

**Case Report****Emperipolesis, Apoptosis and Hemophagocytosis during Acute Epstein-Barr Virus (EBV) infection: A Case Report**

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**Abstract**

*An eight-year-old patient had intermittent high grade fever and pharyngitis since one week. Later, he developed left-sided cervical lymphadenitis. Subsequently, he also developed palatal petechiae. Absolute lymphocyte count (ALC) was raised (ALC 20,262 cells/mm<sup>3</sup>). Hepatosplenomegaly was also detected. Clinically, the current case was diagnosed as a case of infectious mononucleosis. Possibility of infection by Epstein-Barr virus was considered. FNAC was done. Blood-mixed aspirate showed monocytoid cells with mild pleomorphic nuclei, and clumped chromatin. Inconspicuous nucleoli and abundant eosinophilic cytoplasm were seen. Foci of apoptosis were also seen. Frequent mitoses were seen. Findings were suggestive of acute reactive lymphocytic hyperplasia with hemophagocytosis and apoptosis. EBV DNA was detected by RT PCR with linear reporting range of 3.3×10<sup>4</sup> IU/ml. Herewith, we describe a case of infectious mononucleosis with emperipolesis and hemophagocytosis.*

**Keywords:** Epstein-Barr virus associated hemophagocytosis syndrome (EBVAHS).

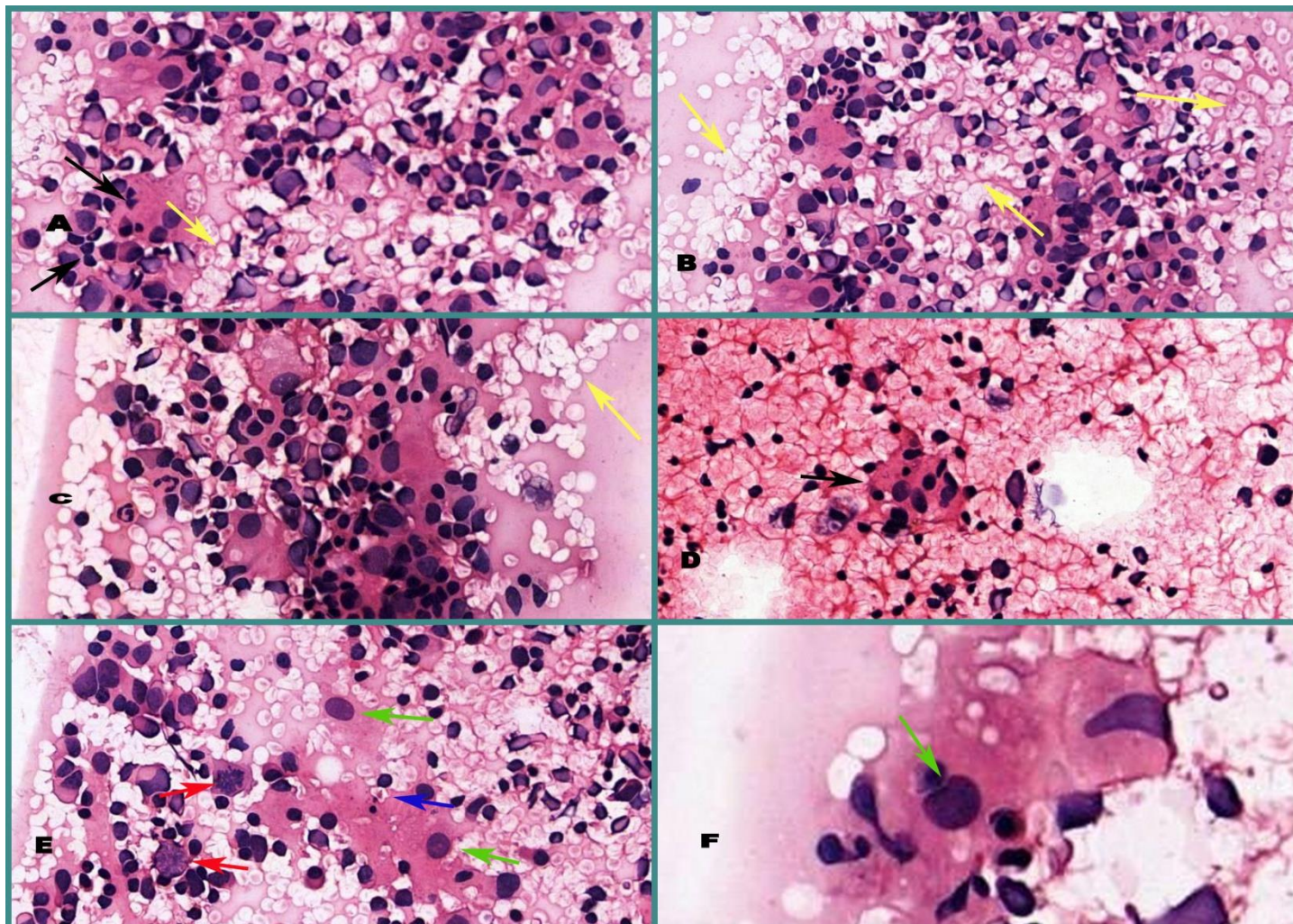
**Case Report**

An eight-year-old patient had high grade fever for the last one week. Subsequently, he developed swelling in left submandibular region since 3 days. Swelling was 1 cm in diameter. Clinically,

he was diagnosed as a case of cervical lymphadenitis. FNAC was done. Smear was suggestive of acute reactive lymphocytic hyperplasia with hemophagocytosis and apoptosis (fig.1E). Later, the patient was diagnosed as a case

of infectious mononucleosis. Absolute lymphocyte count was severally raised (ALC 20,262 cells/mm<sup>3</sup>). Platelets were adequate in number; platelet count was 1,75000/mm<sup>3</sup>. Real time PCR and probe-based assay for EBV were done. EBV DNA was detected with the linear

reporting range being  $3.3 \times 10^4$  IU/ml. Hematoxylin and Eosin (HE)-stained smears were hypercellular and showed lymphoid and monocytoid cells. In addition, lymphoid cells showed mild pleomorphism. Brisk mitoses were also seen (fig.1E)



**Figure 1:** (A) Photomicrograph shows smear from lymph node aspirate. Aspirate is mildly hypercellular and shows polymorphous population of cells. Erythrophagocytosis is also seen with white arrow (HE×200). (B) Photomicrograph shows large number of mature small lymphocytes, few centrocytes and histiocytes along with monocytoid cells. Smear also shows erythrophagocytosis (HE×200). (C) Photomicrograph shows similar findings as in B (HE×200). Nucleomegaly is also seen by green arrow. (D) Photomicrograph shows prominent hemophagocytosis in a hemorrhagic background (HE×200). (E) Shows frequent mitoses. Closed red arrows show mitotic figures. Thin blue arrow (→) shows apoptosis (HE×200). (F) Photomicrograph shows mild anisonucleosis by green arrow (HE×400).

### Discussion

Several viruses, e. g. EBV, Cytomegalovirus (CMV), HIV and few other viruses are known to produce a similar clinical and hematological picture, suggesting a diagnosis of infectious

mononucleosis. Loss of appetite and cough may frequently develop<sup>[1]</sup>. In addition, present patient also showed apoptosis and hemophagocytosis. High fever, pharyngitis and lymphadenitis are known to develop subsequent to EBV-induced



infectious mononucleosis. It has been reported with blood loss in association with neoplasm<sup>[2]</sup>. Palatal petechiae, splenomegaly, palpebral edema and skin rash may also develop<sup>[3]</sup>. However, apoptosis has been rarely reported earlier in EBV infection. It is characterized by the entry of marrow cells into other cells. Later, nuclear condensation, margination, and apoptotic bodies are formed. Subsequently, apoptotic bodies are removed by monocyte macrophages. Another interesting finding was the detection of hemophagocytosis. EBV is probably also associated with acute reactive hemophagocytic syndrome. Moreover, children <9 years of age are relatively less likely to develop anti-EBV antibodies<sup>[2]</sup>.

Most important feature of the present case was the detection of emperipolesis. It is characterized by the entry of bone marrow and hemic cells in megakaryocytes or other cells. Furthermore, it is characterized by 'inside round-about wandering' which describes the penetration of EBV-infected cells by lymphocytes. T lymphocytes attach themselves to monocytes with the help of uropods forming rosettes. Stable rosettes are formed when lymphocytes and monocytes come in contact with antigens. Thus, uropods may play an important role in antigen recognition. Later, lymphocytes in rosettes get stimulated with raised DNA synthesis. Emperipolesis also has been reported in patients with autoimmune hepatitis<sup>[4]</sup>. Lymphocytic infiltration of hepatocytes might show shrinkage of cells with margination and condensed chromatin. All these features were suggestive of apoptosis. Moreover, CD8<sup>+</sup> Ia<sup>+</sup> T lymphocytes were also seen inside hepatocytes<sup>[4]</sup>. Similar features may be observed in EBV infection. EBV-induced CD8<sup>+</sup> T lymphocytes also appear to cause cell death by apoptosis. Another interesting feature of this report was the detection of hemophagocytosis. Hemophagocytosis differs from emperipolesis. In hemophagocytosis, engulfed cells are destroyed in cytoplasm by necrosis. However, secondary hemophagocytic syndrome has been reported in an

immunocompromised host in association with viral infection<sup>[5]</sup>. EBV may induce both primary and secondary forms of hemophagocytic lymphohistiocytosis<sup>[5]</sup>. Further, EBV-associated lymphocyte stimulation leading to proliferation may be another important feature of EBV-induced hemophagocytic lymphohistiocytosis<sup>[6]</sup>. EBV has been shown to proliferate in macrophages<sup>[7]</sup>. Moreover, virus-associated hemophagocytic syndrome (VAHS) has been recognized as fever, splenomegaly, hepatomegaly and hemophagocytosis. In addition, macrophages might ingest hemic cells in reticuloendothelial system. Rarely, these patients may develop severe fatal disease<sup>[8]</sup>. Emperipolesis was first described by Humble et al<sup>[9]</sup>. Suicidal emperipolesis may eliminate autoreactive T cells by apoptosis, resulting in immunological tolerance<sup>[9]</sup>. Further, follicular dendritic cells may save B cells and memory B cells from cell death by apoptosis<sup>[10,11]</sup>. Epstein-Barr virus-induced hemophagocytosis may be a fatal disease. Similar disease model has been reported in rabbits using a related virus-Herpes Virus Papio (HPV). Anti-RBC antibodies in presence of activated monocyte macrophages of reticuloendothelial system may result in phagocytosis of blood cells and lysis of RBC. However, in emperipolesis cell death occurs by apoptosis either by neutrophils or CTL CD8<sup>+</sup> suppressor T cells or other cells. Thus, we propose that hemophagocytic syndrome requires 2 separate events, one of the events require monocyte-macrophage activation and generation of cytotoxic T cells. Another event leads to polyclonal B cell proliferation and memory B cell formation<sup>[12]</sup>. Rarely, erythrophagocytosis by neutrophils and megakaryocytic emperipolesis has been reported<sup>[13]</sup>. Furthermore, it has been concluded that early treatment with Etoposide in EBV associated hemophagocytic lymphohistiocytosis syndrome may be the treatment of choice<sup>[14]</sup>.

Triad of infectious mononucleosis consists of fever, sore throat and lymphadenopathy. In addition, atypical lymphocytes and heterophil antibody may appear in blood. Rarely, unusual

symptoms, e.g. nausea, skin rash, diarrhea and epigastric discomfort may develop. Children, when first exposed may not develop symptoms (asymptomatic). Later in life, the patient may become a reservoir of EBV and spread may occur by saliva by "kissing". Fatigue is another common symptom. If the immunity is compromised, virus may replicate in an uncontrolled manner; causing lymphoma. Moreover, if latent infection develops then memory B cells may persist for life<sup>[15]</sup>.

### Conclusion (s)

A child aged 8 years had high fever and cervical lymphadenitis. He also had lymphocytosis. Blood smear examination showed apoptosis and hemophagocytosis; these features have been rarely described in EBV infection. Hematological examination showed atypical lymphocytes. RT-PCR for EBV DNA was positive. The patient was diagnosed as a case of infectious mononucleosis due to EBV infection.

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