Indian Journal of Medical Microbiology, (2008) 26(3): 252-55

Brief Communication

INCIDENCE OF HUMAN HERPES VIRUS-6 AND HUMAN CYTOMEGALOVIRUS INFECTIONS IN DONATED BONE MARROW AND UMBILICAL CORD BLOOD HEMATOPOIETIC STEM CELLS

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Abstract

This study examined the incidence of human herpes virus-6 (HHV-6) and human cytomegalovirus (HCMV) infections that are potentially transmitted to haematopoietic stem cells (HSC) transplant recipients via bone marrow (BM) or umbilical cord blood (UCB). Bone marrow progenitor cells were collected from 30 allogenic BM donors. UCB HSC were collected from 34 subjects. The extracted DNA was then processed using nested polymerase chain reaction (nPCR) technique. HCMV and HHV-6 serological status were determined by enzyme immunoassay (EIA). Nested PCR identified HCMV in 22 (73%) of 30 samples of BM progenitor cells but in only eight (23.5%) of 34 samples of UBC HSC ($P = 0.001$). HHV-6 DNA was detected in 11 (36.6%) of 30 BM progenitor cells and in only one (2.9%) of 34 UBC cells ($P = 0.002$). Both HHV-6 and HCMV infections were determined in nine (26.5%) of 34 bone marrow samples. The results indicate that, the risk of HCMV and HHV-6 via BM progenitor cells is higher than transmission by UCB cells ($P = 0.04$).

Key words: *BM donors, HCMV, haematopoietic stem cells, HHV-6, nested PCR*

Bone marrow transplantation (BMT) is a special therapy for patients with cancer or other diseases which affect the bone marrow.[1-4] The goal of BMT is to transfuse healthy bone marrow cells into a person after their own unhealthy bone marrow has been eliminated. BMTs are classified as either autologous or allogeneic, based on the source of the haematopoietic stem cells.

In allogeneic transplantations, the stem cells are harvested from a donor patient who is other than the recipient of the BMT. Viral infection of allograft is a serious problem in BMT, for it increases the risks of post-transplant morbidity and mortality.[5] Those most commonly seen in BMT patients are caused by the herpes viruses. The incidence of latent viral infections tends to increase throughout the adult years, so these samples are at greater risk for virus transmission from donor bone marrow (BM). As the number of patients undergoing bone marrow transplantation and, consequently, immunosuppressive therapy rises, the number of individuals at risk from herpes virus infections is increasing.

Human cytomegalovirus (HCMV) and human herpesvirus-6 (HHV-6) infections are common in the general population. Both viruses establish latency in the host and can be reactivated in immunocompromised individuals such as recipients of bone marrow transplant. In immunocompromised individuals, HCMV can cause severe disseminated disease characterized by chorioretinitis, pneumonia, esophagitis, colitis, myelitis, meningitis, encephalitis, leukopenia, lymphocytosis, and hepatitis.[6] HHV-6 infection on the other hand, is associated with bone marrow suppression, interstitial pneumonitis, encephalitis and graft versus host disease (GHVD).[7,8] Human herpesvirus-6 may predispose the transplant recipient to opportunistic infection with CMV or other infectious pathogens as well.[9,10]

Placental or umbilical cord blood (UCB) obtained immediately after birth has been used to harvest stem cells for transplantation.[2]This is primarily being used for allogeneic transplantations in children. The main advantages of UCB over bone marrow stem cells are the relative ease of procurement, the absence of donor risk, the greatly reduced risk of transmitting infection, and the rapid availability of the sample.

In a previous study, we have shown the risk of viral transmission (HCMV in particular) via BM progenitor cells from allogenic bone marrow donors to the recipient individuals.[11] However, using simple polymerase chain reaction (PCR) we were not able to detect HHV-6 genome in any type of clinical samples of the BM donors. In this connection, using different sets of primers and nested PCR, this study examined the incidence of HHV-6 and HCMV infections which might be potentially transmitted to haematopoietic stem cells (HSC) transplant recipients via BM or UCB haematopoietic cells.

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Received: 02-08-07
Accepted: 24-09-07

Materials and Methods

Patients and samples

Specimens of plasma, peripheral blood leukocytes, and BM progenitor cells were obtained from 30 allogenic Bone marrow (BM) donors at Namazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran. Fourteen individuals were men, 16 were women of overall mean age of 24 years. A physician collected each BM specimen into transport medium. From each of 34 subjects a UCB sample collected using a semi-closed system syringe, was transferred to an EDTA tube. UCB haematopoietic stem cells in each sample were isolated using the ficoll-hypaque density-gradient method (Ficoll, Lymphoprep, Nycomed, Netherlands). All the collected samples were frozen at -70°C to be used for DNA detection by polymerase chain reaction (PCR) and enzyme immunoassay (EIA).

Nucleic acid extraction

For each of the three different donor specimens (plasma, peripheral blood leukocytes, and BM progenitor cells) and the UCB haematopoietic stem cell specimens, guanidine thiocyanate (GuScN)) and phenol-chloroform DNA extraction method was used to isolate the total viral DNA. The extracted DNA was then processed using nested PCR technique to detect HHV-6 and HCMV DNA.

PCR amplification

Using specific oligonucleotide primer set, selected from a highly conserved regions of immediate early gene of HCMV and major capsid protein gene of HHV 6 and a thermal cycling program nested PCR assays was preformed to amplify the genomes of the viruses of interest in the BM donor and UCB specimens. The nested primer set, complementary to the gene coding the major capsid protein was used with the aim of recognizing both HHV-6A and HHV-6B variants. The nucleotide sequence of the primers 5'-GCTAGAACGTATTTGCTG-3' and 5'-ACAACTGTCTGACTGGCA-3' (outer pairs); 5'-TCACGCACATCGGTATAT-3' and 5'-CTCAAGATCAA CAAGTTG-3' (inner pairs) were used for the first and the second round of the PCR assay, respectively. The inner primers amplify a 167-bp fragment of HHV-6. The nucleotide sequence of the primers 5′-GTCTACGGATTGCTGACGCT-3' and 5′-TTGCAGGCCACGAAC GT-3′ (outer pairs); 5′-ACCGCTTTCAGCGTACTCAT-3′ and 5′-ACATACAGCG CAAAC ACCAG-3′ (inner pairs) were used for the first and the second round of the HCMV-PCR assay, respectively. The inner primers amplify a 179-bp fragment of HCMV IE gene. Each PCR contained 5 μL of extracted DNA in a final volume of 50 μL with PCR buffer (Fermentas, Lithuania), 0.2 mM of each deoxyribonucleoside triphosphate, 1.5 mM $MgCl_2$, 2U Taq DNA polymerase (Fermentas, Lithuania) and 0.5 μM of each specific primer. Plasmid DNA (kindly provided by Dr. Hans H. Hirsch, Division of Infectious Diseases, University of Basel, Switzerland) was used as the positive control. To exclude the possibility of contamination during the PCR, HHV-6 and HCMV negative DNA, as well as water controls, were included in each experiment. The first round of HHV-6 PCR was carried out at 94°C for 3 minutes, followed by 30 cycles of 94°C for 40 seconds, 51°C for 1 minute, and 72°C for 40 seconds. Terminal extension of 72°C for 5 minutes was carried out after the completion of the 30 cycles. A sample of the first round product (3 μL) was used as template for the second round using the conditions described for the first round.

The first round HCMV-PCR was carried out at 94°C for 3 minutes, followed by 30 cycles of 94°C for 40 seconds, 61°C for 40 seconds, and 72°C for 40 seconds respectively. Terminal extension of 72°C for 5 minutes was carried out after the completion of the 30 cycles. A sample of the first round product (3 μL) was used as template for the second round using the conditions described for the first round.

Detection of amplified products

Conventional gel electrophoresis and ethidium bromide staining were then used to analyze the PCR product

Determination of the PCR sensitivity

To assess the sensitivity of the PCR assay, template DNA was extracted from 10-fold serial dilutions of plasmid DNA that had been spiked into samples that were negative for the viral DNA being investigated in the study.

Anti-HHV-6 IgG antibody

IgG antibodies to HHV-6 in serum samples were determined by EIA according to manufacturer's instruction (Biotrin, Dublin, Ireland). The presence or absence of HHV-6 antibodies was determined in relation to the cut-off calibrator. An index value was calculated with dividing the sample or control absorbance by the cut-off calibrator (COC) value. An index value <0.9 was calculated to be negative, an index value between 0.9-1.1 was equivocal and an index value >1.1 was calculated to be positive.

Anti-HCMV IgG antibody

IgG antibodies to HCMV in serum samples were determined by EIA according to manufacture instruction (Genesis, England). The expected optical density (OD) for value for the negative and positive samples were <3 IU/mL and ≥/= 3 IU/mL respectively.

Statistical analysis

All statistical testing was done using SPSS software version 11.0. *Chi-square* analysis and the two-tail Fisher exact test were used to analyse differences between results

Table: Comparison of HCMV and HHV-6 DNA detection in samples collected from BM and UCB donors

Type of isolated DNA	Bone marrow donors *n* = 30 (%)	UCB cells *n* = 34 (%)	*P* value
HHV-6	11/30 (36.6)	1/34 (2.9)	0.002
HCMV	22/30 (73.3)	8/34 (23.5)	0.001
Both HCMV and HHV-6	9/30 (26.5)	0/34 (0)	0.005

HPSC = Haematopoetic stem cells; ND = Not done

obtained by PCR method.

Results

Sensitivity of the PCR assay

When evaluated by gel electrophoresis and ethidium bromide staining, the detection limit of the PCR was found to be 350 molecules of HHV-6 plasmid DNA and 200 molecules of HCMV plasmid DNA in 100 μL samples.

Clinical sample

Nested PCR identified HCMV DNA in 22 (73.3%) of 30 samples of BM progenitor cells and in 9 (30%) of the 30 BM buffy coats. HCMV DNA was detected in only 8 (23.5%) of the 34 samples of UCB haematopoietic stem cells which is statistically significant when compared with BM progenitor cells ($P = 0.001$). The ratio of HCMV DNA positivity in male BM donors' samples versus female donors was 1.4:1. HHV-6 DNA was detected in 11 (36.6%) of 30 BM progenitor cells collected from seven males and four females, but in only one (2.9%) of 34 UBC cells ($P = 0.002$). Both HHV-6 and HCMV DNA were determined in nine (26.5%) of 34 bone marrow samples, but in any UCB haematopoietic stem cells. Seroprevalence of HHV-6 and HCMV positivity among BM and UCB donors group were 98% and 75% respectively. The results are presented in the table including previous results of other viral DNA detection in UCB and BM samples.

Discussion

Viral infections are a major barrier to transplant success. Implantation of an infected graft is the most important problem faced by patients undergoing BMT because these individuals are already at increased risk for viral infection. Careful BMT donor screening and testing are critical to exclude potential BM donors with a viral infection. Cord blood is a feasible alternative source of haematopoietic stem cells for BMT. The low rate of viral infection at birth is one of the obvious advantages of using these cells for transplantation. Analysis of the specimens in this study together with previous data[11] revealed that the BM donors had a much higher frequency of viral DNA with any of the viruses tested than the UCB donors. Seroprevalence of HHV-6 and HCMV infections among adult population were 75% and 98% respectively. Considering the different sets of specimens we studied, and using DNA amplification method, we found the highest rate of viral DNA was HCMV in BM progenitor cells (73%). It has been shown that the level of HHV-6 DNA in healthy volunteers is low.[12] Simple PCR assay was not able to detect HHV-6 DNA in BM or UCB samples[11] however, using different set of primers and nested PCR, HHV-6 was detected in 36.6% of BM progenitor cells and in 2.9% of UCB haematopoietic stem cells ($P = 0.002$).

It has been reported that physical and psychological stress resulted in decreased virus-specific T-cell immunity and reactivation of EBV.[13] Based on this report, we assume that the higher frequency of viral DNA in BM donors than UCB donors is the result of psychological stress prior to transplantation. This can certainly cause latent viruses to become active. The ratio of HCMV DNA detection in the male BM donors versus the female BM donors was 1.4:1 suggesting infection with HCMV is gender independent. Both HHV-6 and HCMV infections were determined in 9 (26.5%) of 30 bone marrow samples. In conclusion, the results indicate that, the risk of HCMV and HHV-6 via BM progenitor cells is higher than transmission by UCB cells. Our results also indicate that the risk of viral transmission might be greater with male BM donors than with female BM donors. Considering significant risk of viral transmission through BM progenitor cells, we recommend UCB for haematopoietic stem cells transplantation.

Acknowledgement

This project was financially supported by the Shiraz University of Medical Sciences, Shiraz, Iran.

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Source of Support: Nil, **Conflict of Interest:** None declared.