Prevalence of CMV DNA in Blood Donors in a Regional Blood Center in Turkey

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ABSTRACT

Cytomegalovirus (CMV) infections with a high seroprevalence rate in adults are often asymptomatic or mild. The current prevalence of CMV DNA in Turkey is not known. This study aimed to find out the prevalence and quantity of CMV DNA in blood donors by PCR. Totally 1003 samples were collected between March and June 2016 at a regional blood bank for detection of CMV DNA in healthy blood donors by quantitative real-time PCR. CMV-DNA positive samples were tested for the presence of anti CMV IgM and IgG by ELISA. Among 1003 donors, 973 (97.01%) were male and 30 (2.99%) were female. Age distribution of donors was between 18-64; mean age was 27. Most of the donors were between 18-30 years old (75.47%) and male (97.01%). One donor was found as CMV DNA positive (0.099%). The DNA quantity for positive donation was 1.75x10^2 IU/ml. CMV DNA positive sample was positive for IgG, whereas negative for IgM. Although the seroprevalence of CMV is high in our country the CMV DNA prevalence was found very low (0.099%). Testing of CMV DNA in blood donation particularly for some specific patient groups and the clinical follow-up of blood donation with low level of CMV DNA should be considered.

Keywords: CMV DNA, Blood Donors, Transfusion-Transmitted Infection, Blood safety

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ÖZET

Amaç: Yetişkinlerde yüksek seroprevaleans oranına sahip Cytomegalovirus (CMV) enfeksiyonları genellikle asemptomatik veya hafiftir. CMV DNA’nın Türkiye’deki mevcut prevalansını bilmememektedir. Bu çalışma, kan donörlerinde PCR yöntemi ile CMV DNA’nın prevalansını ve miktarını bulmayı amaçlamıştır.

Yöntem:inantitif gercek zamanli PCR ile salgılı kan donörlerinde CMV DNA tespiti için bölgesel bir kan bankasında Mart-Haziran 2016 tarihleri arasında toplam 1003 örnek toplanmıştır. CMV-DNA pozitif numuneler, ELISA ile anti CMV IgM ve IgG varyeti açısından test edilmiştir.

Bulgular: 1003 donörün 973’ü (%97.01) erkek, 30’si (%2.99) kadındı. Bağışçılardan yaş dağılımı 18-64 arasındaki; ortalama yaş 27 idi. Vericilerin çoğu 18-30 yaş (%75.47) ve erkek (%97.01) arasında. Çalışmanın dahil edilen bağışçılarda biri CMV DNA pozitif olarak saptanmıştır (%0.099). Pozitif saptanan bağışçının DNA miktarı 1.75x10^2 IU/ml olarak belirlenmiştir. CMV DNA pozitif saptanmış numune IgG için pozitifken, IgM için negatif bulunmuştur.

Sonuç: Ülkemizde CMV seroprevalansı yüksek olmasına rağmen CMV DNA prevalansı çok düşük (%0.099) bulunmuştur. Özellikle belirli hasta gruplarında kullanılabilecek olan bağışçılarda CMV DNA’nın test edilmesi ve düşük CMV DNA düzeyi ile kan bağışı sonrası klinik takibinin yapılması gereği düşünülmelidir.

Anahtar Sözcükler: CMV DNA, Kan Bağışçıları, Transfüzyonla Bulaşan Enfeksiyon, Kan Güvenliği

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INTRODUCTION

Human cytomegalovirus (CMV) is a human herpesvirus with a seroprevalence rate in adults ranging from 40 to 100% in adult population (1). It is transmitted by direct contact with body fluids such as saliva, breast milk or urine, or following transplantation (bone marrow or solid) as well as blood transfusion and usually causes a wide range of symptoms (2). Infection in the healthy individual is often asymptomatic or a mild, self-limited viral illness, however in immunocompetent individuals such as CMV seronegative infants, it can result in severe CMV disease (3).

Transfusion-transmitted CMV (TT-CMV) is a significant cause of morbidity and mortality in immunocompromised patients, including premature low-birthweight infants born to seronegative mothers, and seronegative recipients of seronegative allogeneic or autologous marrow or peripheral blood progenitor cell transplants. The incidence of TT-CMV in such population was between 13-37% (4). Even for other patients, TT-CMV may constitute an increased risk, because prior CMV infections have been associated with an increased risk of other disorders, such as cardiovascular disease, immunosuppressed and Alzheimer’s disease. Therefore, recent reports concluded that prevention of TT-CMV even in immunocompetent subjects could be an important concern for the transfusion medicine community (5,6).

Two methods are mainly used to reduce the risk of transfusion-transmitted CMV; first is transfusion of leukocyte depleted and the other is CMV seronegative blood components, although not completely eliminate the risk of CMV transmission. Donors with latent CMV infections or during the late phase of primary CMV infections can be recognized by detection of CMV antibodies. Seronegative blood products can be selected for patients at risk of TT-CMV to avoid donations from such donors. However, this selection would not eliminate the risk from donors with primary CMV infection before development of CMV specific antibodies, in window period (7). “Breakthrough TT-CMV” may develop in high-risk patients, possibly due to transfusion of blood from donors in the window period. The source of this risk is mainly due to residual white blood cells remaining in the component after leukodepletion. The other source is infectious viremia, which are detected during the reactivation of primary or latent infection known to be not associated with leukocyte cells and are associated with CMV DNA in plasma (3). In order to reduce the risk of donors in this term, nucleic acid amplification test (NAT) is applied (8). For this reason, we aimed to perform CMV-DNA screening in blood donors by real-time PCR assay, which is one of the best methods used for CMV safe blood supply.

MATERIAL and METHODS

Study group and sample collection

This study was conducted after the approval from the local ethical committee (Gulhane Military Medical Academy, Ankara, Turkey; Decision number: 16/1648–748), and informed consent was obtained from the study subjects who were eligible for blood donation based on blood donor questionnaires, and whole blood count results. All candidates had been questioned in detail for any chronic or acute illness, and clinically examined by using routine methods. Samples were collected for this study from March 2016 through June 2016. Whole blood samples were collected in vacutainer blood collection tubes at the blood collection sites. Sera was separated by centrifugation and were stored at -20ºC until work.

Quantitative Real-time PCR

DNA isolation was performed by using viral DNA/RNA nucleic acid extraction kit (Anatolia, Turkey) according to the direction of manufacturer. The primers and probes targeting the UL20 type 1 membrane protein gene sequences were used. The primers and probe sequences were; Forward: 5’-ggagagatgccgtcctttttct-3’, Reverse: 5’-gccaacagcctcactcctg-3’, and Probe: 5’-FAM-caggtgtctgctactgctgg-BHQ-3’ (9). The primer set amplifies a 118 base pairs fragment. Internal control was the human glyceraldehyde-3-phosphate dehydrogenase gene. The amplicon size was 145 base pairs, and primers and probe sequences for internal control were as Forward: 5’-tcctgcacacacacagtctag-3’, Reverse: 5’-catcaacaccaytgyccag-3’, and Probe: 5’-VIC-agggcatctcgaacttctgyatc-BHQ-3’ (MWG-Biotech, Ebersberg, Germany). The PCR mixture without the template DNA was used as a negative control. Previously prepared standards from laboratory between 10³-10⁸ copies/ml were used to determine the detection sensitivity of the PCR assay. The detection limit of the quantitative real-time PCR was 10² copies/ml (83 IU/ml). Three quantitative standards were included for each PCR assay to determine the viral load. The reaction mixture was prepared for all real-time PCR assays as follows: 1.25 U Hot Start Taq DNA polymerase (Bioron, Germany), 10 pmol of each primer, 2.5 pmol TaqMan probe, 0.2 mM dNTP mix, and 2.5 mM MgCl₂. PCR amplifications were carried out in a final volume of 25 µl of the PCR reaction mixture, after the addition of 5 µl of the sample containing template DNA. The amplification conditions were as follows: initial denaturation for 15 min at 95ºC, followed by 40 amplification cycles of 15 s at 95ºC and 1 min at 60ºC. PCR assays were carried out by using ABI Prism 7500 Sequence Detection System (Applied Biosystems, USA).

Statistical Analysis

The sample size was calculated at a 90% confidence level and a ±5% margin of error and a total of 1003 individuals were enrolled in this study. Unless stated otherwise, means are calculated as arithmetic means ± standard deviation (SD). Calculations were assisted by database and statistical program (Excel, Microsoft Corp., Redmond, WA).

RESULTS

Of the 1003 donors, 973 (97.01%) were male and 30 (2.99%) were female. Age distribution of donors was between 18-64; mean age was 27. The age and gender distribution of blood donors were given in Table1. Most of the donors (75.47%) were between 18-30 years old and male.

Table 1. Donor distribution according to their age group and gender.

| Age Groups | Gender | 18-30 years | 31-40 years | 41-50 years | 51-65 years | Total |
|------------|--------|-------------|-------------|-------------|-------------|-------|
|            | n      | (%)         | n           | (%)         | n           | (%)   |
| Female     | 9      | 0.90        | 6           | 0.60        | 10          | 0.99  |
| Male       | 748    | 74.58       | 111         | 11.07       | 91          | 9.07  |
| Total      | 757    | 75.47       | 117         | 11.67       | 101         | 10.06 |

A total of 1 individual of 1003 blood donors was CMV DNA positive (0.09%). The DNA quantity for positive donation was 1.75x10² IU/ml. CMV DNA positive sample were tested for both IgG and IgM by ELISA. The donor who was positive for CMV-DNA was positive for IgG, whereas negative for IgM. The blood donor was 24 years old and male.

DISCUSSION

Different methods have been applied to prevent the development of transfusion-transmitted CMV infection in patients underwent transfusion. Leukoreduction is the most preferred method among them. It has been reported that leukoreduction reduces the risk of development of TI-CMV by 92% rate (10). However, this method cannot totally prevent transmission of CMV that circulate freely in plasma. Methods used to reduce residual risk are as follows; (i) Supply of seronegative blood component, (ii) Supply of blood components from donors who have not seropositive for a long time, (iii) Supply of CMV-DNA negative components. These test methods applied to minimize the risk of transmission in specific patient groups, i.e. haemopoietic stem cell transplant patients or neonatal patients. (7)
The CMV seroprevalence was estimated in Turkey with a mean of 97% (95%UI: 95-98) which is high comparing with other countries (14). In our study, CMV DNA was found to be positive in one of 1003 participants (0.099%). The CMV IgG of this donor was positive and IgM was negative. According to the current literature, this data is the first data on CMV DNA positivity among blood donors in our country. In a study conducted in the United States, CMV DNA positivity rate was found to be 0.13% (42/31075), regardless of the serological profile of blood donors (14). Therefore, the lower detection limit of quantitative real-time PCR in our study is 83 IU/ml, which is considered to be sufficient for the detection of infections especially in the window period. Serological test results of the blood donor who were detected as positive for CMV by PCR test was not seem to be in the window period.

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Conflict of interest
No conflict of interest was declared by the authors.

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