Quantitative polymerase chain reaction for detection of human herpesvirus-7 infection in umbilical cord blood donors


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Abstract: Objective. Umbilical cord blood (UCB) has been a reasonable alternative to granulocyte colony-stimulating factor-mobilized peripheral blood or bone marrow, as a source of hematopoietic stem cells with a lower risk of graft-versus-host disease. In immunocompromised hosts after transplantation, the risk of viral infection in adults, especially with beta-herpesviruses such as human herpesvirus-7 (HHV-7), may be increased. This virus in immunocompromised patients can be reactivated from latency and converted to an active phase. Therefore, light-upon-extension real-time polymerase chain reaction (PCR) was developed to assess the prevalence and load of HHV-7 in the plasma and buffy coat of donors.

Methods. About 825 UCB samples under standard protocol from donors were collected. Then, DNA from plasma and buffy coat was extracted and quantitative real-time PCR was performed with light-upon-extension primers.

Results. Overall, HHV-7 was detected in 3.64% (30/825) of UCB donors. HHV-7 DNA was detected in 26 (3.2%) buffy coat samples (latent infection), and only 4 (0.48%) of them were positive for HHV-7 DNA in plasma samples (active infection); the mean HHV-7 viral load was $1.31 \times 10^4$ copies/mL in latent infection, and $1.94 \times 10^5$ copies/mL in active infection.

Conclusions. We suggest that real-time PCR in plasma and buffy coat could be a useful method to detect active and latent HHV-7 infection in UCB donors and determine its role in subsequent transmission events.

Since the first successful umbilical cord blood (UCB) transplantation for a patient with Fanconi anemia, UCB has been recommended as a reasonable alternative to granulocyte colony-stimulating factor-mobilized peripheral blood or bone marrow as a source of hematopoietic stem cells (HSC) (1–6). Although UCB is less available, it is a valuable source for earlier stage of HSC and progenitor cells to restore the hematopoietic system in transplant patients (7, 8). In addition, UCB transplantation is associated with lower rates of graft-versus-host disease, one of the main factors of transplant-related mortality.

Abbreviations: CMV, cytomegalovirus; HHV, human herpesvirus; HSC, hematopoietic stem cells; PCR, polymerase chain reaction; UCB, umbilical cord blood
Beta-herpesviruses are widespread pathogens with high seroprevalence in the adult population (9). UCB transplantation is associated with lower risk of transmission of highly prevalent persisting viral infections, such as Epstein–Barr viruses, cytomegalovirus (CMV), and human herpesvirus (HHV)-6A and -6B (10–12). However, little is known about HHV-7 (13–15). These viruses in immunocompromised patients can be reactivated from their latent state (10). Some authors have assumed a potential rise in virulence of HHV-7 during a simultaneous CMV reactivation, resulting in a greater danger of CMV illness after transplantation (14, 16). HHV-7 infection may impair the differentiation and survival of megakaryocytic cells (15) and also affect survival/differentiation of CD34+ hematopoietic progenitor cells (17).

The aim of this study was to investigate the prevalence of HHV-7 infections by using a light-upon-extension real-time polymerase chain reaction (PCR) (LUX Real-Time PCR) in UCB samples as a source of HSC transplantation.

Material and methods

Clinical specimens

In this study, 825 UCB samples from pregnant women with a mean age of 26.5 (range 17–35) years were included. Informed consent was obtained from all subjects, and UCB samples were gathered in Milad Hospital, Tehran, Iran and Shoshtari Hospital, Shiraz, Iran. All mothers passed routine lab screening tests for pregnancy. The exclusion criteria were the existence of any of following conditions: (i) systemic diseases (e.g., diabetes mellitus and autoimmune conditions); (ii) infectious diseases (e.g., patients at high risk for human immunodeficiency virus infection or acquired immunodeficiency syndrome; existence of malaria fever in the past 3 years or taking anti-malaria treatment in the past 6 months; or hepatitis C virus antibody or hepatitis B surface-antigen positivity); (iii) any history of malignancy except for skin and cervix; (iv) history of tattooing in the past year; or (v) organ transplantation.

DNA extraction

In the K2EDTA tube, 10-mL samples of blood were collected from UCB, and DNA was extracted from buffy coat and plasma of the UCB samples using the High Pure Viral Nucleic Acid extraction kit (Roche, Mannheim, Germany) according to the manufacturer’s instructions.

Preparation of plasmid standard DNA for HHV-7

An HHV-7 DNA fragment of approximately 312 base pair (bp) (nucleotides 138976-139287; GenBank accession No. AF037218) in the U95 gene region was selected and synthesized. Then, to construct a plasmid DNA containing the U95 gene region of HHV-7 as a reference for the quantitation of HHV-7, the amplified 312-bp product was cloned into the pCRII plasmid by using the TA cloning kit (Invitrogen Corp, San Diego, California, USA), according to the manufacturer’s instructions and transformed into Escherichia coli TG1. To confirm cloning, recombinant plasmid pCRII-HHV-7 was purified and sequenced by using the BigDye Terminator version 3.1 Cycle sequencing kit (Bioneer Corp, Daejeon, South Korea). The concentration of the plasmid DNA containing the U95 gene region of HHV-7 was quantified by using a Nano-drop Spectrometer (Thermo Scientific, Waltham, Massachusetts, USA). Copy number of cloned plasmid was calculated according to a formula in the Qiagen QuantiFast Probe PCR Handbook (www.qiagen.com/resources/download.aspx?id).

The sensitivity of this method was determined with serial dilutions (1 × 10^1 – 1 × 10^7 copies/mL) of the plasmid DNA containing the U95 gene of HHV-7. The detection limit of this assay was 10 copies/mL.

Real-time PCR for HHV-7

Reaction mix consisted of 1X QuantiFast probe PCR Master Mix without Rox, Lux perimers (forward 139002-139023:5'TCCAACCACAGTTAGCGTTGT3' (reverse 139096-139078:5'CGGGCCTGTCAATGGATAACTCC (FAM) G3') (13); 5 μL extracted DNA was added to 15 μL Master Mix. Thermal profile was optimized for the 7500 Real-Time PCR System (Applied Biosystems/Life Technologies, Grand Island, New York, USA) as follows: 1 cycle of denaturation at 95°C for 3 min, followed by 40X (cycle) of amplification at 95°C for 15 sec, and 60°C for 60 sec. Melting curve analysis was done according to the ABI Real-Time PCR default.

Results

Overall, HHV-7 was detected in 3.64% (30/825) of UCB donors. HHV-7 DNA was detected in 26 (3.2%) buffy...
coat samples (latent infection), and only 4 (0.48%) of them were positive for HHV-7 DNA in plasma samples (active infection), which indicates the presence of latent and active HHV-7 infections in donors, respectively. In this study, the quantitative assay of HHV-7 viral load showed a mean of $1.31 \times 10^3$ copies/mL and $1.94 \times 10^2$ copies/mL in latent and active infections, respectively.

### Discussion

UCB transplantation is increasingly used in children, because the risk of graft-versus-host disease is lower compared with unrelated bone marrow transplantation. Concern exists that a higher risk of opportunistic infections is transferred with UCB. Therefore, some common infections, such as HHV, are checked serologically.

In immunocompromised hosts and in primary infection, the pathogenicity of HHV-7 remains unclear. Interestingly, a potential increase in virulence of both roseoloviruses (HHV-6, HHV-7) in the course of a simultaneous CMV reactivation has been postulated (16). Chapenko et al. (14) showed an association of latent HHV-7 with development of a febrile syndrome post kidney transplantation in 2 patients. This syndrome was independent to polyclonal anti-thymocyte globulin treatment. The risk of reactivation of CMV increased with co-infection of HHV-7 and CMV up to 2.2-fold for HHV-7 and 12-fold for CMV, compared with each of these infections alone (14). Chapenko et al. (14) also demonstrated that HHV-7 should be the cofactor for CMV disease progression, and they believed that dual (CMV and HHV-7) infection is a risk factor for CMV disease. Therefore, they suggested that “Screening diagnosis should include testing for both viral infections in transplant donors as well as in recipients before and after renal transplant” (14).

HHV-7 infection impairs the differentiation and survival of megakaryocytic cells (15). Studies by Miranda et al. (17) indicate that HHV-7 infection may affect survival/differentiation of CD34+ hematopoietic progenitor cells by inhibiting more progenitor cells and disturbing the maturation of myeloid cells. These findings may be important for groups at high risk of HHV-7 infection, such as UCB transplant patients, developing fetuses, or recipients of solid organ transplant or bone marrow transplant.

In 2005, Weinberg et al. (18) studied 362 mononuclear cells and sera from UCB. The incidence of HHV-7 in these samples was zero, which means that none of the samples were positive for HHV-7 DNA. However, among 825 UCB donor samples in our study, 26 (3.2%) were positive for HHV-7 DNA by real-time PCR in buffy coat as a latent infection, and 4 (0.48%) were positive for HHV-7 DNA by real-time PCR in plasma, representing active infection. The prevalence found in this study was different from that in other reports. These differences could be a result of the sample size, PCR sensitivity, or economic status and sanitary situation of the sample donors.

Based on our results, and considering the role of HHV-7 as an opportunistic infection in immunocompromised patients in inhibition of myeloid cell maturation and reactivation of CMV in these patients, we suggest that real-time PCR in plasma and buffy coat could be a useful marker to detect active and latent HHV-7 infection in UCB donors.

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### References


