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Serological Detection of Cytomegalovirus Antibodies among Sudanese Renal Transplant Recipients

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Abstract

Background: Cytomegalovirus (CMV) infection is one of the most common infectious complications subsequent to kidney transplantation and responsible for CMV disease in renal transplant recipients. It has a significant impact on morbidity, mortality and graft survival.
Objective: To perform serological detection of cytomegalovirus antibodies among Sudanese renal transplant recipients.
Materials and methods: One hundred and four selected renal transplant recipients were enrolled in this cross-sectional study. Blood specimens were collected and investigated for the presence of CMV IgG and IgM antibodies using the enzyme-linked immunosorbent assay (ELISA).
Results: From a total number of 104 renal transplant recipients, 27 (26%) out of them were positive for CMV IgM antibody, while 103 (99%) were positive for CMV IgG antibody.
Conclusion: The frequency rate of sero-positive CMV antibodies was high among the Sudanese renal transplant recipients.

Key words: Cytomegalovirus antibodies, Serological detection, Renal transplant recipients.

Introduction

Cytomegalovirus (CMV) infection is one of the major infectious complications in kidney transplantation and is associated with acute rejection or chronic kidney allograft dysfunction and opportunistic infections. Furthermore, several studies had reported that CMV infection increases graft loss and is associated with death resulted from the infection. HCMV (Human CMV) is ubiquitous being endemic worldwide, and prevails throughout the year without seasonal variation[1].
The prevalence rate of antibody to HCMV infection increases with age, the highest prevalence rate being in developing countries and among lower socioeconomic status people of developed nations. CMV seroprevalence rate is close to 100% of the general population in developing countries. After transplantation, the severe immunosuppressive regimes used to prevent rejection of the transplant, make the recipients prone to severe CMV disease. About 60 to 90% of all renal

transplant recipients have latent CMV infections. Symptomatic infection occurs in 20-60% of them. CMV is a significant cause of increased morbidity and mortality in this population[2].
CMV is reactivated in transplanted recipients by immunosuppression and possibly by allogeneic stimulation (transplanted cells). CMV influences the condition of immunocompromised patients are classified into direct effects of invasive viral infection (viral syndrome and organ involvement), and indirect effects by alterations in host immune and inflammatory responses (immunomodulatory). CMV infection increases the risk of other opportunistic infections, including human herpes viruses (HHV-6 and HHV-7), Epstein-Barr virus, *Nocardia*, Mycobacteria, and fungi such as *Aspergillus* and *Candida*[3].
Detection of CMV by serology, culture or other techniques without signs and symptoms of infection denotes CMV infection. If symptoms such as prolonged fever, leucopenia, hepatitis, colitis, retinitis, allograft injury occur, CMV disease is diagnosed. Active CMV infection can be confirmed by specific antibody assays, detection of inclusion bodies within the infected cells, antigen staining by immunohistochemical methods, detection of viremia by polymerase chain reaction (PCR), and CMV-DNA in peripheral blood leukocytes[4].

Materials and methods

This is a descriptive, cross-sectional, hospital based study aimed to determine the frequency rate of CMV IgM and IgG antibodies among Sudanese renal transplant recipients. The study was conducted in the Kidney Transplant Association Hospital and Ahmed Gassim Hospital (Khartoum State) from June 2013 to June 2015. One-hundred and four renal transplant recipients were selected for this study.
Inclusion criteria included renal transplant recipients, who consented to participate in this study. Participants were adults and children, males and females, and with or without signs and symptoms of CMV infection.
The study was approved by the Research Board of Sudan University of Science and Technology. Permission to collect specimens was obtained from the Kidney Transplant Association Hospital and Ahmed Gassim Hospital (Khartoum State). Verbal consent was taken from each renal transplant recipient prior to enrolment in the study. Data and samples were collected after informing the renal transplant recipients about the purpose and importance of the study.
The study based on non-probability convenience sampling technique. Samples were taken from patients during their regular medical checkup. 5 ml of blood specimens were collected in plain containers. Sera were separated and stored at -20 C° until analysis.
All sera were tested for IgM and IgG CMV antibodies by the enzyme-linked immunosorbent assay (ELISA)**.** The CMV-specific IgM/IgG antibodies were analyzed by commercial ELISA kits (G.E.N.E.S.I.S diagnostics, Omega diagnostics group PLC, Cambridge shire, UK) as per the manufacturer's instructions.
For IgG antibody detection, a100 µl of each of negative control, 3 IU/ml standard, positive control and diluted samples (1:100) were pipetted in microplate well coated with CMV antigen and incubated at room temperature for 20 minutes. After washing three times, 100 µl of conjugate reagent were added to each well and again incubated at room temperature for 20 minutes. Then after another washing step, 100 µl of a substrate solution (TMB Substrate) was

added to each well and the plate was incubated for 10 minutes. A blue color develops if IgG CMV antibodies are present. The blue color changes to yellow after adding 100 µl of the stop solution. Optical densities (O.D) were measured using a microplate reader within 10 minutes at 450 – 630 nm. To determine the presence or absence of anti-CMV IgG, the measured O.D is compared to a O.D mean of 3 IU/ml standard as follows:
Negative samples: O.D < O.D of 3 IU/ml standard
Positive samples: O.D ≥ O.D of 3 IU/ml standard.
For detection of IgM antibody, IgG-absorbent is prepared by adding 40 ml of sample diluent to 10 ml IgG absorbent (1:4) and all samples were diluted 1:100. A100 µl of the negative control, 10 IU/ml standard, positive control and diluted samples were pipetted in microplate well coated with CMV antigen and incubated at room temperature for 20 minutes. The wells were then washed three times. Then 100 µl of conjugate reagent were added to each well and incubated at room temperature for 20 minutes. After another washing step, 100 µl/well of substrate solution (TMB Substrate) were added and the plate was incubated for 10 minutes. A blue color develops if IgM CMV antibodies were present. The blue color changes to yellow after adding 100 µl of the stop solution. Optical densities (O.D) of controls, 10 U/ml standard and samples were measured in a microplate reader within 10 minutes at 450-630 nm. To determine the presence or absence of anti-CMV IgM, the measured O.D is compared to a O.D mean of 10 IU/ml standard as follows:
Negative samples: O.D < O.D of 10 IU/ml standard.
Positive samples: O.D ≥ O.D of 10 IU/ml standard.

Data was analyzed by the SPSS (Statistical Package of Social Sciences) software program version (11.5).

Results

A total of 104 renal transplant recipients were investigated in this study. Their age range was from 11 to 72 years and the mean age was 37 years (SD =14.37). Out of them, males were 72 (69.2%) and females were 32 (30.8%).
Also, 50 (48%) of the recipients had received their transplanted organs in Sudan; and 54 (52%) recipients had received their transplanted organs in hospitals abroad.
Most of the renal transplant recipients 79 (76%) had received their transplanted organs form living-relative donors; and only 25 (24%) recipients had received their transplanted organs form from living non-relative donors.
Out of 104 renal transplant recipients investigated, 27 (26%) were found positive for anti-CMV IgM; and 77 (74%) of them were found negative.
Also, 103 (99%) renal transplant recipients were found positive for anti-CMV IgG; and only one (1%) recipient was found negative.
All positive anti-CMV IgM samples were also positive for anti-CMV IgG. Also, 76 of the negative anti-CMV IgM samples (98.7%) were found positive for anti-CMV IgG; and only one sample (1.3%) was found negative for both CMV IgM and IgG antibodies (Table (I).

Table (I): Correlation of seropositivity between CMV IgM and IgG antibodies

Anti-CMV IgM	Anti-CMV IgG		Total
	Positive	Negative	
Positive	27 (100%)	0 (0%)	27 (100%)
Negative	76 (98%)	1 (1.3%)	77 (100%)
Total	103 (99%)	1 (1%)	104 (100%)

Discussion

This study was designed to determine the frequency rate of CMV IgG and IgM antibodies among Sudanese renal transplant recipients. The study found that most of recipients were males with male/female ratio about 2:1. This finding was in agreement with Hasanzamani and his colleagues[4] in Iran, who reported that 41 (62.1%) of their study population were males and 25 (37.9%) were females. Similar results were also observed by Khameneh and his co-workers[2] in Iran who found that 22 (61.1%) of their study population were males, and 14 (38.9%) were females. In the present study most of the population investigated (76%) received their transplanted organs form relative donors. This finding was different from those findings obtained by other workers. For instant, Pourmand and his co-authors[5] in Iran found 85% of their population study had received living-unrelated organs, 8% had received living-related organs, and 7% had received cadaveric organs. Also van Ree and his colleagues[6] in Italy found that 83% of their study population had received cadaveric organs. The present study revealed that the frequency rate of anti-CMV IgG was 99%, which indicated a high serofrequency rate this antibody. This finding was in agreement with that obtained (100%) in Sudan by Enan and his co-workers[7] and that obtained (98(%) by Awad Alkreem and his co-authors[8] in Sudan population. Also, Khairi and his colleagues[9] observed high seroprevalence of CMV antibodies (97.5%) in Sudanese pregnant women. Furthermore, a high prevalence rate of CMV antibodies (95.7%) was detected by Abd Alla and his co-workers[10] among Sudanese hemodialysis patients which indicates an earlier acquisition of the infection. The findings of our study were also in agreement with Bates and Brantsaeter[11], who reported an almost 100% seroprevalence rate of anti-CMV IgG in Africa. This high rate could be due to the lower socioeconomic status, broadly neglected diagnosis, poor treatment of CMV-related diseases, and no randomized clinical trials of anti-CMV drugs.

On the other hand, the detected CMV IgM antibodies among our study population was 26% which indicates reactivation of CMV infection. This finding was higher than that obtained by Enan and his colleagues[7] and Awad Alkreem and his co- authors[8] in Sudan, who reported a 6% frequency rate of anti-CMV IgM among renal transplant recipients. In contrast, Khameneh and his colleagues[2] in Iran obtained higher result (37.5%) of anti-CMV IgM seropositivity among transplant recipients. High frequency rate of CMV infection among transplant recipients may be due to changes in recipient T-cell subgroups.

Conclusion: The frequency rate of sero-positive CMV antibodies was high among the Sudanese renal transplant recipients.

References

1. Heymann, D.L. Control of communicable disease Manual 18. *American Public Health Association,* 2004) 142-146.
2. Khameneh**,** Z.R. Sepehrvand, N. and Aghazadeh, T. Cytomegalovirus infection among Iranian kidney graft recipients, *Transplant Proc,* 45, 2013,178-181**.**
3. Ardalan, M. Rare presentations of cytomegalovirus infection in renal allograft recipients. *Nephro-Urol Mon*, 4(2), 2012, 431-436.
4. Hasanzamani, B. Hami, B. Zolfaghari, V. *et. al.* The effect of cytomegalovirus infection on acute rejection in kidney transplanted patients, *J Renal Inj Prev*, 5(2), 2016, 85-88.
5. Pourmand, G. Pourmand, M. Salem, S. *et al.* Post-transplant infectious complications: A prospective study on 142 kidney allograft recipients, *Urology J*, 3(1), 2006, 23-31.
6.. van Ree, R.M, de Vries, A.J.P., Zelle, M.D. *et. al.* Latent cytomegalovirus infection is an independent risk factor for late graft failure in renal transplant recipients, *Med Sci Monit.* 17 (11), 2011, 609-617.
7. Enan, K.A., El-Hussein, A.M., El Eragi, A. *et al.* Sero-reactivity to human cytomegalovirus in a cohort of Sudanese renal transplant patients. *Khartoum Medical Journal*. 2 (2), 2009, 197-199.
8. Awadalkareem, M. Adam, Ahamed, I.F. and A.I. Khalafalla. Prevalence of IgG and IgM antibodies to human cytomegalovirus among Sudanese renal transplant recipients and haemodialysis patients, *Sudan Med Monit*, 8(4), 2013, 183-185.
9. Khairi, S. I. **,** Intisar, K. S. Enan, K.A. *et al* Seroprevalence of cytomegalovirus infection among pregnant women at Omdurman Maternity Hospital, *Sudan*, J. Med. Lab. *Diag*, 4(4), 2013, 45-49.
10. Abd Alla, . A. E., Altayeb, A. A. Alshareef. *et al.* Seroprevalence of cytomegalovirus antibodies among hemodialysis patients in Gezira State, Central Sudan, *WJPR*, 4 (7), 2015, 19-25**.**
11. Bates, M. and Brantsaeter, A.B. Human cytomegalovirus (CMV) in Africa: a neglected but important pathogen, *J Virus Erad.* 2, 2016, 136-142.