A Comparative Study of Immunological and Molecular Techniques to Diagnose Human Cytomegalovirus in renal Failure Patients in Diyala Governor

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Abstract

Background: Cytomegalovirus (CMV)belongsto the herpes virus family, it has the ability to cause systemic infection and serious diseases in immunocompromised patients such as hemodialysis patients. The aim of this study was to investigate the prevalence of CMV infectionamonghemodialysis (HD) patients.. Renal failure is a condition in which the kidneys fail to remove metabolic end-products from the blood and regulate fluid, electrolytes and pH balance of extracellular fluids.Renal failure diesease is a wide dissemination among kidney patients in*Ba'quba City.*

Aim: the study was carried out toImmunological andMolecular detection of CMV among renal failure patients whom admitted to IbnSina Center for kidney Dialysis in Baquba Teaching Hospital .

Patients and Methods: This study was conducted for the period from 1/12/2019 to 15/6/2020 in Baquba city in Iraq ,The study involved a total of 100 patients (62 males and 38 females) with kidney diseasewith ageand 50 healthy individuals considered as controls.First step includes Human Cytomegalovirus (HCMV) diagnosis in studied groupsbyEnzyme-Linked Immunosorbent Assay technique(ELISA) and second step was detection of Human Cytomegalovirus(HCMV) by Real-time Polymerase Chain Reaction.

Results: The obtained results showed that HCMV antibody was detected in renal failure patients by ELISAIgG (100%) while IgM were (15.0%) . Also,Distribution Of Detection of human cytomegalovirus DNA in serum between patients with renal failure and control by using sensitive molecular techniques, The obtainedresults showed that the HCMV DNA was detected in (6 out of 45) or 6 % in patients, while in control group (0 out of 5) or 0.0 %.Also, the HCMV DNA was detected in males 1(16.66) %. while in females was 5(83.34) %, while in control group (0 out of 5) or 0.0 %. with highly precent differences was noticing among both sexes. These results showed the age group 60-70 showed the highest rate of infection among other groups.

Conclusions: Real time PCR was rapid, sensitive and useful for diagnosing CMV infection in such patients.The results showed that Cytomegalovirus has relationship with chronic and acute renal failure and can affect the patient's immune status. our results can provide an advanced diagnosis of viral infections among patients in hospitals in Iraq.

Key Words: *CMV, renal failure, ELISA,Real time PCR*

Introduction

Renal failure occurs when the kidneys are unable to do their job: to filter wastes from the blood, help regulate blood pressure, and regulate salt and water balances in the body. As blood flows through the kidneys, it is filtered, and wastes are removed and sent to the bladder as urine. If kidney function becomes impaired, acute (rapid) or chronic (gradually developing) renal failure may occur. With acute renal failure, kidney function can return to normal if the underlying cause of the failure is discovered and successfully treated. There are two type of kidney infection: Acute Renal Failure (ARF) and Chronic Renal Failure (CRF).[1] Human Cytomegalovirus (CMV) is a ubiquitous humanspecific .DNA virus, belonging to the Herpesviridae family. Cytomegalovirus (CMV) is a ubiquitousvirus with high worldwide prevalence ranging from 34%-80% in developed countries to 100% in some parts ofAfrica [2]. Human cytomegalovirus(HCMV) belongs to the herpesviridae family, subfamilyBetaherpesvirinae, genus *Cytomegalovirus* andcharacterized by slow replication and clinically causesasymptomatic infection in immunocompetent individuals[3]. *The virus is the most significant infectious cause of congenital disease, an important opportunist in the immunocompromised hostlike renal failure .Cytomegalovirus (CMV) is a member of the human herpesvirus family*.

Herpesviruses are enveloped viruses with an icosahedral capsid that encloses a double-stranded DNA genome, CMV is the largest member of the human herpesvirus family, with a genome of 236 kbp and more than 200 open reading frames (ORFs) encoding more than 80 viral proteins, including glycoproteins (e.g., gB), phosphoproteins (e.g., pp65), and other transcription/replication proteins.[4]*CMV is one of the most successful of human pathogens, since it can be transmitted both vertically and horizontally,* Following primary infection, which is almostalways asymptomatic in people with normal immunity and the virus establishes latency. The spreading of HCMV from person to person is by direct contact such as kissing, sexual contact and getting saliva or urine on your hands and then touching eyes, nose or mouth [5]. The infection can occur through blood transfusion from donors with active or latent infection .*Risk factors for primary CMV infection include blood transfusion (treatment for clotting factors, and etcetera), infected transplants, hemodialysis, and the frequency of dialysis in a week. CMV seroprevalence has been shown to be highest in South America, Africa, and Asia, while it is lowest in Western European countries and the United States*[6]*Globally, between 60 and 90% of the general population is infected with CMV with generally higher rates in developing countries.*

Diagnosis of active HCMV infection by ELISA for HCMV-specific IgM antibodies has been shown to be superior and practical. Detection of HCMV-specific IgG antibodies in blood is an indicator of previous exposure to HCMV while IgM antibodies are associated with active CMV infection [7]*.and Molecular assays for determining the level of CMV replication such* areal-time PCR assay used in our laboratory targets the conserved region that lies upstream of antigenic the gB gene.Patients with *Renal failure* disease have impaired immune response, which may result in high rates of viral infections, including CMV. Infections in these patients may be due to primary infection or, morecommonly, by reactivation of latent virus or re-infectionwith exogenous virus. [8].

CMV infection triggers a forceful immune reaction in the human body, including both antibody- and T-cell-mediated responses. Because of its effective immune evasion strategies[9] *B-cell immune responses Primary CMV infection elicits a transient IgM response within 1-3 weeks that is followed by the development of persistent IgG antibodies. These*

antibodies play a minor role in clearing the infection but are believed to play an important role in reducing the severity of CMV disease in adults and protecting the fetus from congenital infection. The antibodies are directed against at least 15 different proteins; the most immunogenic is pp150, against which nearly 100% of the CMV-seropositive individuals have antibodies. [10] *Another important immunogenic protein is pp65; the antibody response against this protein is very high during the acute phase of the infection. The best characterized protein, however, is glycoprotein B (gB), and up to 50-70% of the host's neutralizing antibody response is accounted for by the response to his protein .* [11]

Materials and Methods

Samples collection: A search was performed through The sample A search was 100 Iraqi patients with renal failure at age range (13-76 years) from IbnSina Center for kidney Dialysis in Baquba Teaching Hospital and 50 healthy controls from Blood donors at the main blood bank in Baqubah at age range (18–45) years during the period from January to March 2020.The patients in this study included 62 males, 38 females , aswellas healthy controls 38 males, and 12 females. First step includes Human Cytomegalovirus (HCMV) diagnosis in studied groups by ELISA *,while study was 45 Iraqi patients with renal failure from IbnSina Center for kidney Dialysis in Baquba Teaching Hospital and 5 healthy controls from Blood donors at the main blood bank in Baqubah during the period from January to March 2020. First step includes* Diagnosis of Human Cytomegalovirus in renal failure patients by ELISA and sconed step Real time PCR *and*

Collection and Preparation of Samples*: 5 ml of vein blood samples were collected , put in gel tube and left 15 min to allow clotting at room temperature about 20 -25 °C , after that the serum was separated by 3000 rpm for 10 minutes in centrifugation. The serum was collected and distributed in small tubes and stored in -20°C until use to diagnosis HCMV in study groups.*

***Serological*detection of Human Cytomegalovirus**:

Serological investigation included detection of CMV-IgG antibodies and CMV-IgM antibodies by using enzyme-linked immunosorbent assay (ELISA) (MyBioSource , USA). The procedure was carried out according to the manufacturer's instructions.

Molecular detection of Human Cytomegalovirus

Extraction of Viral Nucleic Acid

Genomic DNA or RNA was isolated from Serum samples according to the protocol of QIAamp® MinElute® Virus Spin Kit.

Diagnosis of Human Cytomegalovirus in renal failure patientsbyReal time PCR:

Fluorion CMV QNP 3.0 Real-Time PCR Kit CMV-FRT was a kit used in PCR test in lab for amplification of nucleic acid. It could be used for detection of DNA of the human cytomegalovirus in suspected sample urine, saliva, urogenital, and blood.

Data Analysis

By the end of the thermal protocol, the Fluorion Detection System software automatically sets the baseline cycles and the threshold. The standard curve (Threshold Cycle vs. Log Starting Quantity) is plotted using the data obtained from the defined standards.

Results and Discussion

*The sample of study was 100 Iraqi patients with renal failure*during the period from January to March 2020This study was conducted to detect the prevalence of cytomegalovirus infection (CMV) among patients

undergoing hemodialysis by using CMV/IgG, CMV/IgM.

The result of infection current research revealed that CMV-IgG was found in 100 out of 100 (100.0%), while CMV- IgM was detected in 15 out of 100 (15.0%) , Chi-Square(8.333) of hemodialysis patients. The high prevalence of IgG seropositive was probably due to cumulative effect of previous infection ; reactivation or new infection lead to high percentage of seropositivity, because renal failure patients conceder immunosuppressed individuals. A positive test for CMV IgG indicates that a person was infected with CMV at some time during their life when a person was infected The seroprevalence of CMV varies in differentstudies**.** [12]revealed that CMV-IgG was found in 102 out of 116 (87.9%), while CMV-IgM was detected in 10 out of 116 (8.6%) of hemodialysis patients in Tikrit city. [13]found that the rate of CMV infection among hemodialysis patients (HD) was 98% using CMV IgG and 11% using CMV/IgM. Astudy was carried out in Antakya, Turkey to determine the rate of CMV infections revealed that anti-CMV IgG and IgM was found in 99.6% and 0.4% respectively of the HD patients [14]. The rate of anti-CMV IgG (39%) obtained by the current study was similar to that obtained by **(Firouzjahi etal.,2015)**[15](34%),but differ with [16] (69%) . HCMV IgG antibody levels increased by increasing frequency of exposure and transmission viacrowded and poor living conditions. [17]. These variations in the results may be attributed to several factors including endemicity of infection, study population, the techniques used for diagnosis and immune status of the patients.

Table 1: Anti CMV IgMAb& Anti CMV IgGAb frequency and percentage in patients' group by ELISA technique

Groups	Anti CMV IgMAb	
	Patient	Control
	No. (%)	No. (%)
Positive	15 (15.0)	0
Negative	85 (85.0)	50
Total	100 (100.0)	50
Chi-Square	8.333	
Df	1	
P value	0.004	
Positive	100	0 (0.0)
Negative	0	50 (100.0)
Total	100 (100.0)	50
Chi-Square	150.000	
Df	1	
P value	0.000	

Moreover of this study results was found that the CMV-IgG was found in 62% in males. while females ,was detected in 38%.The laboratory investigation concerning CMV- IgM among hemodialysis patients revealed that 33.3% of males and 66.7% of females have CMV- IgM antibodies.

Table 2 : anti-CMV IgG and IgM frequency distribution according to the sex of the patient's group

Groups	Patients group		Control groups	
Anti-CMV IgG status	Positive	Negative	Positive	Negative
	No. (%)	No. (%)	No. (%)	No. (%)
Males	62 (62)	0 (0.0)	0	40
Females	38 (38)	0 (0.0)	0	10
Total	100 (100.0)	0 (0.0)	0	50
Anti-CMV IgM status				
Males	5 (33.3)	57 (67.1)	0	40
Females	10 (66.7)	28 (32.9)	0	10
Total	15 (100.0)	85 (100.0)	0	50

Table 3: Gender distribution and CMV frequency by real-time PCR in patients' group

Gender	CMV frequency by real-time PCR		control	
	Positive	Negative	Positive	Negative
	No. (%)	No. (%)	No. (%)	No. (%)
Males	1 (16.66)	26(66.66)	0	3(60)
Females	5 (83.34)	13(33.34)	0	2(40)
Total	6 (100.0)	39(100)	0	5(100)

Revealed 69 male, CMV-IgG was found in 59 (85.5%) of them. Regarding females, CMV-IgG was detected in 43 out of 47 (91.5%). However, that CMV- IgM among hemodialysis patients revealed that 7 out of 69 (10.1%) of males and 3 out of 47 (6.4 %) of females have CMV- IgM antibodies. In Turkey, gender did not contribute independently to the seroepidemiology of CMV($p > 0.01$). [18] Also, [19] reported that there was no difference in CMV prevalence between males (87.9%) and females (96.3%). [16] showed non- significant relation concerning sex status of the CMV-cases. and [13] in U.S. reported that females had higher seroprevalence than males. It is possible that the gender difference in CMV seroprevalence reflects females' exposure to young children. The relationship of child care to CMV infection has been presumed to be attributable to the presence of CMV at high titers in urine and/or saliva. Nevertheless, a previous study suggested that, similar to females, adolescent males are at an increased risk of CMV infection when exposed to young children in the household.

Varying methodologies perhaps may have contributed to the disparities observed. Although immunocompromised patients are at risk for morbidity due to wide variety of pathogens, few, if any of these are capable of producing such widspread disease as CMV. CMV-related morbidity follows a progressive, relentless course in the absence of effective therapeutic intervention. Thus, rapid diagnosis of active CMV infection is of great importance to avoid over treatment with immunosuppressive drugs and to guide antiviral therapy. In recent years, treatment of CMV infection in high-risk patients prior to the onset of clinical disease is preferred.Seroprevalence of CMV in the study groups according to age An increase in the seroprevalence rates was observed with age . Also, a significant association between increase of the age and increment of the seroprevalence confirmed that according to age, a progressive increase in seropositivity was observed in hemodialysis patients.

CMV- IgM was detected at a highest rate in patients within the age group was found among age group 50-70 years. Many investigators observed that 0lder patients were at higher risk of CMV infection . From the previous studies and our study there was an agreement. This may be due to that patients with highest ages will have the low immune response. *The sample of study was 45 Iraqi patients with renal failure*during the period from January to March 2020This study was conducted detection of Human Cytomegalovirus(HCMV) by Real-time Polymerase Chain Reaction,The result of infection current research revealed that These results, the HCMV DNA was detected in (6 out of 45) or 6 % in patients, while in control group (0 out of 5) or 0.0 % as . Also, the HCMV DNA was detected in males 1(16.66) %. while in females was 5(83.34) %, while in control group (0 out of 5) or 0.0 %. with highly precent differences was noticing among both sexes as shown in Table (3).

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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