

Serodetection of Cytomegalovirus and Epstein - Barr virus Antibodies Among Hemodialysis Patients

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The infection with herpesviruses as Human herpes virus-5 (cytomegalovirus-CMV) and Human herpes virus-4 (Epstein – Barr virus -EBV) is one of the main causes of morbidity and mortality in hemodialysis patients. This study aims to investigate the serostatus of CMV and EBV in patients with renal failure who underwent hemodialysis. The study included 134 cases (55 females and 79 males) with an age range of 35-68 years (mean age 37.43 ± 13.42 years). HCMV-IgG, IgM and EBV-IgG, EBV-IgM were determined in subjects' sera. 87 of 134 (65%) were underwent hemodialysis, while 47 (35%) had normal kidney functions without HCV infection. 68 (78.2%) and 31 (35.6%) cases out 87 hemodialysis patients were positive for CMV-IgG and IgM antibodies, respectively. On the other hand, 56.3% and 20.7% of the 87 hemodialysis patients were positive for EBV-IgG and IgM antibodies, respectively. There is no significant differences were observed between females and males in terms of seroprevalance rates. The rate of positive CMV-IgG among 48-64 years hemodialysis patients was high, Whereas, EBV-IgG were detected among 39-67 years. Our data suggest that seroprevalence of CMV and EBV antibodies among hemodialysis cases is a high and cause complications for these patients.

Keywords: CMV-IgG, CMV-IgM, EBV-IgG and IgM for Hemodialysis Patients.

Cytomegalovirus (CMV) is a worldwide virus which can cause severe morbidity and mortality in immunocompromised patients, such as AIDS patients, allograft recipients and patients with renal failure¹. HCMV (HHV-5) is the prototype of subfamily *betaherpesvirinae*, like all herpes viruses in latency and persistence for lifetime of the individuals^{2, 5}. CMV infection is almost asymptomatic or is followed by mild symptoms³. Reactivation of the virus occurs in immunosuppressed patients such as HIV cases, elderly patients, hemodialysis patients and subjects

subjected to chemotherapy and ionized radiation treatment, and pregnant women, due to suppression of the immune system^{1, 4}.

The most common human infection in immunosuppressed patients is Epstein–Barr virus (EBV) infection and can lead to life-threatening lymphoproliferative diseases. The Epstein – Barr Virus has been implicated in several diseases, including infectious mononucleosis, African Burkitt lymphomas (BL), Hodgkin lymphoma, B-cell lymphomas of immunosuppressed cases, nasopharyngeal carcinomas (NPC)^{6, 8}.

Primary HCMV infection (first time) occurs when an individual without defense against cytomegalovirus. Afterwards, the second type of CMV infection creates latency from which it may reactivate^{7,9}. The third type of HCMV infection is called reinfection when contact with an infectious person who has already been infected, despite their possession of natural immunity^{9,13}.

The transmission of human cytomegalo virus and EBV in patients with renal failure may occur through hemodialysis or transplantation^{5,11}. Among hemodialysis patients, the probability of direct contact with human cytomegalovirus is high¹⁴.

Reactivation of the latent virus in patients with renal failure undergoing hemodialysis can result in severe systemic disease, with high fever, multiple organ dysfunction with leukopenia, and CMV in the blood^{10,16}.

Many procedures for CMV and EBV detection are available, including serology techniques, conventional virus culture, shell-vial, and molecular techniques¹⁵. Among the highly sensitive and specific immunological technique used for the detection of specific human anti-cytomegalovirus, EBV antibody (IgM and IgG) is the microparticle enzyme-linked immunosorbent assay (ELISA)¹⁷. This study was proposed to evaluate the prevalence of CMV and EBV antibodies (IgM and IgG) using serological tests as ELISA in patients with renal failure undergoing hemodialysis.

SUBJECTS AND METHODS

Ethical approval

The Review Board of Ain Shams University approved this research protocol.

Study population

One hundred and thirty four cases were listed in this study. The 134 individuals included 55 women and 79 men, with an age range 35-68 means of (37.43±13.42) years. All blood samples were collected from different hospitals (Waddi El-Nile, Dar El-Fouaad, General Daqahlya, and Qena hospitals). The cases were divided into two types; hemodialysis cases that had a failure and underwent hemodialysis (n= 87) and control cases (n=47) that had normal kidney functions and negative for HCV antibodies. Infected samples (n=87) were selected

from patients treated at nephrology departments of previous hospitals. The consent forms that include (name, age, sex, history of blood transfusion) were obtained from each individual before sampling. All sera samples were separated after centrifugation at 1000 rpm for 10 min. Antibodies against HCMV and EBV (IgG, IgM) were assessed and recorded for all samples.

Serological analysis of HCMV

HCMV-IgM and HCMV-IgG antibodies were determined by enzyme-linked immunosorbent assay (ELISA) technique using commercially available HCMV-IgM and IgG kits (BioCheck, Foster City, CA, USA). Procedures were done according to the manufacturer instructions and results of CMV-IgM and IgG were expressed as optical density (O.D) units.

Serological analysis of EBV

EBV-IgG antibodies were detected using commercially available kits (ATLAS Medical EBV-IgG Kit, UK) according to the manufacturer's instructions. Human EBV-IgM antibodies were detected in all samples by qualitative ELISA test using commercially available EBV kits (Diagnostic Automation, USA). The data from the EBV IgG and IgM measurements were expressed as optical density units.

Statistical analysis

The SPSS software package was used for data analysis and data management. Statistical significance was considered when $p < 0.05$.

RESULTS

Total responses of HCMV-IgG antibodies

68 out of 87 patient cases (78.2%) were positive for CMV-IgG antibodies, while 21.8% were negative for HCMV-IgG. Among the control cases, 24 out of 47 (51%) had detectable HCMV-IgG antibodies, while 49% of the cases were negative for HCMV-IgG. (table.1)

Responses of total HCMV-IgM antibodies

Among hemodialysis cases, 31 out of 87 patient cases (35.6%) were positive for HCMV-IgM antibodies, while 64.4% were negative for HCMV- IgG. Ten out of 47 control cases (21.3%) had detectable HCMV-IgM antibodies, while 78.7% of the cases were negative for HCMV-IgM. (Table 2)

Total EBV-IgG antibody responses

47 out of 87 patient cases (56.3%) were positive for EBV-IgG antibodies, while 43.7% were negative for EBV-IgG. Among the control cases, 19 out of 47 (40.4%) had detectable EBV-IgG antibodies, while 59.6% of the cases were negative for EBV-IgG. (table.3)

Responses to total EBV-IgM antibodies

Among hemodialysis cases, 18 out of 87 patient cases (20.7%) were positive for EBV-IgM antibodies, while 79.3% were negative for EBV-IgG. Eight out of 47 control cases (17%) had detectable antibodies to EBV-IgM, while 83% of the cases were negative for EBV-IgM. (Table 4)

DISCUSSION

Human Herpes Virus-5 (HHV-5, also known HCMV) is one of the herpes viruses which cause infection for 75-90% of the world population. HCMV infections are regulated by the effective immune system, but without the virus' ultimate clearance^{17,22}.

During periods of down-regulation of the immune system, such as treatment with pharmaceutical products and stress associated with illness, or viral co-infection, CMV and EBV can be reactivated¹⁸.

CMV was first identified as a one of mortality in elderly, organ allograft recipients, and

Table 1. HCMV-IgG rates in hemodialysis and control cases

	Total No. of Cases	HCMV-IgG				P-value
		Positive No.	Positive %	N	Negative %	
Hemodialysis cases	87	68	78.2%	19	21.8%	<0.001
Control cases	47	24	51%	23	49%	>0.5

Table 2. HCMV-IgM antibodies rates in hemodialysis and control cases

	Total No. of Cases	HCMV-IgM				P-value
		Positive No.	Positive %	N	Negative %	
Hemodialysis cases	87	31	35.6%	56	64.4%	<0.01
Control cases	47	10	21.3%	37	78.7%	<0.02

Table 3. EBV-IgG rates in hemodialysis and control cases

	Total No. of Cases	EBV-IgG				P-value
		Positive No.	Positive %	N	Negative %	
Hemodialysis cases	87	49	56.3%	38	43.7%	>0.1
Control cases	47	19	40.4%	28	59.6%	>0.1

Table 4. EBV-IgM antibodies rates in hemodialysis and control cases

	Total No. of Cases	EBV-IgM				P-value
		Positive No.	Positive %	N	Negative %	
Hemodialysis cases	87	31	35.6%	56	64.4%	<0.01
Control cases	47	10	21.3%	37	78.7%	<0.02

hemodialysis patients¹⁹. Another study shows the presence of infection due to activated HCMV in the population presenting alterations of the immune system^{20, 27}.

In this investigation, we study the seroprevalence of HCMV in Egyptian patients with renal failure who underwent hemodialysis. The results showed that the percentage of seropositive CMV-IgG was significantly higher ($P < 0.01$) in hemodialysis cases than in control cases (normal kidney function without HCV infection). Similarly, CMV-IgM was detected in 35.6% of hemodialysis patients (69% of them had positive IgG antibodies) compared to 12% of the control group to negative CMV-IgG. The results of this study showed that the seropositivity of CMV-IgG, and IgM was higher in elderly hemodialysis patients. Our results were in agreement with other studies concluded on, the correlation between CMV seropositivity and prevalent frailty in older people²¹.

Other studies illustrated that, the source of infection in the majority of allograft recipients (60–75%) during kidney transplantation is a kidney from a seropositive donor²⁴. The remaining infections (25–40%) are due to transfusion of leukocyte-containing blood products from CMV-positive donors [20, 28, 30]. CMV infection is a frequent complication and the main cause of death, of end-stage renal failure disease^{25, 30}. Acquired immunity is suppressed in uremic cases^{26, 27}.

Other studies suggested that, the number of circulating T cells was reduced and suppressor cells increased, where hemodialysis does not recover the weakness of the immune response in patients with renal failure^{12, 28}. These factors contribute to the suppression of the adaptive immune response and increase the incidence of CMV infection among hemodialysis patients^{16, 29}. Likewise, our results displayed insignificant difference between female and male hemodialysis cases with seropositive HCMV antibodies. These results are resemble to other data concluded that, there is no significant variation in CMV seroprevalence by gender²³.

Sagedal *et al.*,¹⁷ reported a highly significant prevalence of CMV antibodies in chronic hemodialysis patients compared to healthy individuals.

In the current study, the rate of positivity for anti-IgG antibody was 49/87 (43.7%). Also, EBV-IgM was detected in 18/87 (20.7%). No

significant variation was observed in EBV IgG antibody in male and female hemodialysis cases. These results are in agreement with Vilibiæ-Èavlek *et al.* (2017) who observed a seroprevalence of EBV-IgG antibodies among hemodialysis patients³¹. In another study carried out in Cyprus, the seroprevalence of EBV IgG antibodies between hemodialysis patients was very high³². Saghafi *et al.* reported that the prevalence of EBV IgG and IgM antibody is 100% among adult potential donors and recipients³³.

In our findings, the percentage of hemodialysis patients positive for anti-CMV IgG was found to be significantly higher than in the healthy volunteers. The risk of CMV infection increases with increasing the time for dialysis treatment. In addition, seroprevalence of EBV antibodies among hemodialysis is high.

CONCLUSIONS

Based on our results, 78.2%, 56.3% of seropositivity was found for HCMV-IgG and EBV-IgG, respectively. On the other hand, 35.6% for HCMV-IgM and 17% for EBV-IgM in hemodialysis patients, showing a high morbidity rate by cytomegalovirus in these individuals. Therefore, it is important to estimate the route of transmission of the CMV and EBV and verify the risk of the transmission through hemodialysis to seropositive and seronegative cases and define strategies of selection. Therefore, we recommended that patients with renal failure who undergo hemodialysis should be tested for HCMV and Human EBV before dialysis and a viral survey should be performed periodically to avoid the transmission of viral infection through the dialysis procedure.

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Conflict of interest

All the authors declare no conflict of interest in this work.

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