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Transfusion-Transmitted Cytomegalovirus Infection (TT-CMV): A Pilot Study on Safety of Whole-Blood Derivatives

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Abstract

Background: Transfusion-transmitted cytomegalovirus infection (TT-CMV) is known to cause significant morbidity and mortality in immunosuppressed patients particularly among allograft recipients and infants born with birth weights less than 1.5 kg.
Objectives: This is the first report in Iran showing the prevalence of CMV-DNA in whole blood/red cell components to evaluate safety for patients.
Patients and Methods: 153 units of whole blood or red cell components [CPDA1 RBC (n = 88), washed RBC (n = 50), whole blood CPDA1 (n = 1), whole blood low volume (n = 1) and leukocytes reduced (n = 13)] were selected for the presence of CMV-DNA from two different hospitals in Gorgan. Detection of CMV-DNA in plasma was performed by nested polymerase chain reaction (Nested PCR) using specific primers selected from highly conserved regions of major capsid protein (MCP) gene of human cytomegalovirus. In addition, CMV-IgM antibody of plasma was analyzed by serological methods. Data was analyzed using SPSS software (version 18).
Results: Totally, 2 of 153 (1.3%) whole blood or red cell components had positive results for CMV infection. Both viremia and anti-IgM CMV positivity were 0.65% (1/153), respectively. CMV-DNA was detected in 2/88 CPDA1 RBC, but not in other products.
Conclusions: Unscreened whole-blood derivatives can act as a vehicle for transmission of CMV infection, thus, screening for cytomegalovirus infection should be performed at least for special groups of patients.

Keywords: Blood Products, Cytomegalovirus, Iran

1. Background

Red blood cell (RBC) transfusion is necessary for prevention and treatment of a variety of life-threatening injuries and diseases. However, contamination of these products with viruses is a great threat to patients (1).

Due to implementation of a variety of strategies such as careful selection of donors and routine screening of blood donors (whole blood) and plasma derivatives, the risk of transmission of blood-borne viruses, especially HBV, HCV and HIV, through plasma and plasma products has been greatly reduced or eliminated in many areas of the world (1).

In developing countries, screening to improve the virus safety of whole blood or red cell components is limited to above viruses and screening of cytomegalovirus (CMV) antibodies is performed only for special groups of patients (1).

Cytomegalovirus (CMV) is a ubiquitous DNA virus belonging to the herpes family infecting people from different age groups, races and socioeconomic conditions. It is transmitted by direct person-to-person contact, through tissue and organ transplantation and via transfusion of blood products (2).

Cytomegalovirus infection is usually asymptomatic or self-limiting, but the risk of acquiring the infection through blood transfusion from asymptomatic donors is a major problem for immunocompromised CMV seronegative patients (3).

2. Objectives

The current prevalence of CMV transfusion transmission in Iran is unknown, thus the present study was designed to determine the frequency of CMV infection in red cell products in our area, Gorgan.

3. Patients and Methods

3.1. Study Design

This pilot study was conducted by the department of virology, Golestan university of medical sciences, Gorgan,

Iran, between October and November 2013. The study protocol was approved by ethics approval committee of faculty of medical sciences, Golestan university of medical sciences with code of 20199207149 on September 2013.

153 units of whole blood or red cell components [CPDA1 RBC (n = 88), washed RBC (n = 50), whole blood CPDA1 (n = 1), whole blood low volume (n = 1) and leukocytes reduced (n = 13)] were selected to assess the presence of CMV-DNA from two different hospitals in Gorgan. Plasma from all 153 units were aliquoted and stored at −70°C until processing.

3.2. Serological Markers

CMV-IgM antibody of plasma was measured by an enzyme linked immunosorbent assay (IgM-Dia.Pro Inc; third generation ELISA kit-Italy). Results were interpreted precisely, according to the manufacturer's instructions.

3.3. DNA Extraction

Briefly, DNA was extracted from 200 μL of EDTA (Merck, Germany) anticoagulated plasma by a commercially available kit (high pure extraction kit; Roche diagnostics GmbH, Mannheim, Germany). Negative (CMV-DNA negative plasma) and positive (CMV-DNA positive plasma) controls were included in each run.

3.4. Nested- PCR Reaction

polymerase chain reaction (PCR) amplification was performed for the presence of CMV-DNA with the primers pairs 5'-GAGCGCGTCCACAAAGTCTA-3' and 5'-GTGATCCGACTGGGCGAAAA-3' (264 bp) from highly conserved regions of major capsid protein (MCP) gene of human cytomegalovirus; NCBI Reference Sequence: M25411.1, as described elsewhere (4).

Polymerase chain reaction procedure was carefully optimized. The first-round PCR amplification was performed in a 25 μL reaction volume, containing 1μL (1.5 - 2.5 μg) DNA, 10 pmol of each of the universal outer forward and reverse primers, 0.1 mM of each deoxynucleotide (dNTP) (Genet Bio (A type), Korea), 2.5 U of Taq DNA polymerase (Genet Bio (A type), Korea), 2.5 mM $MgCl_2$ (Genet Bio (A type), Korea) and 2.5 μL 10X PCR buffer (Genet Bio (A type), Korea). In the samples with low or undetectable concentration of CMV DNA, 1 μL of reaction, as template, was re-amplified in a second round of PCR with the same primers. Reactions were performed in a Peq Lab thermal cycler (Primus Advanced 96 thermal cycler, USA) programmed as follows; 95°C for five minutes; 35 cycles of 94°C for 40 seconds, 50°C for 20 seconds, 72°C for 20 seconds and 72°C for 2 minutes.

3.5. Detection

PCR products were electrophoresed in a 2% Agarose gel, stained with ethidium bromide and visualized by UV illuminator (Figure 1).

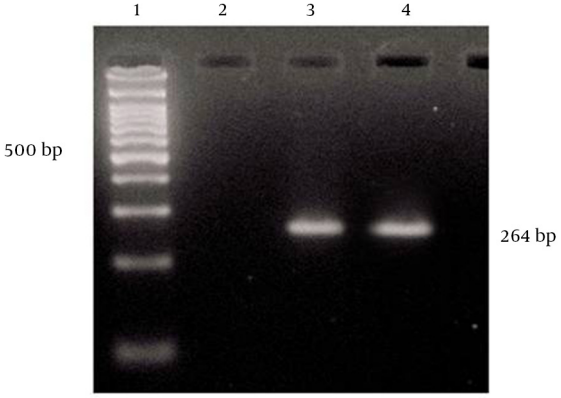
3.6. Statistical Analyses

Prevalence and 95% confidence intervals (CI) were calculated. Chi-square test or Fisher's exact test was used to evaluate distribution of characteristics associated with CMV infection. P value below 0.05 was considered statistically significant. Data was analyzed using SPSS software, version 18 (Chicago: SPSS Inc.).

4. Results

Totally, 2 of 153 (1.3%) whole blood or red cell components had positive results for CMV infection. Both viremia and anti-IgM CMV positivity were 0.65% (1/153), respectively. As shown in Table 1, there was no correlation between CMV contamination with kind of product.

Figure 1. Agarose Gel with PCR Products



From left, Lane 1: DNA ladder (100 bp); Lane 2: negative control; Lane 3: positive control; Lane 4: CMV-positive specimen.

Table 1. Positive and Negative Results for CMV Infection in Different Blood Products, Gorgan[a]

Variable	Total CMV (+), n = 2 (100%)	Total CMV (−), n = 151 (100%)	P Value
Kind of Product			.97
CPDA1 RBC	2 (2.27)	86 (97.72)	
Washed RBC	0	50 (100)	
Whole blood CPDA1	0	1 (100)	
Whole blood low volume	0	1 (100)	
Leukocytes reduced	0	13 (100)	

[a]Values are presented as No. (%).

5. Discussion

Transfusion-transmitted cytomegalovirus infection (TT-CMV) is known to cause morbidity and mortality in immunosuppressed patients, particularly among allograft recipients and infants born with birth weights less than 1.5 kg (2, 5).

Approximately 20 - 100% of the blood donor populations around the world are CMV seropositive, the highest in South America, Africa and Asia and the lowest in Western European countries and the United States (6).

It is important to prevent exposure to CMV using safe blood transfusion, thus, all seropositive donors (IgG+/IgM+) could be regarded as potentially infectious for at-risk patients (2).

Diagnosis of acute HCMV infection is complicated and difficult. Currently, several diagnostic tests including virus culture, shell-vial, serological tests, antigenemia and nested PCR are available for direct or indirect detection of HCMV infection (7). Although, nested PCR proved to be more sensitive in diagnosis of infection than any other test (8).

In the present study, 2 of 153 (1.3%) whole blood or red cell components had positive results for CMV infection by both enzyme linked immunosorbent assay and polymerase chain reaction. Therefore, the prevalence of CMV infection in our study might be underestimated, when other assays are used.

It is not possible to rely entirely on the results of the tests alone for the following possible reasons:

1- In some cases there is a time between CMV infection and production of IgM antibody due to delayed immune system response, thereby, a negative IgM CMV antibody result may occur.

2- Some seronegative donors (false-negative) might have an increased risk for transfusion-transmitted CMV infections (TT-CMV) due to window period donations (9).

3- PCR test may give a false-negative result, if CMV DNA concentration in whole blood and plasma samples is low (9).

However, due to the importance of donor serostatus for a CMV-seronegative patient, a rapid, reliable, accurate and cost-benefit method is essential to screen donors' blood for previous or active (primary/reactivated)CMV infection (5, 10).

Until these methods are introduced, CMV transmission via blood transfusion can be strongly prevented using the following alternate methods:

1- Diagnosis of acute CMV infection by presence of IgM and low IgG avidity.

2- Testing donors' urine instead of blood for CMV by nested-PCR.

3- Adoptive transfer of HCMV-specific T cells after transfusion in immunosuppressed patients (11).

4- Filtering or irradiation of CMV seropositive blood products (10).

5- Leukoreduction of blood products (2).

Abovementioned strategies seem appropriate, although, some even speculate that these strategies cannot prevent reactivation of preexisting CMV in recipients, especially in immunosuppressed patients (12).

Although removal or inactivation of viruses in blood products is a worthy goal, it is difficult in practice. Thus, efforts to develop new strategies for screening and inactivation would be required to make whole blood or red cell components, safe.

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Footnotes

Authors' Contribution: Alijan Tabarraei contributed to project management, interpretation of data, critical revision and final approval of the study, and writing the manuscript. Mishar Kelishadi was involved in all steps of experimental work, manuscript preparation, interpretation of data, statistical analysis and writing the manuscript. Mohammad Mojerloo and Abdolvahab Moradi contributed to interpretation of data, statistical analysis, critical review and comments. Masoud Bazouri, Pezhman Hashemi, Sobhan Samadi, and Atefeh Saeedi contributed to data collection and sampling.

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