Epstein Barr Virus Associated Hemophagocytic Syndrome
- A Case Report -

Virus associated hemophagocytic syndrome (VAHS) are a heterogeneous group of disorders in which viral infection is associated with a proliferation of hemophagocytic histiocytes through the reticuloendothelial system. We report the case of a 21-year-old Korean man who presented to us with high fever, marked hepatosplenomegaly, severe hepatic dysfunction, coagulopathy, pancytopenia and marked panhypogammaglobulinemia. Bone marrow aspiration and biopsy showed histiocytes proliferation with active phagocytosis of red cells and neutrophils. Primary Epstein-Barr (EB) viral infection at presentation was confirmed by the presence of IgM antibody to viral capsid antigen (VCA) with absence of antibody to EB viral nuclear antigen (EBNA). A liver biopsy performed one month after the presentation showed erythrophagocytic histiocytes within the sinusoids. EB virus was demonstrated in the liver biopsy tissue by DNA PCR method, and EBER mRNA in situ hybridization.

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Key Words : *VAHS; EB virus infection; Herpesvirus 4; Human*

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INTRODUCTION

The term VAHS was introduced to describe a disorder characterized by a benign generalized histiocytic proliferation with marked hemophagocytosis associated with systemic viral infections (1). Subsequently several other diseases and agents have been associated with this histiocytic reaction, including tuberculosis, leishmaniasis, gram negative bacteria, histoplasmosis, systemic lupus and several types of malignant neoplasms, and it is referred to as reactive hemophagocytic syndrome (2~6). Although most VAHS affected patients have acquired immunosuppression or an established immune deficiency syndrome, VAHS occurring in normal persons has been reported (7, 8).

A few cases of VAHS have been reported in Korea, yet EB viral etiology is not well documented. We report a 21 years old Korean man with no known prior illness, presenting with fulminant EB viral infection and marked panhypogammaglobulinemia.

CASE REPORT

A 21 years old man was admitted to the hospital because of fever and epigastric discomfort of 3 days duration. Past medical history was unremarkable except for dystonic spells following head trauma being followed by a neurologist. Physical examination showed an acutely ill looking man with body temperature of 38.7℃. His sclera was icteric and the liver was palpable 4 cm below the right costal margin with diffuse tenderness. The spleen margin was about 10 cm below the left costal margin. Hemogram showed hemoglobin 7.7 gm/dl, hematocrit 22.5%, white blood cells 2,100/μl and platelet count 38,000/μl. Reticulocyte count was 2.4%, serum iron was 51 μg/dl, total iron binding capacity was 363 μg/dl and serum ferritin 225 ng/ml. Liver function test was markedly abnormal with total protein of 3.9 g/dl, albumin 2.1 g/dl, total bilirubin 6.5 mg/dl, ALT 130 U/L, AST 332 U/L and LDH of 509 U/L. Quantitative immunoglobulins were markedly diminished with IgG 569 mg/dl, IgA 66.1 mg/dl, and IgM 41 mg/dl. Follow up one month later showed IgG 379 mg/dl, IgA 15.7 mg/dl and IgM 25.3 mg/dl. Coagulation profile showed prothrombin time of 45%, activated partial thromboplastin time 53 seconds (normal control 27 sec), fibrinogen 50 mg/dl and FDP was below 10 μg/ml. Urinalysis showed 1+ albumin with 3+ bile. Chest x-ray did not show any abnormality. Initial blood cultures were negative. AIDS antibody was negative. IgM antibody to EBV VCA was positive with titer of 1:10, but IgG antibody to EBV VCA was negative as was antibody to EBNA. Bone marrow aspiration and biopsy showed

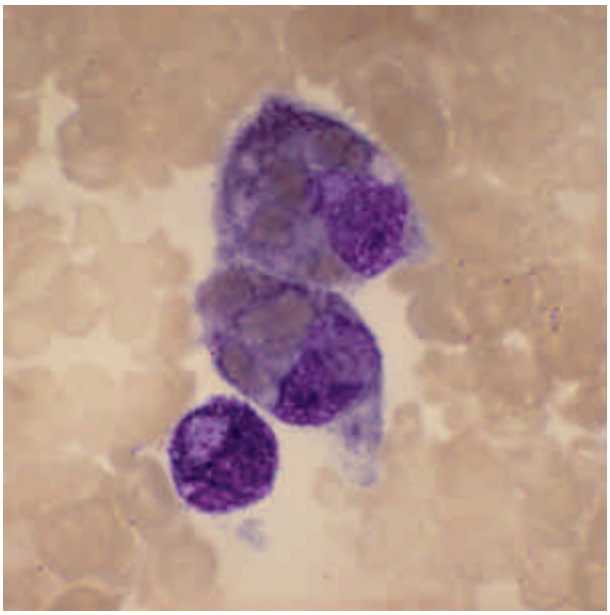

Fig. 1. Two macrophages ingest numerous erythrocytes in aspirated bone marrow smear (Wright, ×400).

increased histiocytes some of which were actively phagocytizing red cells and neutrophils (Fig. 1). About one month after the acute presentation, a liver biopsy was performed. The Liver biopsy showed markedly dilated sinusoids containing erythrophagocytic histiocytes and a few lymphocytic infiltrate in the lobules (Fig. 2A). These histiocytes were positive for CD68 immunostaining (Fig. 2B). Infiltrating lymphocytes were positive for pan T-cell markers (CD 45RO and CD43) and negative for pan B-cell markers (CD20 and MB-2). No NK cells were detected with CD56 immunostaining (Fig. 3). A few fat vacuoles and bile plugs were also noted. DNA was extracted from liver biopsy tissue using a QIAamp tissue kit (QIAGEN, Germany).

For PCR amplification of EBV genome, 20 base primers are designed to amplify 138 bp segment in the EBNA-1 region and designated EBNA 1-PF: 5'-TGA TAA CCA TGG ACG AGG AC-3' and EBNA 1-PR: 5'-GCA GCC AAT GCA ACT TGG AC-3'. PCR was performed in 1 cycle of 3 min at 95℃, and 35 cycles of 30 sec at 94℃, 1 min at 58℃, 2 min at 72℃, and final extension of 15 min at 72℃. PCR amplified products were analyzed by 2% Nusieve agarose gel electrophoresis and showed discrete single band of 138 bp fragment in this case (Fig. 4). Known EBV negative and positive DNAs from lymphoma patients were used as a control.

To detect EBV nuclear RNA transcript in formalin-fixed paraffin embedded liver biopsy, in situ hybridization (ISH) was performed with a fluorescein-conjugated oligonucleotide probe, EBER (YO17, Dakopatts, Denmark). ISH demonstrated a few EBER positive T lymphocytes (Fig. 3A). During acute episodes, supportive transfusions were given with packed red cells, platelet concentrates, fresh frozen plasmas as well as human immunoglobulins

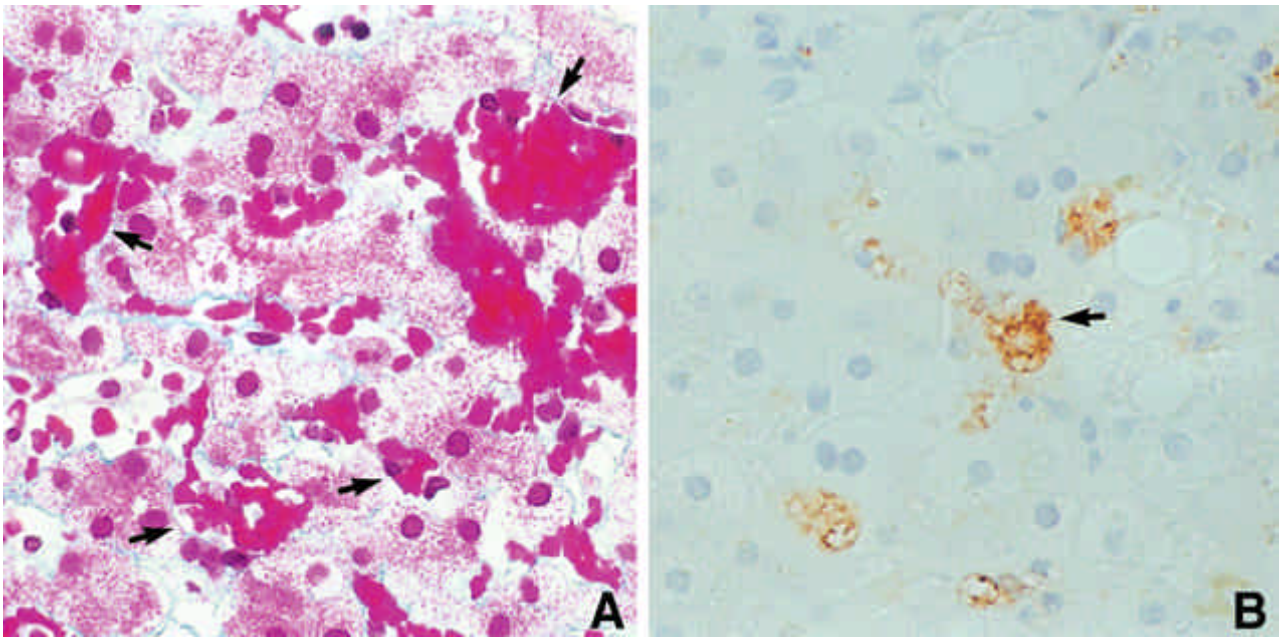

Fig. 2. The liver shows erythrophagocytic histiocytes(arrows) within the sinusoids (A, Masson-trichrome, ×400). These histiocytes are positive for CD68 immunostaining (B, ×200).

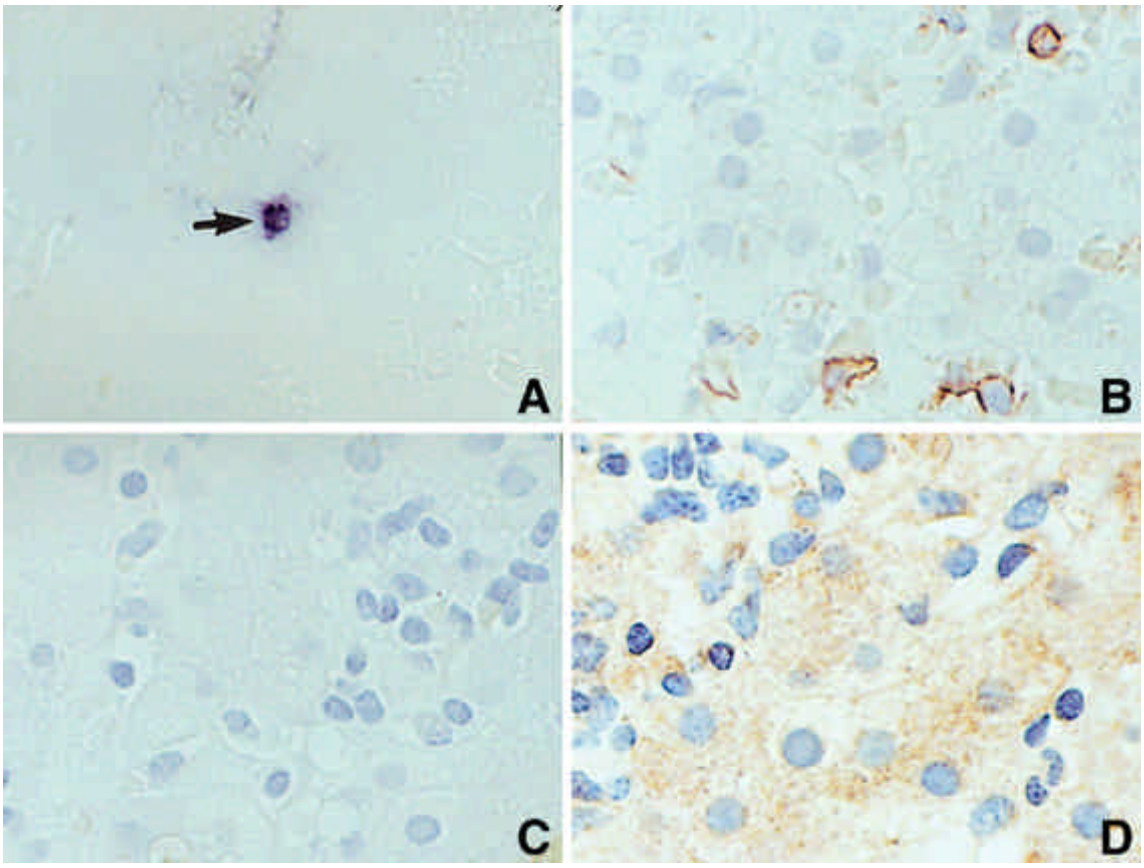

Fig. 3. EBER in situ hybridization of liver biopsy showing a positive signal in lymphocyte (A). Infiltrating lymphocytes are positive for CD45RO(B) and negative for CD20(L26)(C). No NK cells were detected with CD56 immunostaining (D, ×200).

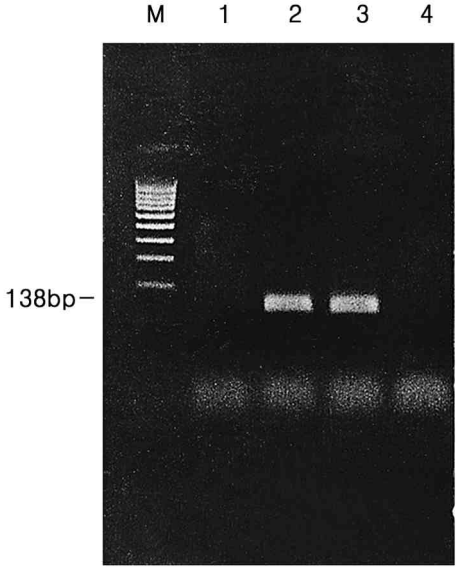

Fig. 4. 2% Nusieve-gel electrophoresis of PCR products show a discrete single band of EBNA-1 gene at 138bp.
Lane 1 : negative control — known EBV-negative lymphoma case
Lane 2 : positive control — known EBV-positive lymphoma case
Lane 3 : patient sample

and a short term trial of prednisone.

Although hemogram improved briefly, the patient developed a series of infections including herpes zoster of the right lower leg and coagulase negative staphylococcus which were controlled with parenteral ayclovir and vancomycin injection and removal of intravenous catheter. The patient's liver function deteriorated and total bilirubin was up to 19.8 mg/dl with persistence of coagulopathy and the patient expired 2 months after admission.

DISCUSSION

It is becoming clear that hemophagocytic syndromes are a heterogeneous group of disorders that may have diverse etiologies and clinical courses. The EB virus has been associated with many of the VAHS. The most common histopathologic finding in patients with this syndrome is a proliferation of cytologically benign yet actively phagocytic histiocytes. The histiocytosis generally involves the bone marrow, the sinusoids and medullary

cords of the lymph nodes, the red pulp of the spleen and the sinusoids and portal tracts of the liver. In our case, we confirmed hemophagocytosis in bone marrow biopsy at presentation and later in the liver biopsy. Primary EB viral infection was obvious with positive IgM antibody to EBV VCA and absence of antibody to EBNA. About one month after the presentation, EB virus was detected from liver biopsy tissue by DNA PCR method, and EBER mRNA ISH.

The pathogenesis of VAHS remains unclear. Generally, infectious mononucleosis (IM) is a benign, self-limiting disease associated with a primary EB viral infection and EBV-infected B cells trigger polyclonal proliferation of cytotoxic T cells, which themselves are free of EBV. However, rare cases develop severe or fatal IM; additionally, severe or fatal IM is commonly accompanied by VAHS (9). Recently, monoclonal proliferation of cytotoxic T cells containing EB virus genome has been reported in a fatal mononucleosis patient (10). Others have documented clonal proliferation of natural killer cells containing EB virus genome in a fulminant hemophagocytic syndrome (11). It seems that unlike in common EB virus infection in B lymphocytes leading to a clinical picture of mild IM, EB viral infection in NK cells or T lymphocytes may lead to a fulminant infection. Studies to ascertain the pathogenetic mechanism of VAHS and fatal IM have focused on a possible imbalance of various cytokine networks (9, 12). Increased levels of interferon-gamma or interferon-alpha have been detected as well as soluble interleukin (IL)-2 receptor, macrophage colony stimulating factor (MCSF), tumor necrosis factor (TNF)-alpha and IL-6 (9). MCSF is produced by T cells, and stimulates monocytes/macrophages. Activated monocytes/macrophages secrete IL-6 and TNF-alpha. Therefore, an overproliferation of activated T cells appears to be part of the syndrome of hemophagocytic syndrome and immune deregulation, abnormal feedback control, or failure of normal shutoff mechanisms may lead to the development of diffuse histiocytic activation and phagocytosis throughout the reticuloendothelial system (9).

In our case EBER ISH showed a few positive signals in T lymphocytes. No NK cells were detected with CD56 immunostaining. Infiltrating lymphocytes were all positive for pan T-cell markers and negative for pan B-cell markers. Therefore in this case an overproliferation of activated T cells, probably cytotoxic T cells, not NK cells, appears to be involved in pathogenesis of VAHS and immune deregulation.

The clinical course varies from a mild to a very severe form with mortality rates of 30% and probably higher in immunocompromised patients. In our patient, besides the usual clinical picture of high fever, lymphadenopathy, hepatosplenomegaly with hepatic dysfunction, coagulopathy and pancytopenia, severe panhypogammaglobulinemia was striking at the time of admission and persisted till death. We interpreted this as secondary to disseminated EB viral infection since the patient did not have a family history nor any serious infection prior to this admission and had normal total protein and normal albumin values before admission. Acquired agammaglobulinemia as seen in our case has been reported in three children with clinical and laboratory features with IM and this was postulated as abnormal T-cell response to transformation of B cells by EB virus, leading to B-cell dysfunction and leading to agammaglobulinemia (13). Although reported cases are few, VAHS with hypogammaglobulinema and/or agammaglobulinemia may predict a fatal outcome.

Many therapies have been attempted against severe EBV infection. These treatments include antiviral agents (such as acyclovir, ganciclovir, and adenine arabinoside), high-dose gammaglobulins and immunomodulating agents, such as IL-2, IFN-alpha, IFN-gamma, and corticosteroids (14, 15). However, no beneficial effects have been clearly demonstrated from any of these treatments. Recently, etoposide was given to a patient with severe IM accompanied by VAHS who had X-linked lymphoproliferative disease resulting in a clinical remission (16). Additionally, cyclosporin A may be useful in controlling the proliferation of T cells in spite of decreasing activity of EBV-specific cytotoxic T lymphocytes (17).

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