Epstein-Barr Virus DNAemia in Iranian Liver Transplant Recipients and Assessment of Its Variation in Posttransplant Lymphoproliferative Disorder Patients by Quantitative Polymerase Chain Reaction Assay

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Abstract

Objectives: Epstein-Barr virus primary infection and/or reactivation may play a major role in the incidence of posttransplant lymphoproliferative disorder in organ recipients. We assessed Epstein-Barr virus viral load in liver transplant patients suspected of having Epstein-Barr virus/post-transplant lymphoproliferative disorder at specified times after transplant and evaluated the clinical findings and posttransplant complications.

Materials and Methods: In the 696 patients who underwent liver transplant in this retrospective study, Epstein-Barr virus viral load was examined intermittently in 127 liver transplant recipients who were suspected to have Epstein-Barr virus infection/disease. Sampling was performed during 4 years from July 2009 to May 2013 using real-time polymerase chain reaction assay. Clinical and pathologic data were gathered by reviewing medical records.

Results: There were 78 of the 127 suspected patients (61%) who exhibited Epstein-Barr virus DNAemia and 19 patients had posttransplant lymphoproliferative disorder. The median EBV viral load of posttransplant lymphoproliferative disorder patients was significantly higher than unaffected patients. Posttransplant lymphoproliferative disorder was diagnosed clinically in 34 subjects (4.9%). Estimated mortality rate of posttransplant lymphoproliferative disorder patients was 35% during 1.5-year follow-up after transplant.

Conclusions: Monitoring Epstein-Barr virus load may enable detection of Epstein-Barr virus infection/disease in liver transplant patients suspected of having the virus, even several weeks before the onset of any clinical manifestations, especially in pediatric patients who have high incidence and mortality from posttransplant lymphoproliferative disorder.

Key words: DNA, Hepatic failure, Immunosuppression, Virology

Introduction

Epstein-Barr virus (EBV) is a member of Herpesviridae family, Gammaherpesvirinae subfamily, and Lymphocryptovirus genus and is known as Human herpesvirus 4.1,2 It can infect smooth muscle cells, natural killer cells, B lymphocytes, and T lymphocytes. This ubiquitous virus 90% adults worldwide.3-5 It is associated with different malignancies and disorders such as Hodgkin lymphoma, non-Hodgkin lymphoma, Burkitt lymphoma, nasopharyngeal carcinoma,
gastric carcinoma, and posttransplant lymphoproliferative disorders (PTLDs). 

In immunocompetent individuals, EBV causes infectious mononucleosis, an acute but self-limited disease that affects children and young adults. Although the severity of infectious mononucleosis is not correlated with EBV viral load, immunocompromised patients such as patients undergoing organ transplant may have EBV DNAemia that may cause a variety of clinical conditions including a nonspecific viral syndrome, mononucleosis, or PTLD. 

The PTLD is a life-threatening condition that requires urgent treatment and occurs up to 10 years after transplant. The highest morbidity of this complication is within the first year after transplant, with a high incidence in the first 6 months. The disease is associated with a wide range of clinical symptoms and may range from a self-restricted proliferation to a severe fulminating disorder. It is classified histologically into 4 groups: (1) early lesions, (2) polymorphic PTLD, (3) monomorphic PTLD, and (4) classic Hodgkin-lymphoma-type PTLD.

Based on clinical and histologic features, the diagnosis of PTLD and its differentiation from organ rejection often are difficult, and use of a sensitive test such as real-time polymerase chain reaction (PCR) is recommended. The high EBV viral load in the plasma and/or peripheral blood mononuclear cells can serve as a reliable marker for development of PTLD. Different studies show a positive correlation between EBV viral load and severity of PTLD.

In this study, we quantified the EBV viral load by real-time PCR in patients after liver transplant who were suspected to have EBV/PTLD at the only center for liver transplant in Iran. We report demographic data and outcome of patients associated with PTLD.

Materials and Methods

Study setting, transplant patients, and samples
Medical records of 696 patients who underwent liver transplant at Nemazee Hospital, Shiraz, Iran, from July 2009 to May 2013, were examined retrospectively in this study. Liver transplant accounted for 75% transplants performed during this period at Nemazee Hospital. Routine immuno-suppressive regimen after transplant was triple therapy with tacrolimus, mycophenolate mofetil, and prednisolone.

There were 510 specimens in 127 patients that were assessed for EBV DNAemia at Professor Alborzi Clinical Microbiology Research Center, the major referral center for this examination. Sampling time varied in different patients based on clinical manifestations from 1 day to 1.5 years after transplant. Blood samples (average, 4 samples; range, 1-10 samples) were tested for suspected patients. Medical record review showed that 34 patients were affected by PTLD based on clinical data and World Health Organization criteria.

Nucleic acid extraction and measurement of Epstein-Barr virus viral load by real-time polymerase chain reaction
Viral DNA was extracted from 200 μL serum by a simple and effective column-based DNA extraction kit (Invisorb Spin Virus DNA Mini Kit, Stratagene Biomedical, Birkenfeld, Germany), according to instructions from the manufacturer. Real-time PCR was used as a sensitive and specific method to determine EBV serum sample loads against a serial dilution of a standard that had known EBV DNA content. To quantify EBV DNA, we used a commercially available system (7500 Real-Time PCR System, TaqMan platform, and PrimerDesign kit, Life Technologies, Carlsbad, CA, USA). Each 25 μL reaction volume contained: 1 × reagent (TaqMan Universal Master Mix), forward and reverse primers (15 pmol each), probe (TaqMan; 10 pmol), 5 μL DNA template, and water added up to 25 μL. Thermocycling conditions were 50°C for 2 minutes; 95°C for 10 minutes; 95°C for 15 seconds, and 60°C for 1 minute for 40 cycles. The sensitivity of the test was 10 copies/μL specimen.

Statistical analyses
Data analyses were performed with statistical software (SPSS for Windows, Version 16.0, SPSS Inc., Chicago, IL, USA). Statistical significance was defined by P < .05.

Results
The mean age of 696 patients undergoing liver transplantation was 28.53 ± 0.66 years (range, 1 mo to 75 y). The male: female ratio was 1.74 (442 males;
254 females). Underlying diseases leading to liver transplant were varied, most commonly end-stage hepatitis B virus infection (Figure 1). In 39% patients, the reasons for liver transplant were unknown.

The EBV genome was detected in 78 of 127 patients suspected of having EBV/PTLD, including 19 PTLD and 59 non-PTLD patients. A significant difference was observed between the median of maximum EBV loads in PTLD patients (4035 copies/mL) and non-PTLD patients (500 copies/mL; SE = [standard error]; P ≤ .05). Evaluation of the changes in EBV load in 8 PTLD patients (at least 5 measurements per patient) showed that the peak viral load varied between 13 and 360 days after transplant (Figure 2). In the PTLD cases, 13 patients were living and 6 patients died; in the non-PTLD patients, 57 patients were living and 2 patients died. A 2-year-old boy with maximum EBV load 234576 copies/mL was among the dead non-PTLD patients.

The incidence of PTLD was 34 patients in 696 transplant patients (4.9%); in 15 patients, there were no medical records with data about EBV load. In the 34 patients, 30 were aged < 12 years (88.2%). Death occurred in 11 of 34 patients (32%) and only 1 patient who died was an adult (Table 1).

According to histopathology reports, most lesions in the PTLD patients were not classified (77%) which 1 was associated with non-Hodgkin lymphoma (4.3%). Among the classified lesions, 6 were monomorphic PTLD including 5 diffuse large B-cell lymphoma (DLBCL) (22%) and 1 mucosa-associated lymphoid tissue (MALT) B-cell PTLD (4.3%). There was 1 Hodgkin lymphoma PTLD (4.3%), and no patients had polymorphic PTLD or early lesion (Table 1).

**Discussion**

There are 40,000 organ transplants performed annually worldwide, most frequently kidney, liver, lung, and heart transplant in decreasing order of frequency. In our center, the only referral center for liver transplant in Iran, liver transplant is performed more commonly than other organ transplants.

Widespread consumption of immunomodulating agents may occur to prevent organ rejection, but this may cause unwanted opportunistic infections including different parasitic, fungal, bacterial, and viral infections, particularly *Herpesviridae* members such as...
Table 1. Characteristics of Patients with Posttransplant Lymphoproliferative Disorder Lesions

<table>
<thead>
<tr>
<th>Complication After Liver Transplant</th>
<th>Patient Number</th>
<th>Mean Viral Load (No. of Samples)</th>
<th>Sex/Age (y)</th>
<th>Underlying Disease</th>
<th>Range of EBV Load</th>
</tr>
</thead>
<tbody>
<tr>
<td>PN1*</td>
<td>17 014 (6)</td>
<td>M/1</td>
<td>Tyrosinemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PN2+</td>
<td>17 014 (6)</td>
<td>M/1</td>
<td>Tyrosinemia/HCC</td>
<td>0-27 029</td>
<td></td>
</tr>
<tr>
<td>PN3*</td>
<td>17 014 (6)</td>
<td>M/1</td>
<td>Tyrosinemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PN4*</td>
<td>3 000 (2)</td>
<td>M/2</td>
<td>Biary ata</td>
<td></td>
<td>1500-3000</td>
</tr>
<tr>
<td>PN5*</td>
<td>26 412 (8)</td>
<td>M/2</td>
<td>Crigger-Naj</td>
<td></td>
<td>0-100 000</td>
</tr>
<tr>
<td>PN6</td>
<td>35 000 (3)</td>
<td>M/2</td>
<td>F5</td>
<td></td>
<td>0-200 000</td>
</tr>
<tr>
<td>PN7</td>
<td>57 369 (5)</td>
<td>M/2</td>
<td>F5</td>
<td></td>
<td>0-200 000</td>
</tr>
<tr>
<td>PN8</td>
<td>0 (1)</td>
<td>F/8</td>
<td>Biary ata</td>
<td></td>
<td>0-286 844</td>
</tr>
<tr>
<td>PN9</td>
<td>3 497 (4)</td>
<td>M/5</td>
<td>Biary ata</td>
<td></td>
<td>0-16 234</td>
</tr>
<tr>
<td>Unclassified PTLD (n = 26) (74.4%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PN11</td>
<td>0 (1)</td>
<td>F/3</td>
<td>BudChiari</td>
<td></td>
<td>0-4370</td>
</tr>
<tr>
<td>PN12</td>
<td>-</td>
<td>M/2</td>
<td>Tyrosinemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PN13*</td>
<td>1 (0)</td>
<td>F/9</td>
<td>Crigger-Naj</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PN14*</td>
<td>1 (0)</td>
<td>F/9</td>
<td>Crigger-Naj</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PN15*</td>
<td>1 (598 266)</td>
<td>M/3</td>
<td>Biary ata</td>
<td></td>
<td>598 266</td>
</tr>
<tr>
<td>PN22*</td>
<td>35 000 (3)</td>
<td>M/2</td>
<td>F5</td>
<td></td>
<td>0-200 000</td>
</tr>
<tr>
<td>PN30*</td>
<td>2500 (1)</td>
<td>M/2</td>
<td>Wilson</td>
<td></td>
<td>2300</td>
</tr>
<tr>
<td>PN31*</td>
<td>40 240 (9)</td>
<td>M/2</td>
<td>PHC</td>
<td></td>
<td>0-198 720</td>
</tr>
<tr>
<td>DLBCL (n = 5) (22%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PN11</td>
<td>2300 (1)</td>
<td>F/8</td>
<td>Wilson</td>
<td></td>
<td>2300</td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma (n = 1) (4.3%)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PN31*</td>
<td>40 240 (9)</td>
<td>F/8</td>
<td>Wilson</td>
<td></td>
<td>0-198 720</td>
</tr>
<tr>
<td>Hodgkin-lymphoma-type PTLD (n = 1) (4.3%)</td>
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</tbody>
</table>

**Abbreviations:** DLBCL, diffuse large B-cell lymphoma; EBV, Epstein-Barr virus; F, female; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; M, male; MALT, mucosa-associated lymphoid tissue; PHC, progressive familial intrahepatic cholestasis; PTLD, posttransplant lymphoproliferative disorder

*These patients died.

cytomegalovirus, and EBV. At present, it is proven that quantitative PCR is important in the early diagnosis of EBV-associated disorders, even several months before the onset of clinical manifestations. In the present study, according to the protocol in our center, EBV load was assessed in 127 suspected patients of 696 patients who received liver organ transplant between 2009 and 2013. The EBV loads in these patients were monitored 1 to 10 times after transplant, based on the clinical presentations. The data showed that the median of maximum viral loads were significantly higher in the PTLD patients (4035 copies/mL) than nonaffected patients (500 copies/mL), consistent with several previous studies.

As we detected in this study, a wide range of EBV loads were observed in liver transplant patients. This was because of involvement of multiple host factors such as age of transplant, active primary infection, different underlying disease, immunosuppressive drug regimen, and intensity. Therefore, it is more acceptable to monitor EBV load than consider a cutoff value to treat patients, as recommended in other studies.

In 8 PTLD patients who had serial viral loads available, large variation in maximum load (4370 to 286 844 copies/mL) and time of maximum load (13-360 d after transplant) were observed, which is consistent with other studies.

Infection with EBV causes a wide range of clinical manifestations including a nonspecific viral syndrome, infectious mononucleosis, and PTLD. The PTLD, which is the most important EBV-associated disorder, can be prevented by modulation of the immune system, autologous expanded T-cell infusion, and/or use of anti-EBV drugs such as rituximab. The incidence of PTLD in liver recipients in this study was 4.9% (34 of 696 patients); this is in agreement with other studies (1.6%-15%). In another study conducted in our center from 2003 to 2010, the incidence of PTLD was 0.9% (5 patients with PTLD in 550 liver transplant
patients). This difference may be explained by the use of more potent immunosuppressive regimens in the later years and higher index of suspicion of our clinicians to diagnose PTLD.

The mortality of our patients with PTLD was high (11 of 34 patients (35%)), which is within the reported range of 15% to 55%. High mortality indicates that current treatment modalities such as immunomodulation and antiviral therapy did not reverse the immunopathologic background that caused PTLD. The mortality rate of PTLD among liver transplant recipients was somehow similar to those in previous report [11 of 696 patients (1.58%) vs (3 of 550 patients (0.54%)].

As shown in several studies, the incidence of PTLD is higher in pediatric than adult liver transplant patients. The Scientific Registry of Transplant Recipients (SRTR) reported that 82% of PTLDs occur in children aged <17 years. In the current study, more than 88% (30 of 34 patients) of PTLD cases occurred in children aged <12 years. This may have been caused by EBV seronegativity, primary infection occurring from a latent EBV-infected donor passenger leukocyte, or by close contact with healthy individuals in the community after transplant.

Although it is difficult to subcategorize PTLD lesions, the most common PTLD lesion in our patients was monomorphic (5 of 34 patients), which is similar to data from other studies including a previous study in our center (3 of 5 patients). A previous study reported that the monomorphic type occurred in 31%, polymorphic type in 19%, and hyperplastic form of early lesions in 1% patients.

Because the highest PTLD incidence and mortality were seen in pediatric liver transplant recipients, timely diagnosis of this complication before appearance of clinical symptoms is important. Preventing PTLD by monitoring EBV loads after transplant should be considered, especially in infants, young children, and other patients who are at high risk of developing EBV infection.

References


