Fatal Epstein-Barr virus infection in a case of familial hemophagocytic lymphohistiocytosis with syntaxin-11 mutation

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Familial hemophagocytic lymphohistiocytosis (FHL) is a fatal disease of early infancy caused by defective natural killer cell activity and is characterized by fever, organomegaly, pancytopenia, and coagulopathy. Disease-causing mutations have been found in perforin, Munc 13-4 and syntaxin-11 genes. We herein describe a case of late-onset FHL with syntaxin-11 mutation in a six-year-old boy in whom only partial response was obtained by immunochemotherapy (HLH-94 protocol) and who died with persistent Epstein-Barr virus (EBV) infection. The role of EBV infection in the prognosis of FHL is discussed.

Key words: familial hemophagocytic lymphohistiocytosis, syntaxin-11 mutation, Epstein-Barr virus, child.

Hemophagocytic lymphohistiocytosis is a rare disorder characterized by a sepsis-like clinical picture and proliferation of benign lymphocytes and histiocytes with hemophagocytosis[1]. It can be caused by a genetic or acquired defect of lymphocyte cytotoxicity that results in incomplete elimination of intracellular microorganisms and a hyper-inflammatory response due to uncontrolled cytokine release. Familial hemophagocytic lymphohistiocytosis (FHL) is a fatal disorder that has four types (FHL1, 2, 3, 4) due to mutations in certain genes[2,3]. The gene defect responsible for FHL1 is unknown. FHL2 (20–50% of FHL cases), which is particularly common in Turkey, consists of mutations in the perforin gene in which resultant imperfect perforin production leads to defective apoptosis triggering[4,5]. FHL3 is composed of Munc 13-4 mutations that are required for priming of granules for membrane fusion and exocytosis. Gene encoding syntaxin-11 mutations were identified in FHL4 patients and caused malfunctioning syntaxin-11 protein with consequent incomplete intracellular vesicle trafficking[6].

Acquired or secondary FHL is seen in association with a variety of infections and malignant or autoimmune disorders[7,8]. Epstein-Barr virus (EBV)-associated FHL is a fulminant disorder occurring mostly in healthy individuals[9,10]. We herein report a fatal EBV infection in a six-year-old boy with syntaxin-11 mutation.

Case Report

A six-year-old boy presented with a three-month history of high fever exceeding 39°C several times a day and abdominal distention. His past history was unremarkable with no frequent infections. He was the first child of a healthy couple who were first cousins. He had no sibling and no family history consistent with hemophagocytic lymphohistiocytosis. Physical examination revealed 39.5°C fever and hepatomegaly and splenomegaly of 3 cm and 11 cm below the right and left costal margins, respectively. Laboratory investigations showed pancytopenia, hyperferritinemia, hyperlipidemia, hypofibrinogenemia, and mildly elevated liver enzymes (Table I).

Table I. Clinical and Laboratory Features of the Patient at Diagnosis and During the Treatment

	Fever	SM/HM (cm)	Hb g/dl	WBC x10³/ml	ANC x10³/ml	Plt x10³/ml	TG mg/dl	Fer ng/ml	Fib mg/dl	AST/ALT U/dl	EBV PCR	Treatment
At diagnosis	+	15/10	7.5	5.45	0.8	34.6	454	2000	50	255/110		IVIG, BsAb
3rd week	+	11/3	5.9	1.2	0.1	23	220	2000	70	14/28	5000 copies	HLH–94 (Ind), acyclovir
6th week	–	5/1	12	13.4	6	190	128	98	200	20/82	500 copies	HLH–94 maintenance
24th week 1st react	+	8/3	8.4	3	0.9	78	244	678	172	192/206	25000 copies	HLH–94 (Ind), BsAb, acyclovir
32nd week	–	8/2	8.1	1.1	0	86	60		247	146/315	2000 copies	HLH–94 maintenance
40th week 2nd react	+	15/8	7.5	5.3	0.2	34	454	2000	50	255/110	5000 copies	HLH–94 (Ind), BsAb
44th week	+	15/7	5.8	3	1.4	14.7	73	2000	207	328/101	1500 copies	Etoposide, steroid, acyclovir
45th week	+	15/8	10	4	1	120	159	1500	125	29/40		High–dose steroid, acyclovir
50th week (before death)	+	16/9	10	3.3	0.7	36		38000	66	96/134	300.000 copies	Steroid, CsA, acyclovir

SM: Splenomegaly. HM: Hepatomegaly. Hb: Hemoglobin. WBC: White blood cell count. ANC: Absolute neutrophil count. Plt: Platelet count. TG: Triglyceride. Fer: Ferritin. Fib: Fibrinogen. AST: Aspartate aminotransferase. ALT: Alanine aminotransferase. EBV: Epstein-Barr virus. HLH: Hemophagocytic lymphohistiocytosis. IVIG: Intravenous immunoglobulin. BsAb: Broad spectrum antibiotics. Ind: Induction. CsA: Cyclosporine.

Abdominal ultrasonography showed diffuse hepatosplenomegaly. Chest X-ray was normal. Multiple bacterial and fungal cultures from blood, urine, throat, pan fungal polymerase chain reaction (PCR) and EBV PCR (using PCR Kit) and serological studies for EBV, cytomegalovirus (CMV), hepatitis A virus (HAV), human immunodeficiency virus (HIV), human herpesvirus (HHV) type 1 and type 2, brucellosis, and toxoplasmosis were all negative, while anti HBs IgG was positive. The serum levels of immunoglobulins were normal. Differential count in peripheral blood revealed: 56% granulocytes, 40% lymphocytes and 4% monocytes. Bone marrow aspiration showed increased number of benign histiocytes and occasional hemophagocytosis. A diagnosis of FHL was made and intravenous immune globulin 400 mg/kg per day for five days and broad spectrum antibiotics for 10 days were given. After a transient improvement with defervescence of fever, reduction in both liver and spleen size and normalization of blood chemistry and blood counts within two weeks, the clinical picture deteriorated and HLH-94 chemotherapy protocol had to be commenced. Clinical and laboratory abnormalities disappeared during induction therapy except for 2 cm splenomegaly but reactivation occurred shortly after the start of the maintenance therapy. Because EBV PCR titer increased to 50,000 copies, acyclovir at a dose of 1500 mg/m^2 was given for two weeks followed by 750 mg/m^2 as prophylaxis during the follow-up. Reinduction therapy with HLH-94 protocol produced only partial remission again and the patient died 53 weeks after diagnosis during preparation for stem cell transplantation from his one antigen mismatched mother at reactivation in association with an EBV PCR titer of 300,000 copies. Mutation analysis revealed a 5 bp deletion in the exon 2 of the syntaxin-11 gene. There is a 2 bp and a 3 bp deletion separated by three normal nucleotides. The mutation found in our patient with FHL is as follows: c.369-370delAG/c.374-376delCGC, which leads to a protein exchange Val124fsX60 and a preliminary stop codon in the syntaxin-11 gene.

Discussion

The patient presented herein had prolonged fever, massive splenomegaly, hepatomegaly, pancytopenia, hyperferritinemia, hyper-

triglyceridemia, and hypofibrinogenemia. These clinical and laboratory findings fulfill the diagnostic criteria of FHL proposed by the Histiocyte Society[11]. Parental consanguinity and lack of an underlying infection, malignancy or autoimmune disorder suggested a clinical diagnosis of FHL.

Familial hemophagocytic lymphohistiocytosis is an autosomal recessive disease that presents as a severe hyper-inflammatory syndrome with activated macrophages and T lymphocytes. It is caused by mutations in certain genes like perforin, Munc 13-4 and syntaxin-11. Genetic analysis revealed that our patient was homozygous and both parents were heterozygous for a 5 bp deletion in exon 2 of the syntaxin-11 gene.

Syntaxin-11 is a widely expressed member of the family of membrane trafficking proteins. Syntaxin-11 is expressed mostly in lymphoid organs, particularly abundant in T cells and natural killer cells, as well as macrophages. Syntaxin-11 has been attributed a role in secretory lysosome exocytosis, as cytotoxic lymphocytes from patients with mutations in dytaxin-11 demonstrate intrinsic deficiency-like defective degranulation. However, others suggest an indirect impairment of lymphocyte function during interactions with antigen-presenting cells[12].

Syntaxin-11 mutation has previously been described in seven patients from three families, all of whom were of Turkish origin[3]. Rudd et al.[6] reported syntaxin-11 mutations in six Turkish children (20%) from four of the 28 unrelated perforin-negative families. In addition, zur Stadt et al.[14] analyzed a large group of 63 unrelated patients with FHL of different geographic origins (Turkey: 32; Germany: 23; others: 8) for mutations in syntaxin-11, perforin and Munc 13-4. Of the 32 patients from Turkey, 14 had mutations in perforin, six had mutations in Munc 13-4 and six had mutations in syntaxin-11. Syntaxin-11 mutations accounts for 19% of patients from Turkey, whereas it has not been found in patients of German origin.

Of note, the onset is late in most of the patients with syntaxin-11 mutations of FHL, while FHL due to perforin or Munc 13-4 mutations typically starts in early infancy in 70-80% of the cases[14]. Our patient seemed perfectly healthy until the age of six years, supporting that late onset is a common feature of FHL associated with syntaxin-11 mutations. However, more patients are needed for a better correlation of the outcome of different genetic mutations.

Infections, predominantly with viruses, can precipitate both the inherited and acquired form of FHL[4]. In a study of 18 patients with primary FHL, an infection was documented in 5 (28%) at diagnosis and in 10 (56%) during the course of the disease. EBV was found in only two of these patients with positive serology in both and PCR in one[15]. The true incidence of EBV infection in children with FHL is not known, since the serological diagnosis may be inconclusive and routine viral screen with PCR was not available until recently. In contrast to FHL, EBV is the most common cause of secondary hemophagocytic lymphohistiocytosis due to infections, particularly in Eastern Asia[16,17]. On the other hand, in Turkey, Gürgey et al.[19] reported a total of 18 children between 2 weeks-72 months of age who were diagnosed as having secondary hemophagocytic lymphohistiocytosis, with only one patient (5.5%) related to EBV infection.

Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis is a fulminant, potentially fatal disorder, mostly occurring in patients without an underlying immune deficiency and characterized by proliferation of EBV-infected T and natural killer cells leading to hypercytokinemia and tissue damage. The pattern of EBV serology is consistent with a previous infection in two-thirds and reactivation in one-third of the patients[10].

In our patient, EBV-DNA level was elevated at onset and remained so throughout the 50-week follow-up period. Decrease in the copy number of EBV DNA during partial remission and marked increase before death (Table I) suggest that uncontrolled EBV infection was responsible for the disease activity and eventual death. Anti-viral capsid antigen (VCA) and anti-early antigen (EA) remained undetectable during the course of disease, indicating the inability to mount immune response to EBV. Normal levels of serum immunoglobulins and lymphocyte subpopulations as well as uncomplicated childhood vaccinations excluded the possibility of a preexisting immune deficiency disorder.

Immunochemotherapy with HLH-94 protocol can prolong life in the majority of patients with FHL, and the prognosis is uniformly fatal

without stem cell transplantation[11]. Our patient showed only a short-term, partial response to treatment with HLH-94 protocol combined with antiviral therapy and frequent intravenous immune globulin administration, and he died one year after diagnosis. It is possible that persistent EBV infection may be responsible for the treatment failure. Addition of therapeutic agents to the immunochemotherapy protocol that can eliminate EBV may improve the prognosis in patients with FHL.

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