Case Report

Chronic Active Epstein–Barr Virus Infection

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Chronic active Epstein-Barr virus (CAEBV) infection is a systemic Epstein-Barr virus (EBV) positive lymphoproliferative disease characterized by fever, lymphadenopathy, splenomegaly, unusual pattern of anti-EBV antibodies, and/or increased EBV genomes in affected tissues. Most cases are from Asia. So far, there is hardly any adult case reported from mainland of China. We herein presented a 33-year-old man with fever, facial erythema and rash, lymphadenopathy, lower limbs weakness, splenomegaly and liver lesion. EBV VCA, EA and EBNA were all positive. EBV DNA could be found in serum and PBMC. In situ hybridization of EBV encoded RNA in skin and liver biopsy was positive. Viral load in serum decreased under interferon alpha therapy. To our knowledge, it's the first adult case reported from mainland of China.

Key words: Epstein-Barr virus (EBV); Chronic active EBV infection (CAEBV); Infectious mononucleosis (IM)

Epstein-Barr virus (EBV) is a member of the family of herpes virus that was originally identified in a human Burkitt lymphoma cell line. It is now known that EBV infects more than 95% of adults worldwide.¹ Primary EBV infection is usually asymptomatic, only 10% of the patients present typical infectious mononucleosis. EBV remains in a latent state following primary infection. Persistent infection causes chronic active EBV infection (CAEBV) that characterized by chronic or recurrent infectious mononucleosis-like symptoms for over 6 months and presenting an unusual pattern of anti-EBV antibodies or EBV DNA in the blood and EBV RNA or protein in lymphocytes in tissues.² EBV had been detected in T, B, NK cells and epithelial cells of CAEBV patients.³ Although the mechanism of CAEBV has not been well recognized, discrepancy lymphocytes subtype plays a role in CAEBV. Increased number of NK cells was found in CAEBV patients from Japan. On the contrary, patients from USA had decreased NK cell numbers.⁴ The complicated pathogenesis of CAEBV results in therapy dilemma and disappointed prognosis.⁵

CAEBV is more prevalent in Asia,⁵ while there is no adult case report from mainland of China. We herein present a young male with CAEBV infection. The patient first showed in our hospital for his skin rash and weakness, the 2nd time for the intermittent fever, and the 3rd time for a mass in the middle of his upper abdomen. The symptoms and signs recurred in 2-year's follow-up. We also reviewed the literature about CAEBV infection.

CASE REPORT

A 33-year old male first showed (March 5, 2007) in our hospital for "intermittent facial erythema with systematic rash and fever for 1.5 years, deterioration with lower limbs weakness for a month". No-pitting facial erythema was obvious around his eyes and lips with burning pain around mouth. No such erythema was found apart from facial part. Red maculopapules intermittently appeared on his body and limbs, no pain and no itching. The facial erythema around his mouth and rash in his body were even worse for a month, accompanying lower limb weakness. He had been treated with "prednisolone 40 mg/day" and tapered for 3 months before admission, with no significant effect. Two weeks before admission, erythema around his mouth aggravated with pain, there were small blisters and several ulcerations in his mouth's mucosa and his tongue. His weakness became worse and worse, he could not walk for 1 week before admission. He reported a history of "tuberculosis" (treated for 6 months with quadruple therapy) 10 years ago and "perforation repairing" of his stomach 5 years ago. He denied other medication history, blood transfusion, and drug abuse or alcoholism. He had smoked for over 20 years, 20 cigarettes per day. He was clear mind and cooperated very well at examination. His temperature was 37.85°C, P 96/min, R 20/min, BP 125/70 mm Hg. Enlarged lymph nodes were found in bilateral cervical, submandibular, and axillary parts, which were soft and movable, no tenderness, and the diameter was around 1-2 cm. No-pitting erythema showed around eyelids, lips, and lower jaw. The dark red congestive rash could be seen all over his body, much intenser in his face and limbs with bran-like desquamation. Spleen was palpable under left costal arch (I line was 5 cm), tenderness and soft. Muscle force of bilateral distal lower limbs, proximal lower limbs, bilateral upper

Table 1. Blood test results of the patient during interferon alpha therapy

	ALT (U/L)	AST (U/L)	ALB (g/L)	ALP (U/L)	GGT (U/L)
Normal	0-40	0-45	35-50	40-160	0-50
1 st admission	47	37	31.6	333	95
Before IFN- α therapy	51	23	30	355	374
IFN- α therapy for 6 weeks	53	34	41	93	165
IFN- α therapy for 30 weeks	19	24	44.9	126	38

	LDH (U/L)	CK (U/L)	WBC ($\times 10^9/L$)	Lymphocyte (%)	PLT ($\times 10^9/L$)	RF (U/ml)
Normal	100-240	25-195	3.5-9.5	20-40	100-300	<30
1 st admission	295	181	2.85	18	142	27
Before IFN- α therapy	322	10	3.31	14	114	624
IFN- α therapy for 6 weeks	ND	ND	4.1	21.5	116	67.5
IFN- α therapy for 30 weeks	ND	85	3.7	11.4	150	ND

EBV-IgM	±	±		—
EBV-VCA-IgG	1 : 2560	1 : 20480		1 : 5880
EBV-EA-IgG	+	+		
EBV-NA-IgG	+	+		

Figure 1. EBV DNA level of serum and PBMC and antibodies.

Notes: Upper panel, EBV DNA was detected in patient serum and PBMC collected in different time point (real time PCR, the lowest detectable limit < 500 copies/ml). Lower panel, anti-EBV antibodies detected in the corresponding time point by ELISA. NA, not available.

limbs were respectively IV⁺, IV and V. Laboratory findings included slightly abnormal liver function, elevated LDH and leucopenia, as shown in Table 1. There were no positive findings in immunology examination, such as circulating immune complexes, IgA, IgM, IgG and autoantibodies (ANA, SMA, AMA, LKM-1). Negative reactions were confirmed in anti-HSV-IgM, anti-CMV-IgM, anti-parvovirus B19-IgM, anti-Coxsackie virus-IgM and hepatitis virus (A, B, C, E). Anti-EBV-IgM was weakly positive, which led us to investigate more: EBV DNA in serum was 2100 copies/ml (real time PCR, the lowest detection limit is 500 copies/ml), EBV-VCA-IgG was 1 : 2560, EBV-EA and EBV-NA were positive (Figure 1). Skin biopsy (April 13, 2007) showed: perivascular lymphocytic infiltration (Figure 2). Muscle biopsy (Figure 3) presented inflammatory cells infiltration with skeletal muscle fiber regeneration/atrophy. Type B ultrasound indicated splenomegaly (4.4 cm $\times$ 18.7 cm), spleen vein diameter (SVD) was 1.2 cm. His diagnose was "connective tissue diseases (possible dermatomyositis), chronic active EBV infection was

suspected". Prednisone (30 mg/day for 10 days, followed by 60 mg/d, and then tapered) combined with intravenous acyclovir (1.0 g/day for 22 days) was prescribed. After 22 days therapy, his body temperature was normal, his facial erythema and rash were significantly relieved. He could staggeringly walk and was discharged.

Forty days later (June 7, 2007), he was admitted because of "fever for 2 days". He had got a cold with high temperature (39°C) and catarrhal symptoms, significant hair loss for a month and anorexia for 10 days. Only slightly erythema around his mouth was found at examination. There were no obvious rash and no palpable superficial lymph nodes. Enlarged spleen was as the same as his first appearance. Muscle force maintained at grade IV. Liver biopsy (July 6, 2007) indicted EBV induced liver injury (Figure 4), which pointed us to carry out in situ hybridization in both liver and skin biopsy sample (Figure 5). Positive result for EBV encoded RNA (EBER) confirmed the diagnosis of CAEBV. "Interferon- α -2b, 3 MIU, 3 times a week" was given in order to suppress EBV

Figure 2. Skin biopsy sample in the first admission.

Note: Perivascular lymphocytic infiltration was found in patient's derma. Diffuse infiltration of inflammatory cells also be found in derma (hematoxylin-eosin staining, $\times 200$).

Figure 3. Inflammatory cells infiltration, muscle fibers regenerated and atrophy in muscle biopsy sample.

Note: hematoxylin-eosin staining, $\times 100$.

Figure 4. Inflammatory cell infiltrated in liver biopsy sample, with focal interface activity.

Note: hematoxylin-eosin staining, $\times 200$.

replication from June 22, 2007. Serum EBV DNA decreased slowly under interferon- α pressure for 30 weeks as shown in Figure 1, while EBV load in PBMC changed a little. Liver function was normal at the 10th week of interferon therapy. At the 17th weeks, muscle force recovered to grade V and his spleen shrank to the edge of left costal. He was discharged after 4 week interferon- α therapy on July 24, 2007.

Six months later (January 22, 2008), he was admitted to our hospital for "epigastrium pain for 2 weeks". Examination found a hard, round, unmovable, 4 cm $\times$ 5 cm mass in the middle of upper abdominal with tenderness. Gastroscopy revealed "gastrolith". Endoscopic lithotomy was done for 3 times and was discharged. He reported that he had eaten too

Figure 5. In situ hybridization of skin biopsy sample showed positive for EBV encoded RNA.

Note: hematoxylin-eosin staining, $\times 200$.

many "hawthorns" (at least 2 pounds within 2 hours) 2 weeks ago.

He was admitted to a local hospital for "hemorrhage of upper digestive tract" and died.

DISCUSSION

The diagnosis criteria of CAEBV infection had been updated for several times since its first description by Straus in 1988.⁶ The latest proposal characterized CAEBV infection as persistent or recurrent infectious mononucleosis-like symptom, high titer of anti-VCA and anti-EA, and increased EBV genomes in affected tissues.² The patient we here presented was a 33-year-

old man with intermittent fever, facial erythema and rash, lymphadenopathy, lower limbs weakness, splenomegaly and liver lesion. Antibodies against EBV VCA, EA and EBNA were all positive. EBV DNA was positive not only in serum but also in PBMC. In situ hybridization of EBV encoded RNA in skin and liver biopsy was positive. All of these evidence resulted in the diagnosis of CAEBV infection. Although most of the CAEBV infection cases are from Asia, there has not been any adult case reported from mainland of China.

Typical symptoms and signs combined with virological evidence are critical to confirm the diagnosis of CAEBV infection, while high titers of EBV-related antibodies are not always a prerequisite. Detection of EBV DNA in peripheral blood by PCR is one of the convenient methods that indicate active EBV replication.² Cell-free circulating EBV DNA (in patient's serum or plasma) could be detected in 9 of 14 fatal CAEBV patients (62%), which was much higher than nonfatal CAEBV patients (2/19, 11%), and infectious mononucleosis patients (2/18, 11%). None of the healthy controls got positive results.⁷ Those results raised the possibility that serum/plasma EBV DNA load could be one of predictors for CAEBV prognosis. Real-time quantitative PCR (RQ-PCR) was used to detect EBV DNA in whole blood samples from patients with EBV associated non-Hodgkin lymphoma (NHL).⁸ EBV DNA levels showed synchronous changes with disease severity: undetectable in complete remission patients compared to sustained or elevated levels in disease progress or recurrence patients. It must be cautious in interpreting the results of viral level detected by PCR. Different PCR methods using different primers and reaction conditions resulted in different sensitivity and specificity.

One of the other methods to support active EBV replication is in situ hybridization (ISH) of EBV encoded RNA (EBER) and immunofluorescence/immunohistological staining of EBNA/LMP.² A detailed standard protocol for detection of EBER in biopsy sample by ISH method was released in 2009.⁹ In liver biopsy samples from 8 patients with EBV hepatitis, 6 samples presented active EBV replication confirmed by both PCR for EBV DNA and ISH for EBER. On the contrary, immunohistochemical staining for EBV LMP gave negative results in all 8 samples.¹⁰ They claimed that ISH for EBER and PCR for EBV DNA were equally sensitive in detecting EBV replication in routinely processed liver biopsy sample. ISH for EBER in liver and skin biopsy samples strongly support active EBV replication in current case. We could not determine the interaction between EBV and decreased muscle force due to lack of biopsy sample. We speculated that EBV itself or EBV induced abnormal immunological status resulted in patient's weakness of his limbs.

Patient with CAEBV infection was incurable. Immunosuppressive agents, antiviral medicine (interferon- α or acyclovir), rituximab, autologous cytotoxic T cells and chemotherapy could only lead a short-term remission. The patient we reported here achieved improvement in his skin rash, fever, facial erythema, muscle force and

serum viral load under interferon- α pressure. However, the patient died of "hemorrhage of upper digestive tract" in the local hospital. We could not exclude the link between his death and chronic EBV infection. A meta-analysis demonstrated that 8.8% of the gastric carcinoma patients could find EBER by ISH from their tumor cells,¹¹ which indicted a directly association between gastric carcinoma and persistent EBV infection. An earlier study claimed that digestive tract ulcer/perforation is one of the mortality risk factors for CAEBV infection patients.¹²

Hardly any adult CAEBV reported from mainland of China, it is possibly not due to lack of patient, but lack of awareness. When patient presented with intermittent fever, rash, lymphadenopathy and splenomegaly, we could investigate whether there was chronic EBV infection by detecting anti-EBV antibodies and EBV genomes in affected tissues.² Further researches in pathogenesis of CAEBV must decrease the current high mortality.

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Competing interest

Jun Li and Shi-hong Li contributed equally. And the authors declare no conflict of interests.

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