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Original Article

Serological and Molecular Diagnosis of Human Cytomegalovirus among Hemodialysis Patients in Kirkuk/Iraq

Eman Ammar Ihsan, Lezan Medhat Mohammed, Waleed Mohammed Ali[1]

Medical Laboratory Techniques Department, Northern Technical University, College of Health and Medical Techniques, [1]Kirkuk Teaching Hospital, Kirkuk Directory of Health, Kirkuk, Iraq

Abstract

Background: Cytomegalovirus (CMV) is a common herpes virus and is usually asymptomatic in healthy individuals. CMV has a worldwide distribution, infecting about 40% to 90% of adults, leading to lifelong latent infection. Hemodialysis patients' weakened immune systems have long been known to contribute to greater prevalence rates of viral infections like CMV. **Objectives:** The purpose of the current study was to estimate the rate of prevalence of human CMV among patients with hemodialysis and also to detect CMV in its acute phase. **Materials and Methods:** About 50 men and 41 women, all receiving hemodialysis treatment at Kirkuk General Hospital and 50 apparently healthy individuals as the control group, were included in the current study. This study was carried out during the period November 2022 to March 2023. Participants' sera were examined for the presence of CMV-IgM and CMV-IgG antibodies, as well as DNA by real-time polymerase chain reaction (PCR). **Results:** While CMV-IgM was only discovered in 5.5% of hemodialysis patients, CMV-IgG was found in 50.5% of them. Only 4% of hemodialysis patients had CMV DNA found. In contrast to females, males had a higher likelihood of having CMV-IgM (60% vs. 40%) and CMV-IgG (55.7% vs. 32.7%, respectively). The highest CMV prevalence was found in older patients, which is related to their weakened immune systems. **Conclusion:** Detection and prevalence of CMV IgG was greater in HD patients than that of CMV IgM. Patients exposed to CMV during dialysis may cause virus reactivation by immunosuppression and inflammation, suggesting that screening patients is necessary to avoid complications like kidney transplant rejection. As a result, PCR can detect extremely small amounts of DNA, it has a high detection rate in the early stages of CMV infection.

Keywords: Cytomegalovirus, hemodialysis, polymerase chain reaction

Introduction

Chronic kidney disease (CKD) is characterized by persistent abnormalities in kidney structure or function that endure for a duration exceeding 3 months, thereby exerting implications on an individual's overall health.[1] In the population of young individuals who are in good health, the typical glomerular filtration rate (GFR) is estimated to be around 125 mL/min/1.73 m^2. Consequently, kidney disease improving global outcome (KDIGO) guidelines define a GFR of less than 60 mL/min/1.73 m^2 as an indication of diminished kidney function.[2]

End-stage renal disease (ESRD), also known as irreversible kidney function, is the final stage of CKD, which affects 8%–16% of the world's population and is on the rise. ESRD is defined as the loss of all kidney function. Patients who have reached the end stage of renal disease (ESRD) are candidates for renal replacement therapy (RRT) known as hemodialysis (HD). This treatment is very common. The International Society of Nephrology reports that there were 2.62 million individuals all over the world who underwent RRT as a treatment for ESRD. Treatment for HD was administered to the vast majority of them.[3] Cytomegalovirus (CMV) is a common human virus. Contact with infected individuals is the primary

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Address for correspondence: Mr. Eman Ammar Ihsan, Medical Laboratory Techniques Department, Northern Technical University, College of Health and Medical Techniques, Kirkuk, Iraq.
E-mail: Emanammar994@gmail.com

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route of transmission for most diseases. A wide variety of physiological fluids, including saliva, urine, blood, cervicovaginal secretions, sperm, and breast milk, have tested positive for the presence of CMV.[4]

The human CMV (HCMV) is a double-stranded DNA (dsDNA) virus with a diameter of 120–200 nm that belongs to the herpesviridae family subfamily *Betaherpesvirinae*.[5,6] CMV, the human herpesvirus family's most extensive member possesses a genome of 236 kbp and over 200 open reading frames (ORFs) that encode more than 80 viral proteins. These proteins include glycoproteins (such as gB), phosphoproteins (such as pp65), and other transcription and replication proteins.[7] The viral pathogen exhibits a wide range of cellular tropism. The extensive cellular tropism observed is attributed to the ubiquitous presence of entry receptors, including integrins and the epidermal growth factor receptor. These are also present in pancreatic cells, which makes them potential targets for CMV infection.[8] CMV infection has a complicated natural history, which can be broken down into three main kinds. Primary infection takes place when a person who is immune to the virus contracts the illness for the very first time. The virus will then enter a dormant condition from which it will later be able to reactivate and cause a second type of infection. The third type of infection is known as re-infection, and it occurs when an individual who has already been infected comes into contact with an infectious person, which results in the individual becoming superinfected despite their inherent immunity.[9] The majority of people contract CMV in childhood, but it rarely results in illness. Usually, 30%–40% of children aged 1–3 years in childcare settings excrete CMV (sometimes can be up to 70%). By coming into close touch with biological fluids like blood, urine, or saliva, CMV can be transferred from one person to another.[10] The transmission of cytomegalovirus through blood transfusions can result in severe infections among individuals with compromised immune systems. In immunocompromised patients and transplant recipients, prior and quiescent CMV infection can lead to severe illness. Following the initial infection, the virus can remain latent and infrequently cause recurrent disease or significant complications, unless the patient has an immunodeficiency disorder due to medication or illness.[5]

In accordance with the Centers for the Control of Disease and Prevention (CDC) and the World Health Organization, CMV is a pathogen that has the potential to infect individuals across all age groups. Approximately 50% of individuals have contracted the infection by the age of 40, while approximately 33% of children in the United States have been infected by the age of 5.[11] Major illness or even deaths brought on by HCMV is frequently associated with inflammation, particularly the creation of pro-inflammatory cytokines, and is typically restricted to circumstances where the body's immune response is severely repressed or yet developing.[12] Today, a variety of diagnostic techniques are available, antibody serum detection is the most common type of diagnosis. Primary infection is characterized by the presence of immunoglobulin M (IgM) antibodies in the patient's serum, which are detected as early as 4 weeks following primary infection and may persist for a maximum of 20 weeks. Blood viral DNA is commonly found in positive patients together with IgM antibodies. Real-time PCR has greater efficacy, specificity, and sensitivity than the aforementioned diagnostic techniques, hence it is not constrained by issues like sample contamination risk.[13] RT-PCR is used to detect genetic targets of viruses in respiratory samples.[14] As PCR can detect extremely tiny quantities of DNA, it has an excellent rate of detection in the early phases of CMV infection. Real-time PCR can aid in the identification and quantification of viruses as well as is frequently utilized for disease diagnosis and severity evaluation. Consequently, real-time PCR can also be used in order to assess the efficacy of a treatment.[15]

Materials and Methods

Study design

A study involving 91 ESRD patients receiving hemodialysis ranging in age from 20 to 70 years. The control group, which was matched with the patients, consisted of 50 individuals with regular kidney function and apparently healthy.

Setting and time of the study

The study was conducted at Kirkuk General Hospital from the month of November 2022 to March 2023.

Study samples and methods

A total of 5 mL of blood was drawn. After allowing the blood sample to clot, it was spun in a centrifuge at (3000 rpm) for 15 min in order to separate the serum from the packed RBCs (using an automated pipette). The serum was stored at −80°C in sterile Eppendorf containers for genetic and serological tests. Enzyme-linked immune sorbent assays (ELISA) were used to determine the levels of virus-specific CMV IgG and IgM antibodies in all of the serum samples, following the manufacturer's instructions (Sunlong**/**China kit) and reading the optical density (OD) at 450 nm.

DNA extraction for genetic detection of HCMV

The procedure was carried out as directed by the manufacturer of the DNA Extraction Kits (Zybio**/**Germany) in order to isolate viral DNA.

Real-time PCR for CMV detection

Polymerase chain reaction (PCR) is used to identify CMV DNA by amplifying a genomic area unique to the infection using particular primers. Fluorescent dyes are employed to track out the amplified product in real-time

PCR. Thermos elective amplification requires the use of dyes that are connected to oligonucleotide probes that bind only to the amplified product. The accumulation of product can be detected during real-time PCR without having to reopen the reaction tubes after each PCR cycle by monitoring the fluorescence intensities in real-time.

Calculation of the PCR value

On the basis of the fluorescence curve crossing the threshold line, the instrument software interprets the data.

Statistical analysis

In this study, the data were examined using the *t* test and the Chi-square test to determine the *P* value of each group sample; significant *P* values were found to be more than 0.05.

Ethical approval

The director of health/Kirkuk provided ethical permission for the carrying out of this study. Each participant's information was collected, and the local ethics committee evaluated and approved the study protocol, as well as the data on the participants and the permission form on November 2022 to March 2023 (764).

Results

This study revealed that the highest frequency of HCMV IgM (60%) was found in the 51–65. The age groups 20–35 and 36–50 had the same rate of prevalence 20% as shown in Table 1.

The rate of CMV IgG also was highest in the oldest age group (34.6%) with significant differences with the control group (*P* value = 0.002) as shown in Table 2.

Tables 3 and 4 show that the HCMV IgM test result tended to be higher in males than in females (60% vs. 40%), while IgG was 55.7% in males and 32.7% in females [Figure 1].

Figure 2 shows that the HCMV DNA was discovered in only four HD patients (4%), while the remaining patients were negative for HCMV (96%), with a significant difference (*P* value of 0.0001) between positive and negative results.

Table 1: Relation of HCMV IgM Ab in HD and control group with age

Groups	Age	CMV/IgM				Total
		+ve	%	−ve	%	
Patients	20–35	1	20	21	15.4	22
	36–50	1	20	28	20.6	29
	51–65	3	60	37	27.2	40
Control	20–35	0	0	21	15.4	21
	36–50	0	0	16	11.8	16
	51–65	0	0	13	9.6	13
	Total	5	100	136	100	141

Non-significant Chi-square = 7.122, *P* value = 0.231

Table 2: Relation of HCMV IgG Ab in HD and control group with age

Groups	Age	CMV/IgG				Total
		+ve	%	−ve	%	
Patients	20–35	15	28.9	7	7.8	22
	36–50	13	25	16	18	29
	51–65	18	34.6	22	24.8	40
Control	20–35	1	1.9	20	22.5	21
	36–50	2	3.9	14	15.7	16
	51–65	3	5.7	10	11.2	13
Total		52	100	89	100	141

Moderately significant *P* value = 0.002

Table 3: Relation of HCMV IgM in HD and control group with sex

Groups	Sex	CMV/IgM				Total
		+ve	%	−ve	%	
Patients	Male	3	60	47	34.5	50
	Female	2	40	39	28.6	41
Control	Male	0	0.00	27	19.8	27
	Female	0	0.00	23	16.9	23
Total		5	100	136	100	141

Non-significant Chi-square = 1.455, *P* value = 0.636

Table 4: Relation of HCMV IgG in HD and control group with sex

Groups	Sex	CMV/IgG				Total
		+ve	%	−ve	%	
Patients	Male	29	55.7	21	23.6	50
	Female	17	32.7	24	26.9	41
Control	Male	2	3.8	25	28.1	27
	Female	4	7.7	19	21.3	23
Total		52	100	89	100	141

**Chi-square = 16.966, *P* value = 0.001

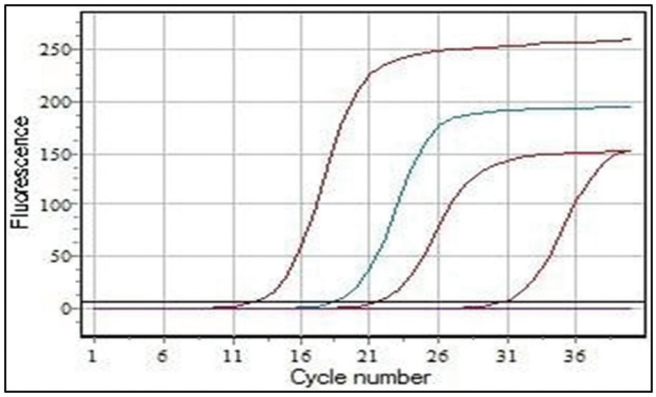

Figure 1: Real-time polymerase chain reaction, the dependence of FAM channel fluorescence on cycle number

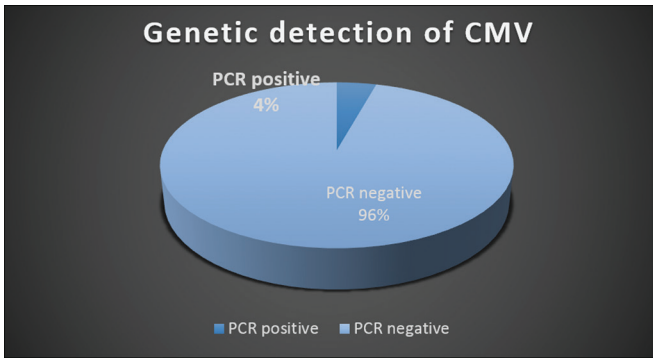

Figure 2: Genetic detection of CMV

Discussion

Consistent with previous research, our findings show that the virus's seroprevalence rises with age. This may be because of a weakened immune system or a less effective infection response in the elderly, both of which are associated with lower naive T cell and lymphocyte counts.[16] Mahmood and Al-Ghazal,[17] also show that most people get CMV between the ages of 50 and 59 (30.5%), and this is likely because CMV infections cause the expansion of CMV-specific T-cells, which can suppress immunity to other pathogens and speed up the immune response in older people. Mohsin *et al*.,[7] also reflect that CMV-IgM was detected at the highest rate among the age group of 50–70 years, this could be because the elderly typically have a weaker immune system. Regarding seropositivity according to sex, the results of this study corresponded with data from a study done in Iraq showing that CMV distribution among hemodialysis patients was 57% among males and 42% among females.[18] Tofiq *et al*.[19] show that CMV in males was more than in females and also in older age from the previous study and present study there was an agreement. A study done at Shahid Beheshti Hospital of Babol found no relation between gender and CMV infection in patients with renal failure undergone hemodialysis.[20] It is well known that the immune system responds differently in males and females, with females often producing more antibodies and experiencing less inflammation in response to infection. The gender differences in CMV are likely caused by the hormones estrogens in females and androgens in males.[21] In Diyala/Iraq, Mohsin *et al*.[7] show that CMV was detected in only 6% of hemodialysis patients. Mahmood *et al*.[22] show that the prevalence of HCMV by PCR was 6.6%. A study in Gorgan, Iran, found that 2.68% of samples contained the CMV-DNA.[23] Another study found that CMV IgM was 7.1%, but this was not confirmed by PCR, this is because IgM antibodies in the serum of immunocompromised dialysis patients can give false positive results. This makes serological methods unreliable for detecting an active cytomegalovirus infection, so confirmation of CMV DNA by PCR is needed. Studies of immunocompromised patients showed that PCR detection of CMV DNA is used for reliable prognosis of further disease course and its clinical outcome and it also enables monitoring of antiviral therapy efficacy and indirect assessment of virus resistance to given therapy.[24] Although PCR is a quick and accurate technique for CMV detection, serological testing is useful in detecting CMV seroprevalence and could validate PCR results. As a result, neither of these two approaches is effective enough for detecting active CMV infection when used alone.[25]

Conclusion

Patients undergoing hemodialysis in Kirkuk City were found to have a high seroprevalence of CMV and EBV infection in comparison to the control group. CMV infection is more common in older people, and the rate of infection in men was found to be higher than that in women. The use of PCR can be an effective approach for diagnosing acute infections.

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Conflicts of interest

There are no conflicts of interest.

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