Seroprevalence of Cytomegalovirus in Haemodialysis Patients

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Abstract

Cytomegalovirus (CMV) is prevalent worldwide. It belongs to the β-herpesvirinae subfamily of Herpesviridae and comprises a double-stranded linear DNA genome and capsid, surrounded by an envelope. CMV infection is most prominently found in patients with kidney failure caused by various possible reasons such as urinary tract infection or systemic disease and are undergoing dialysis. The present study was conducted during the period of March 2020 to April 2021. It included 96 patients with chronic kidney disease undergoing hemodialysis (44 of patients were women and 52 men) within the age range of 11-70 years. Five-mL of the venous blood sample was drawn from each patient to conduct the rapid antibody test for the presence of CMV-specific antibodies (both IgG, and IgM). This study showed that the seroprevalence of CMV infection among haemodialysis patients was 75%. The seropositivity for CMV-IgG was 72.9% which was significantly higher than that for CMV-IgM (2.1%) for both sexes. The present study further demonstrated that the prevalence of positive CMV-IgG in males was higher than that in females (38.5% and 34.4%, respectively). In addition, the positivity of CMV-IgM was highest in the age group 61–70 years old (2.1%), while the positivity of CMV-IgG was highest in patients age groups 41–50 years (24%). The present study revealed a high seroprevalence of CMV infection among haemodialysis patients in Basrah City. The elevated seroprevalence could be related to many factors, including the endemicity of the virus, public health, patient immunity, environmental factors, and geographical location. CMV infection increases with age, and the infection rate in men was higher than that in women. The seroprevalence rate of CMV-IgG antibodies was higher than that of CMV-IgM antibodies, indicating a previous infection or reactivation of CMV virus among haemodialysis patients, leading to a high risk of CMV infection.

Keywords: Cytomegaloviruses, Hemodialysis, Renal failure, CMV-IgM, CMV-IgG

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INTRODUCTION

Cytomegalovirus (CMV) is prevalent worldwide. It belongs to the \textit{β-herpesvirinae} subfamily of \textit{Herpesviridae} and comprises a double-stranded linear DNA genome and capsid, surrounded by an envelope.\textsuperscript{1} The virus persists in a latent state after primary infection of mononuclear cells, is frequently involved in the formation of giant cells, and can cause relatively mild infections such as mononucleosis-like clinical symptoms and other febrile illnesses.\textsuperscript{2} CMV transmission occurs via close personal contact, blood and blood products, bodily fluids, and blood transfusions.\textsuperscript{3} CMV infection is also common in organ transplant recipients, such as kidney transplant patients, taking immunosuppressive drugs.\textsuperscript{4} Most frequently, infection occurs opportunistically in patients who either are immunosuppressed due to prior infections or are immunocompromised, such as those with renal failure and cancer, patients with HIV, those with pneumonitis, encephalitis, retinitis or colitis, and those undergoing chemotherapy.\textsuperscript{4} The prevalence of CMV has become a major public health concern and is a serious threat to dialysis patients who are already immunocompromised. Due to a weakened immune state, the severity of the infection in these patients could be high, leading to an increased mortality rate and other serious complications.\textsuperscript{5} In recent years, a high prevalence of CMV infection was reported in patients with renal failure in haemodialysis units. These patients are more susceptible to CMV reactivation due to multiple blood transfusions and impaired immune responses due to T cells producing fewer inflammatory cytokines.\textsuperscript{6-8}

Renal failure can be an acute or chronic disorder occurring when the kidneys fail to remove waste products and electrolytes from the blood and are thus unable to regulate fluid and pH balance.\textsuperscript{9} There are various causes of kidney failure, such as renal disease, urinary tract disorders, or systemic diseases. The accumulation of urea, a nitrogenous waste, in the blood is an early sign of kidney failure and increases with increasing disease severity. The normal concentration of urea in the plasma is less than 20 mg/dL, but in the case of renal failure, the level is increased to 800 mg/dL.\textsuperscript{10} Most cases of kidney failure require alternative treatment such as dialysis (haemodialysis), which is the most widely used treatment to remove excess fluid accumulation and harmful substances in the body, or through kidney transplantation.\textsuperscript{11} This study aimed to determine the seroprevalence of CMV-IgM and CMV-IgG antibodies in haemodialysis patients.

Methods

Ethics Statement

Written informed consent was obtained from all the patients who participated in the study. The Ethical Committee of Al-Kunooze University College/Department of medical laboratory techniques approved this study under protocol No.2305.

Study design and setting

The study was conducted between March 2020 and April 2021. Samples were collected from Basrah Teaching Hospital. This study included 96 patients ranging from 11 to 70 years who were diagnosed with chronic kidney disease and were undergoing haemodialysis (44 patients were women and 52 were men). Data on the onset of renal failure and haemodialysis history were obtained by reviewing medical records and from personal interviews.

Samples collection

Five millilitres of venous blood was drawn from each patient using disposable syringes, collected in plain tubes without any anticoagulant, and labelled with the patient’s name and date of collection. The samples were immediately processed. Serum was separated by centrifugation (Benchtop centrifuge 5430 series 5430 model incl. microlitre rotor, non-cooled) at 10,000 rpm (3600x980 g) for 5 min at 4°C to test CMV-IgG and IgM antibodies.

The samples were tested using a rapid antibody test (Human Anti-Cytomegalovirus IgG ELISA Kit (CMV) [Abcam, Cat. No. ab108724], and Human Anti-Cytomegalovirus IgM ELISA Kit (CMV) [Abcam, Cat. No. ab108725], NY , USA) for the presence of CMV-specific antibodies (CMV-IgG and IgM) as previously reported.\textsuperscript{12}

Reasons for not doing the PCR or Real-time PCR tests are looking for post exposure prevalence of CMV disease infection, according to recent kidney transplant guidelines, we do CMV serology rather than CMV PCR for risk stratification for CMV infection post kidney transplantation and CMV viral load not available in governmental hospitals which is very costly in private labs sector.
Statistical analysis

Statistical analysis was performed using the SPSS software version 20 (International Business Machines Corporation (IBM), NY, United states). The mean values and standard deviations (SDs) were calculated for the characterisation of the study population. The statistical significance between groups was assessed using a Student’s t-test. P values < 0.01 were considered to be statistically significant.

RESULTS

A total of 96 haemodialysis patients were included in this study, of which 54.2% were men and 45.8% were women. The recruited patients had a minimum age of 11 years old and a maximum age of 70 years old and were divided into six age groups: the first group (11–20 years) included 6.3% of the patients, the second group (21–30 years) had 11.5%, the third group (31–40 years) had 9.4%, the fourth group (41–50 years) had 22.9%, the fifth group (51–60 years) had 26%, and the final group (61–70 years) comprised 24% of the patients. The mean age of the patients was 48.9 ± 16.8 years, with a significant difference in seroprevalence among the groups (P < 0.01) (Table 1).

This study showed that the seroprevalence of CMV infection among haemodialysis patients was 75%. The seropositivity for CMV-IgG was 72.9% which was significantly higher than that for CMV-IgM (2.1%) for both sexes (Fig. 1). The present study further demonstrated that the prevalence of positive CMV-IgG in males was higher than that in females (38.5% and 34.4%, respectively). In addition, the positivity of CMV-IgM was highest in the age group 61–70 years old (2.1%), while the positivity of CMV-IgG was highest in patients age groups 41–50 years (24%) (Table 2).

DISCUSSION

Human CMV infection occurs worldwide. CMV infection is characterised by its year-round presence, and it causes many human diseases. There are three pathways of CMV infection, including the primary infection, reactivation, and superinfection pathways. Sagedal et al. reported that 60% of CMV infections in kidney transplant

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**Table 1.** Baseline clinical characteristics of the hemodialysis patients in this study according to age and gender

| Age Group (years) | Male | Female | Total |
|------------------|------|--------|-------|
|                  | No.  | %      | No.   | %     | No.  | %    |
| 11-20            | 4    | 4.2    | 2     | 2.1   | 6    | 6.3  |
| 21-30            | 4    | 4.2    | 7     | 7.3   | 11   | 11.5 |
| 31-40            | 3    | 3.1    | 6     | 6.3   | 9    | 9.4  |
| 41-50            | 14   | 14.6   | 8     | 8.3   | 22   | 22.9 |
| 51-60            | 12   | 12.5   | 13    | 13.5  | 25   | 26.0 |
| 61-70            | 15   | 15.6   | 8     | 8.3   | 23   | 24.0 |
| total            | 52   | 54.2   | 44    | 45.8  | 96   | 100  |

**Table 2.** The seroprevalence CMV IgM and IgG antibodies in hemodialysis patients according to age and gender

| CMV antibody | Gender | Total (%) | Age group years (%) |
|--------------|--------|-----------|---------------------|
|              | Male (%) | Female (%) | 11-20 | 21-30 | 31-40 | 41-50 | 51-60 | 61-70 |
| IgM positive | 1.04    | 1.04      | 2.1   | 0.0   | 0.0   | 0.0   | 0.0   | 2.1   |
| IgG positive | 38.5    | 34.4      | 72.9  | 4.2   | 9.4   | 10.4  | 19.8  | 16.7  | 12.5  |
recipients are active infections, while 20% develop asymptomatic disease.\textsuperscript{14} Haemodialysis is essential for the survival of patients with kidney failure. However, this process is associated with the risk of CMV infection through blood transmission.\textsuperscript{5} The high seroprevalence of the virus in patients with renal failure and haemodialysis is associated with repeated blood transfusions.\textsuperscript{15} Several studies documented that haemodialysis patients have lower immunity due to impaired immune response, which may resulting from higher prevalence rates of viral infections or other organisms, including CMV.\textsuperscript{16-18} Infections in hemodialytic patients may be due to primary infection with CMV or, more commonly, by reactivation of latent CMV or re-infection with exogenous virus, which may be introduced by frequent blood transfusion or kidney transplant.\textsuperscript{19,20} The seroprevalence of CMV varies in many countries, ranging from 30% to 100%.\textsuperscript{21} Wall et al. stated that CMV seropositivity is closely related to early-stage chronic kidney disease.\textsuperscript{22}

The results of the present study showed that the seroprevalence of CMV infection among haemodialysis patients was 75%. These results are comparable to the data reported in previous studies. Vilibic-Cavlek et al. reported a seroprevalence of 74.4\% in Croatia,\textsuperscript{23} whereas a 77\% seroprevalence was reported in Portugal\textsuperscript{24} and a 79\% seroprevalence in Morocco.\textsuperscript{25} The results from European countries showed a variation in this percentage in different countries. Some studies reported a lower prevalence rate of CMV infection, such as in the Netherlands, where it was 45.6\%,\textsuperscript{26} while CMV infection seroprevalence was 49.5\% in France,\textsuperscript{27} 57.25\% in Germany,\textsuperscript{28} and 62.8\% in Spain.\textsuperscript{29} On the contrary, other studies reported higher prevalence rates of CMV infection, such as studies from Hungary, Turkey, Brazil, and Russia, where seroprevalence rates were 86\%, 89.4\%, 80.9\%, and 94.8\%, respectively.\textsuperscript{30-33} This difference may be due to many factors, including the endemicity of the virus, public health, patient immunity, environmental factors, geographical location, and differences in the assay methods used to diagnose CMV.\textsuperscript{34,35}

The current study recorded that the rate of infection among men was higher than that among women (54.2\% and 45.8\%, respectively), and the mean age of the patients recruited in the present study was 48.9 ± 16.8 years old. These results corresponded with data from studies conducted in Iraq by Salman et al\textsuperscript{36} and Tofiq et al\textsuperscript{34} However, many European studies found the seroprevalence of CMV in women to be higher than in men.\textsuperscript{29} In contrast, a study conducted by Saadon\textsuperscript{37} showed no significant difference in CMV seropositivity between males and females.\textsuperscript{35} In addition, Firouzjahi et al\textsuperscript{38} noted no association between sex and CMV infection in patients on haemodialysis.\textsuperscript{38}

Our study found that CMV infection was more prevalent in older adults, whose ages ranged from 50 to 70 years. This finding is in line with the results from other studies demonstrating increased seroprevalence of the virus with age.\textsuperscript{39-41} This could be due to an impaired immune system in older adults or inefficiency in infection response due to decreased numbers of naïve T cells and lymphocytes. These older individuals are more susceptible to CMV infection. The reactivation of the infection is also strongly related to age, especially in patients undergoing haemodialysis due to multiple blood transfusions, and it is believed that there is a defect in the T cells of these patients due to an intrinsic T cell abnormality.\textsuperscript{42} Contrary to these findings, a study conducted by Hanif showed that there was no correlation between seroprevalence and sex or age.\textsuperscript{43}

We noted that 2.1\% and 72.9\% of haemodialysis patients were seropositive for CMV-IgM and CMV-IgG antibodies, respectively, which differed from the prevalence reported previously in other countries. The study from Iraq revealed 8.6\% and 87.8\% seropositivity for CMV-IgM and CMV-IgG antibodies, respectively,\textsuperscript{37} while studies from Brazil revealed 4.9\% and 96\% seropositivity, respectively,\textsuperscript{44} from Iran revealed 7.1\% and 77.4\% seropositivity\textsuperscript{45} and Turkey showed 0.4\% and 99.6\% seropositivity.\textsuperscript{40} The elevation of CMV-IgG antibodies indicates a previous infection or reactivation of the CMV virus among haemodialysis patients. Some authors attributed this elevation to patients taking therapeutic drugs, leading to a high risk of CMV infection.\textsuperscript{36} The presence of CMV-IgM antibodies often confirms the presence of a primary, recent, or active CMV infection.

CONCLUSION

The present study revealed a high seroprevalence of CMV infection among
haemodialysis patients in Basrah City. The elevated seroprevalence could be related to many factors, including the endemicity of the virus, public health, patient immunity, environmental factors, and geographical location. CMV infection increases with age, and the infection rate in men was higher than that in women. The seroprevalence rate of CMV-IgG antibodies was higher than that of CMV-IgM antibodies, indicating a previous infection or reactivation of CMV virus among haemodialysis patients, leading to a high risk of CMV infection.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS’ CONTRIBUTION

MBJ and MYNA designed the experiments. MBJ analyzed the data. Both authors wrote the manuscript. Both the authors read and approved the manuscript for publication.

FUNDING

None.

DATA AVAILABILITY

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee, Al-Kunooze University College/Department of medical laboratory techniques with protocol No 2305.

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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