, hypertriglyceridemia or hypofibrinogenemia, hyperferritinemia, increased soluble CD25, absent NK cell function and demonstration of hemophagocytes.) five should be present. Diagnosis can be established directly without necessitating such criteria if a known genetic defect has been identified. In our case we got the clinical and laboratory criteria as well as were able to demonstrate the UNC13D mutation.

Genetic HLH can be divided into two subgroups: first, the familial HLH (FHL), an autosomal recessive disease, and second, the immune deficiency syndromes like Chediak–Higashi syndrome (CHS), Griscelli syndrome-2 (GS-2), and X-linked proliferative syndrome (XLP). Four genetic defects have been identified in FHL (FHL2–FHL 5) [5]. The specific gene involved in FHL-1 has not yet been identified. The first genetic defect, mutations in the perforin (PFR1) gene (FHL-2), was described in 1999. Perforin is an essential component of the secretory granules of cytotoxic cells; together with granzymes, mediate apoptotic death of target cells. Perforin mutations account for 13–58 % of FHL cases, depending on ethnic origin [6]. Genes mutated in FHL-3, FHL-4, and FHL-5 are involved in cytotoxic granule exocytosis.

FHL-3 accounts for 10–32 % of genetic HLH cases. It occurs due to mutation of UNC13D gene that ultimately leads to a defective Munc13-4 protein formation. The chromosomal location is usually 17q25 [7]. Our index case was found to be compound heterozygous for two nonsense mutations in UNC13D gene and thus confirms the diagnosis as FHL-3. The parents were found to be heterozygous for the mutation. Though UNC13D mutations has been described previously, these mutations are novel one and never described before. FHL-3 is clinically indistinguishable from other

FHLs, but Central nervous system (CNS) involvement seems to be more common in FHL-3 [8].

Untreated, FHL is a rapidly fatal disease. Most patients die from multiorgan dysfunction due to bacterial or fungal infections [9]. There is also substantial mortality in acquired HLH, depending on the underlying disease. The immediate aim in the treatment of any patient with HLH is to suppress the severe hyperinflammation that is responsible for the life threatening symptoms. If a treatable organism can be identified, therapy against this pathogen should be employed. Immunosuppressive agents are the mainstay of therapy to bring the remission. HLH 2004 protocol promotes the combination 8 weeks chemotherapy comprising of Dexamethasone, Cyclosporine A and Etoposide with or without intrathecal Methotrexate for severe CNS involvement [1, 4, 9]. But familial cases tends to relapse even after achieving the remission and only HSCT results in permanent control of the disease. Unfortunately, transplant mortality with myeloablative conditioning treatment has been high. Recently, reduced-intensity conditioning in HLH was shown to be associated with lower toxicity and increased survival [10].

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