Prevalence of Active Cytomegalovirus Infection in Hemodialysis Patients

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ABSTRACT

Background and Objectives: Human cytomegalovirus (HCMV) is the most common viral cause of morbidity and mortality in immunocompromised patients**.** The aim of this study was to evaluate the frequency of active CMV infection in hemodialysis patients in Gorgan, Iran.

Methods: Plasma samples were obtained from 149 hemodialysis patients at Hemodialysis Unit of Panje-Azar Medical Centre in Gorgan, Iran. Presence of CMV-DNA in plasma samples was evaluated by polymerase chain reaction (PCR) using specific primers for highly conserved regions of major capsid protein gene of HCMV. In addition, level of CMV-IgM antibody was measured by serological testing. Demographic information and past medical history of patients were also recorded. Data was analyzed by SPSS software (version 18).

Results: Total prevalence of CMV infection was 6.7% (10/149) among the patients receiving hemodialysis. CMV-DNA and anti-CMV IgM antibody were detected in 2.68% and 4.69%, of the samples, respectively. One case was found positive for both CMV-DNA and anti-CMV IgM antibody. CMV infection did not have any correlation with gender, age, ethnicity, duration of hemodialysis, and history of blood transfusion.

Conclusion: A notable proportion of hemodialysis patients in Gorgan have active CMV infection. Accurate detection of these individuals is important for preventing infection spread, especially in immunocompromised individuals. Simultaneous diagnosis of CMV infection using serological testing and PCR assay could help reduce the risk of infection spread.

Keywords: HCMV, Hemodialysis, PCR, Iran.

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INTRODUCTION

Human cytomegalovirus (HCMV) is a member of the *Betaherpesvirinae* subfamily. Approximately 40%-100% of all adults worldwide are asymptomatic carriers of this virus (1, 2). Major transmission routes of HCMV include direct person-to-person contact, tissue and organ transplantation and transfusion of blood products (3). HCMV is also the most common viral cause of morbidity, graft loss, and mortality in immunocompromised patients, transplant recipients and frequent blood transfusion recipients (such as hemodialysis patients), respectively (4). CMV infection during pregnancy is of great clinical importance since it can affect the mother's health or even cause mortality and infection-related congenital abnormality in fetus and newborns (3). The virus can also cause pneumonitis, enterocolitis, nephritis, diabetes, hepatitis and cardiac complications (5, 6). Similar to other members of the *Herpesviridae* family, CMV can persist in the host in a latent state following primary infection, and increase the risk of other opportunistic infections such as Epstein-Barr virus and human herpesvirus 6 (1, 3, 7).

Kidney transplantation is considered the treatment of choice for majority of patients with end-stage renal disease. It is safer to match CMV-seronegative donors with CMV-seronegative recipients (blood/organs) to reduce the risk of CMV infection (3). Since evaluation of patients for CMV infection is not part of the routine procedures at blood transfusion and hemodialysis centers, a high seroprevalence of CMV among hemodialysis patients could increase the spread of the infection (2). Numerous laboratory techniques such as virus culture, shell-vial, serology, antigenemia and polymerase chain reaction (PCR) are available for detection of CMV infection (3). However, PCR has been demonstrated to be more sensitive than the other techniques (8). Currently, there is no information available on the prevalence of CMV among the hemodialysis patients in Gorgan, Iran. Hence, the present study aimed to determine the frequency of active CMV infection in these patients.

MATERIAL AND METHODS

This cross-sectional study was conducted by the Department of Virology (Golestan University of Medical Sciences) between October and November 2013. Approval was obtained from the ethics committee of the university, and informed consent was obtained from participants. Blood samples were taken from 149 hemodialysis patients at Hemodialysis Unit of Panje-Azar Medical Centre in Gorgan. Plasma was separated from whole blood, aliquoted and stored at -70°C until processing. Demographic information and medical history of patients were recorded;

Plasma level of CMV-IgM antibody was measured by enzyme linked immunosorbent assay kits (IgM-Dia.Pro Inc; Third Generation Elisa Kit- Italy) according to the manufacturer's instructions. DNA was extracted from 200 µL of EDTA-anticoagulated plasma using a commercially available kit (High Pure Extraction Kit; Roche Diagnostics GmbH, Mannheim, Germany). Presence of CMV-DNA was assessed by PCR amplification using Peq Lab thermal cycler (Primus Advanced 96 thermal cycler, USA) and specific primers (5'-GAGCGCGTCCACAAAGTCTA-3' and 5'-GTGATCCGACTGGGCGAAAA-3') from highly conserved regions of major capsid protein gene of HCMV (NCBI Reference Sequence: M25411.1) (9).

Polymerase chain reaction procedure was carefully optimized. Reaction mixture (25µL) contained 1µL of DNA (1.5-2.5µg), 10 pmol of each primer, 0.1 mM of deoxynucleotide (dNTP) (Genet Bio, South Korea), 2.5 U of Taq DNA polymerase (Genet Bio, South Korea), 2.5 mM $MgCl_2$ (Genet Bio, South Korea) and 2.5µL 10X PCR buffer (Genet Bio, South Korea). In the samples with low or undetectable concentration of CMV DNA, 1 µl of the reaction solution was re-amplified by PCR using the same primers. The amplification program consisted of an initial denaturation at 95°C for five minutes; 35 cycles at 94 °C for 40 seconds, 35 cycles at 50 °C for 20 seconds, 35 cycles at 72 °C for 20 seconds; and 35 cycles at 72 °C for 2 minutes. Negative (CMV-DNA negative plasma) and positive (CMV-DNA positive plasma) controls were included in each run. PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide, and then visualized by an UV illuminator. The frequency of CMV infection was calculated using a 95% confidence interval (CI). Data were analyzed using SPSS software (version 18, Chicago,

USA). Chi-square test or Fisher's exact test were used for comparison of proportions. P-values less than 0.05 were considered as statistically significant.

RESULTS

The study was done on 149 hemodialysis patients (74 males and 75 females) with mean age of 56 ± 15.92 years (age range: 15-90 years). The mean duration of hemodialysis was 3.99 ± 0.11 years. Total prevalence of CMV infection was 6.7% (10/149) among the patients receiving hemodialysis. CMV-DNA and anti-CMV IgM antibody were detected in 2.68% (4/149) and 4.69% (7/149) of the samples, respectively. Only one sample (0.67%) was found positive for both CMV-DNA and anti-CMV IgM marker. There was no correlation between CMV infection and demographic variables or past medical history (Tables 1-2).

Table 1- Comparison of variables between patients positive and negative for CMV in unit of hemodialysis treatment in Gorgan

variable	Total- CMV (+) n=10 (%100)	Total- CMV (-) n=139 (% 100)	p-value
Sex			
male	6(% 60)	68(% 4892)	0.36
female	4(% 40)	71(% 51.08)	
Ethnicity			
Fars	9(% 90)	108 (% 77.69)	
Turk	0	1(% 0.71)	
Turkman	1(% 10)	6(% 4.31)	0.62
Sistani	0	22(% 15.82)	
Cossack	0	2(% 1.43)	
History of blood transfusion			
Yes	8(% 80)	119 (%85.61)	0.45
No	2(% 20)	20(% 14.38)	
Number of dialysis per week			
2	3(% 30)	62(% 44.60)	0.65
3	7(% 70)	77(%55.39)	
Age(years)			
	mean: 62.9±17.92 median: 67	mean: 54.53±15.69 median: 56	0.23

Table 2- Frequency of CMV markers in hemodialysis patients in different countries

Country/year/reference	CMV- IgG	CMV- IgM	CMV- DNA	Seropositivity- CMV	Acute- CMV
Egypt/2008/(10)	98%	11%	30%	98%	32%
Urmia, Iran /2010/(2)	77.4%	7.1%	--	84.5%	At least 7.1%
Tehran, Iran /2006/(7)	91%	18.5%	--	91%	18.5%
Sudan /2011/(11)	98.12%	6.87%	--	98.12%	6.87%
Turkey/2006/(4)	99/6%	0/4%	--	99/6%	0/4%
Egypt /2008/(12)	93%	--	--	93%	--
Turkey / 2002/(13)	77.4%	7.1%	31.7%	77.4%	At least 31.7%

DISCUSSION

Despite the reduced acute allograft rejection rates due to improved immunosuppression, surgical techniques and living kidney donation, HCMV infection remains a major health threat to kidney transplant recipients (3, 7). CMV serostatus and lack of CMV-specific immunity are crucial factors for increased incidence of CMV infection among patients with renal failure (10). Hence, serological and molecular

methods have been used for both routine diagnosis and surveillance of CMV infection in these patients (1). Similar to other developing countries, screening for CMV infection in Iran is carried out only for special groups of patients, and is mainly based on serological testing.

Limited data are available on the prevalence of CMV infection among hemodialysis patients in the area studied (11). Based on our results, 6.7% of the subjects had active CMV infection. Anti-CMV IgM antibodies were found in 4.69% of the samples, while 2.68% of the samples contained the CMV-DNA. Only one case was found positive in both tests. Therefore, the prevalence of CMV active infection could vary depending on the type of test used (Table 1).

According to studies, there is a time lag between primary infection and IgM antibody production, which could be due to delay in immune response. Thus, CMV IgM antibodies might be undetectable in immunocompromised individuals with primary CMV infection. In addition, IgM antibody may remain positive for up to a year after an acute infection. Since PCR assay may produce false-negative results in such cases, it is recommended to verify the results of PCR by serological testing (3, 7).

The frequency of CMV markers in hemodialysis patients varies widely among different populations. This could be related to epidemiological factors, methodological differences (use of different primers and PCR techniques), duration of dialysis, socio-economic factors, etc. As shown in Tables 3, more than 70% of patients are CMV-seropositive, indicating the widespread previous exposure to CMV and increased risk of CMV reactivation in the hosts. In this study, almost all CMV infected patients had a history of blood transfusion. There was no relationship between history of blood trans-fusion, duration of hemodialysis, and CMV infection. Several studies have reported that frequent blood transfusion increases the risk of CMV infection (3).

However, blood transfusion and latent infection can be risk factors for CMV infection in hemodialysis patients receiving immunosuppressive regimens. This indicates the importance of donors' serostatus for CMV-seronegative patients. Therefore, matching serologic status of donor and recipient is an ideal way of reducing the risk of CMV infection. However, because of the scarcity of organ donors and CMV-seronegative blood tests, allocation of organ/blood based on CMV serologic compatibility has not been widely implemented. Nevertheless, accurate diagnosis of active CMV infection could reduce the incidence of CMV infection among the patients at risk (12). Similar to previous studies, we demonstrated that although PCR is a rapid and accurate assay for detection of CMV, serological testing is helpful in determining the seroprevalence of CMV and could verify the results of PCR. Thus, none of these two techniques is efficient enough for detection of active CMV infection if used solely (13).

CONCLUSION

A considerable proportion of hemodialysis patients in Gorgan have active CMV infection. Accurate detection of these individuals is important for preventing infection spread, especially in immunocompromised individuals. Simultaneous diagnosis of CMV infection using serological testing and PCR assay could help reduce the risk of infection spread.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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