Cytomegalovirus infections are the most common infection among patients with renal failure at Al-Najaf province

Al-Khaweledy Ahmed Jassim\textsuperscript{a}, Al-Ammar Mahdi Hussein\textsuperscript{a}, Alkhozai M Ziad\textsuperscript{b}

\textsuperscript{a} Department of Biology, Faculty of Science, Kufa University, Iraq
\textsuperscript{b} Faculty of Dentistry, Al-Qadissyia University, Iraq

Authors receive Thomas Edison Award-2014 in Microbiology for Inspiration and Knowledge Distribution among young research scholars.

**Article history:**
Received: 27 February, 2014
Accepted: 02 March, 2014
Available online: 01 April, 2014

**Keywords:**
HCMV, cytokine; ESRD, IgG, IgM

**Corresponding Author:**
AL.khaweledy A.J.*
Email: jassimahmed68@yahoo.com
Al-Ammar M.H.
Email: mahdi.alammar@uokufa.edu.iq

**Abstract**
Renal failure disease is a wide dissemination among kidney patients in Al-Najaf city, the study was carried out to detect the HCMV among 150 patients suffering from acute or chronic renal failure whom admitted to the hospital (kidney center) at AL- Najaf governorate during the period extended from December 2012 to August, 2013, with age ranged between (1-88) years. The samples collected from renal failure patients were diagnosed by serological and molecular tests. The obtained results showed that HCMV antibody was detected in renal failure patients by ELISA IgG (100%) while IgM were (18.66%). Also, HCMV genome were detected in 22\textsuperscript{14.66\%} of the urine samples in all age groups with viral load ranged (40-28050) Copy/ml. The overall finding results assumed that HCMV has a relation in chronic renal failure and can affect the patient's immune status. Our results could provide an advanced method for diagnosing viral infection among hospitalised patients in Iraq.

**Citation:**

**All Rights Reserved with Photon.**
Photon Ignitor: ISJN53497294D666001042014

**1. Introduction**
Renal failure is a condition in which the kidney fails to remove metabolic end products from the blood and regulate the fluid, electrolyte, and pH balance of the extracellular fluids (Ricci et al., 2012). The underlying cause may be renal disease, systemic disease, or urologic defects of nonretail origin. Renal failure can occur as an acute or a chronic disorder. The accumulation of nitrogenous wastes is an early sign of renal failure, usually occurring before other symptoms become evident. Urea is one of the first nitrogenous wastes to accumulate in the blood, and the blood urea level becomes increasingly elevated as renal failure progresses. The normal concentration of urea in the plasma is usually less than 20 mg/dl, in renal failure, this level may rise to as high as 800 mg/dl (Katzung and Bertram, 2007). Cytomegalovirus (CMV) is a common virus with no known seasonal predominance and with a prevalence that ranges between 50\% and 85\% of adults (Staras et al., 2006).

The epidemiology of CMV varies in different regions of the world and may cause a wide variety of disease manifestations depending on the patient's age and immune status. CMV diseases predominantly occur as an opportunistic infection in patients with severe immunosuppression, such as cancer and renal failure patients, but rarely occur in immunocompetent patients (Drew, 2011).

The virus is excreted through body fluids, and the most common modes of transmission are via the or pharyngeal and genital tract, although transmission can also occur through breast milk, organ transplant or blood transfusions, HCMV inclusions are found in the liver, lungs, and kidneys of HCMV vermeil...
patients examined by biopsy or at autopsy (Cannon et al., 2010).

2. Objectives

Because, the incidence of infection caused by HCMV is increasing and littlest studies related to renal failure in Al Najaf city, the aim of study was achieved by the following objectives:

Assessment of anti- HCMV IgM and anti-HCMV IgG ELISA among renal failure patients and identification viral genome by real time – PCR (RT-PCR) as a confirmed test for diagnosis and to solve this problem rapidly.

3. Methodology

3.1 Patients groups

This study was carried out at Renal dialysis Unit /Najaf during the period from December 2012 to August 2013. A total (150) patients were (93 males and 57 females) with renal failure in age ranged between (1- 60) and 24 healthy individuals as control group.

Five ml of 150 (blood and urine) samples was collected from each patient. Two ml of blood samples were placed in tubes with (EDTA) as anticoagulants, the plasma was separated by centrifugation at 4000 rpm for 10 minutes , and three ml of blood left at room temperature, then they were centrifuged for 5 min at 3000 (rpm) to separate serum (Green Wood et al., 2002).

3.2 Sample processing

All blood samples were subjected to centrifugation at 3000 (rpm) for 10 minutes. The serum was separated and then stored at -20° C in multiple eppendorf tube to avoid multiple thawing. All samples were tested for HCMV antibodies (IgG and IgM) using (ELISA) techniques according to Wall et al. (2013). The human CMV IgM&IgG ELISA is base on the classical ELISA technique using HCMV IgM, IgG ELISA kit from HUMAN Company/ Germany.

3.3 HCMV diagnostic by molecular technique

In this study, Real time PCR technique was used for detection of CMV in patient’s plasma and urine according to method described by Accu Power®CMV Quantitative PCR Kit from Bioneer Company. 2.4 Viral DNA extraction kit. Viral DNA was extracted from plasma and urine of patient samples by using Viral Nucleic Acid Extraction Kit III from Geneaid co.

3.4 Estimation of DNA extracted.

The extracted viral DNA and total DNA of serum and urine patient samples were estimated by using Biodrop spectrophotometer (UK Company) that used to measure the DNA concentration and purity at absorbance 260/280 nm.

3.5 Quantitative Real-Time PCR QRT-Real

Time PCR was preformed for detection and quantification of viral loads of CMV DNA virus by using AccuPower® CMV Quantitative PCR Kit from Bioneer Company that contains specific primers and probe for CMV DNA virus. This technique was carried out according to Kit Company.

4. Justification of Research

The need to diagnose infections rapidly is an essential process in providing effective care. Renal failure patients are prone to various infections while being hospitalised this is due to certain procedures carried out. The ability to diagnose viral infections can be a lengthy process while this can have a dramatic impact on the health condition of the patient. Providing a rapid diagnostic procedure can be an effective solution in managing kidney failure patient which was the aim of this study.

5. Results

5.1 HCMV clinical samples distribution

A total (samples) which distributed according to gender that were 57.38% females and 93.62% males, with age ranged between (1 to 88) years old divided into four groups, (1-20), (21-40), (41-60), (> 60) respectively, compared with control group 24 subject.

5.2 CMV diagnostic by ELISA test

5.2.1 Seropositivity of anti-HCMV IgG and IgM of renal failure

Out of the 150 samples (100%) were positive for anti-HCMV IgG antibodies, where 28 (samples) out of 150 (18.66%) samples were positive for anti-HCMV IgM antibodies by ELISA test, From figure (1) it was shown that the highest percentage of anti-HCMV IgG antibodies positivity was detected in all age group (100%). For anti-HCMV IgM antibodies, it was shown from figure that the highest age group in regards to anti-HCMV IgM antibodies was the age group (1-20) and it represent (37.5%) from the total anti-HCMV IgM antibodies cases while the age group (21-40) years showed the lowest percentage which was 5 (14.7 %) from the total positive anti-HCMV IgM antibodies.
5.3 Detection of viral DNA by RT-PCR techniques

The results of PCR amplification for presence of CMV DNA in plasma & urine samples, The HCMV genome was detected in 22 (14.66 %) of the 150 urine samples tested from patients with viral loads ranging from 20 to 543840 copies/ml, including in age groups. Also the result showed that the ratio as high as (25%) in 1-20 years and decreased to (9.615%) in age 41-60 years, table (1),while in plasma samples were gave negative result for CMV DNA in all age groups.

The finding of RT-PCR amplification of CMV DNA in urine samples of patients including in female groups the result showed the high ratio in as (21.052%) with viral loads ranging fro 20 to 543840 copies/ml while in males (10.752%), with viral loads ranging from 40 – 28050 (copies/ml), figure (2).

Table 1: Detection of CMV nucleic acid by RT-PCR amplification in different age groups

<table>
<thead>
<tr>
<th>Age group(y)</th>
<th>Detection of CMV nucleic acid (DNA)</th>
<th>Total</th>
<th>Range Viral load Copy/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>1-20</td>
<td>6</td>
<td>18</td>
<td>24</td>
</tr>
<tr>
<td>21-40</td>
<td>7</td>
<td>27</td>
<td>34</td>
</tr>
<tr>
<td>41-60</td>
<td>5</td>
<td>47</td>
<td>52</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>4</td>
<td>36</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>128</td>
<td>150</td>
</tr>
</tbody>
</table>

Figure 1: Seropositivity of anti-HCMV IgG and IgM among of renal failure in regards to their age groups

Figure 2: Real-time PCR amplification of commercial standards (blue) and specimens (orange), internal control positive (violate) for the quantitative detection of cytomegalovirus DNA

Figure 3: Standard curve generated using known amounts of standard cytomegalovirus DNA relating log concentration to cycle number of amplification
5.4 Correlation between HCMV DNA and the prevalence of anti-HCMV IgG and IgM among age groups.

In the samples taken from all groups suspected to have a disease, it had been shown that 52 (100%) were seropositive anti-HCMV IgG in patients group (41-60) years, while 9 (37.5%) seropositive anti-HCMV IgM in patients group (1-20) years, and 7 (17.5%) PCR positive in patient group (21-40) years. From the results, it had been shown that 93 males (100%) were anti-HCMV IgG, 11 (11.827%) seropositive IgM and 10 (10.752%) PCR positive, while in female samples 57 (100%) were positive anti-HCMV IgG results, 17 (29.824%) anti-HCMV IgM positive and 12 (21.052%) PCR positive table (2).

Table 2: Correlation between HCMV DNA findings and the prevalence of anti-HCMV IgG and IgM among age groups and genders

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age groups (years)</th>
<th>Female</th>
<th>Male</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-20</td>
<td>Total</td>
<td>9</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>2(22.22%)</td>
<td>4(26.66%)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>IgM</td>
<td>3(33.33%)</td>
<td>6(40%)</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>9(100%)</td>
<td>15(100%)</td>
<td>24</td>
</tr>
<tr>
<td>21-40</td>
<td>Total</td>
<td>13</td>
<td>21</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>5(38.46%)</td>
<td>2(9.523%)</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>IgM</td>
<td>4(30.769%)</td>
<td>1(4.761%)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>13(100%)</td>
<td>21(100%)</td>
<td>34</td>
</tr>
<tr>
<td>41-60</td>
<td>Total</td>
<td>19</td>
<td>33</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>2(10.256%)</td>
<td>3(9.09%)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>IgM</td>
<td>5(26.315%)</td>
<td>1(3.030%)</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>19(100%)</td>
<td>33(100%)</td>
<td>52</td>
</tr>
<tr>
<td>&gt; 60</td>
<td>Total</td>
<td>16</td>
<td>24</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>3(18.75%)</td>
<td>1(4.166%)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>IgM</td>
<td>5(31.25%)</td>
<td>3(12.5%)</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>16(100%)</td>
<td>24(100%)</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>Total</td>
<td>57</td>
<td>93</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>PCR</td>
<td>12(21.052%)</td>
<td>10(10.75%)</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>IgM</td>
<td>17(29.824%)</td>
<td>11(11.827%)</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>57(100%)</td>
<td>93(100%)</td>
<td>150</td>
</tr>
</tbody>
</table>

In the samples taken from females (57 female) suspected to have a disease, it had been shown that (100%) were seropositive anti-HCMV IgG, 3(33.33%) seropositive anti-HCMV IgM in age group(1-20) years and decreased to (26.315%) in age group(41-60) years. PCR positive to (38.46%) in age group, (21-40) while decreased to (10.256%) in age group (41-60) years, table (2). All serum samples were taken from males suspected to have a disease, it had been shown that (100%) were seropositive anti-HCMV IgG, (40%) seropositive anti-HCMV IgM in age group (1-20) years and decreased to (3.030%) in age group (41-60) years. PCR positive to (26.66%) in age group (1-20), while decreased to (4.166%) in age group (> 60) years.

6. Discussion

6.1 Cytomegalovirus prevalence

Human cytomegalovirus is a species of the cytomegalovirus family of viruses, which in turn is a member of the viral family known as Herpesviridae or Herpesviruses. Serious complications from infection are documented in many populations including infants, transplant recipients, and patients with acquired immunodeficiency syndrome, researchers have paid increasing attention to
HCMV as an etiologic agent for chronic diseases and as a marker of immune dysfunction, the ability of HCMV to cause disease is dependent upon its capacity to establish and maintain latent infections. Wall et al. (2013) documented an increased CMV seropositivity is associated with a cohort of patients with early stage chronic kidney disease (CKD).

The incidence and spectrum of disease in newborns, in organ transplant recipients and in AIDS and HIV- virus symptomatic individuals establish this virus as an important and significant human pathogen. The samples of CMV was collected from urine, and plasma of patients with renal failure, the high frequency of positive samples in urine compared with plasma, this results agreed with that found by Jennifer, et al., (2012) which may be found in urine, saliva, tears, semen, stool, vaginal or cervical secretions and breast milk. The lack of detectable CMV DNA in serum samples obtained during latency with the presence of highly differentiated CMV- specific cells that depend on antigen for survival indicates that CMV induces local, low-grade infection, possibly occurring in salivary glands and kidney (Schleiss, 2008).

Among women ages 20 -49 years, severity of infection appeared to be independent of age, suggesting that risk of infection during pregnancy is fairly constant during these ages, and that interventions to prevent congenital CMV must target all women of childbearing age, this study have paid increasing attention to CMV as an etiologic agent for chronic diseases and as a marker of immune dysfunction, (Eric et al., 2010).

6.2 Cytomegalovirus diagnosis
6.2.1 Cytomegalovirus sera diagnosis
HCMV IgG antibodies among the age groups were significantly higher than control group. The age groups gave a high percentage of IgG seropositive, increased to (100%), IgG- positivity was equally common in both sexes in all age groups figure (1). 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 concedes 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 (Drew, 2011; Enan et al., 2011). Eric et al. (2010) were found that increasing CMV IgG antibody titers were associated with all-cause mortality even after adjusting for a number of important covariates such as age, gender, education, and baseline health conditions. A high prevalence of CMV seropositivity (100%) in their cases, the overall longer duration of dialysis treatment and the lack of stratification for underlying kidney disease (Michiel et al., 2007), HCMV IgG antibody levels increased by increasing frequency of exposure and transmission via crowded and poor living conditions, (Kotton et al., 2010). With regard to IgM seropositive results, 28 (18.66%) out of 150 sample were positive for anti-HCMV IgM antibodies and the highest incidence was among the age group (1-20) years about (37.5%) and age group (> 60) about (20%), whereas the age group (41-60) about (11.53%) reported the lowest incidence and these differences are statistically significant. These results are highly distributed among young and the older age groups involved patients, figure (1), was more likely to be IgM-positive than other age groups, suggesting that the immune system weak in children, breastfeeding is widely practiced and plays an important role in the epidemiology of CMV infection most mothers are seropositive, children and older and renal failure patients concedes an immunocompromised individuals. The ELISA test results showed that the majority of IgG seropositivies had negative IgM results, where there few cases of all total positive IgG had a concomitant positive of both anti-HCMV IgG and IgM serology, may be anti- CMV IgM antibodies indicate current or recent CMV infection. These result in table (2) were in agreement with Abdolreza et al. (2010).

In the genders groups the females were more likely to be IgM-positive than males, table (2). The statistically significant difference between males and females in the prevalence of individuals with CMV IgM could be explained by the fact that women may have more contact with children. Also the results showed significant increased within the females age groups, may be reactivated of HCMV might be present as latent infection specially in older womens, the intermittent reactivation that occurs within Herpesviridae family and the fact that it increases with immunocompromised patients. Women of reproductive age (from 15 to 20 and 20 to 40 years, respectively) were susceptible to CMV, which led us to conclude that there is a considerable risk for congenital infection due to maternal primary CMV infection, women who care for children and immunocompromised individuals these individuals in whom exposure to CMV can be
most detrimental will be the target groups (Yasir, 2012).

6.3 Molecular characterization & Viral load
In studying the results of Quantitative Real-Time PCR measurement of CMV-DNA levels, PCR amplification for CMV in study groups duration both the pp65 antigenemia assay and quantitative CMV viral load testing can be utilized in preemptive protocols. To diagnose of CMV infection in patients developing a primary CMV infection, the presence of CMV DNA was monitored weekly until no CMV DNA could be detected anymore, to determine CMV serostatus, anti-CMV IgM and IgG antibody levels were measured in serum samples, and to guide management (Kim et al., 2011).

Estimation of viral load results declared that viral genome copies in patients groups were ranging from (20 - 543840 copy/ml), 14.66% out of 150 sample, table (2) in related studies Enan et al. (2011) showed that viral loads ranging from <20 to 42932 copies/ml, HCMV detection by real-time PCR indicated a high prevalence among renal transplant patients in Khartoum.

Levels of CMV DNA were significantly higher at the time of CMV disease than levels 4 to 12weeks prior to initiation of symptoms or than levels associated with asymptomatic CMV, which possibly due to long period of viral genome amplification and possibly other viral as had been proved by Alexopoulos et al. (2012). In other hand according to age groups, the patient within (1-20) years had gave the highest rate of appearance of CMV 25% of the cases, while in gender groups increased percentage of females 21.052% compared to males up to 10.752%, urine assays often have higher viral loads than plasma samples, infected with HCMV shed virus in urine and saliva, virus genome not detected in plasma may be because the CMV found in whole blood within lymphocyte as a latent stage. Pablo et al. (2010) study also showed the lack of detectable CMV DNA in serum samples obtained during latency with the presence of highly differentiated CMV-specific cells that depend on antigen for survival indicates that CMV induces local, low-grade infection, possibly occurring in salivary glands and kidney. Presented results were in agreement with Al-azzawi (2012) young women were more likely to have an infant with congenital CMV infection than women who were older. Yasir (2012) studies explained that women are known to have higher CMV seroprevalence than men, possibly the combination of an age-related factor with recent exposure to the virus in young women enhances CMV infection during pregnancy and increases the risk for transmission to the fetus. Other studies Fowler and Pass, (2006) found that the age group of (36-42) years was the higher incidence group (98%) of infection, suggesting that CMV exposures occurred frequently in these populations.

Previous studies have shown that children who excrete CMV may spread infection to a parent and to other adults in the household. In general, the highest viral loads are associated with tissue-invasive disease, while the lowest are seen with asymptomatic CMV infection Thomas et al., (2007).

Conclusions
1. HCMV is one of many causes among renal failure patients.
2. Anti- CMV IgM antibodies indicate current or recent CMV infection in such patients.
3. RT-PCR indicates the circulation of the virus in blood stream of some seropositive individuals and gives confirmative results in relatively short time.

Research Highlights
This study is aimed at identifying the need of a rapid and accurate diagnostic procedure for viral infections among kidney failure patients in Iraq. Highlighting the role of HCMV in renal failure patients and how it can be managed effectively.

Limitations
This project was limited by certain factors. One of the main limitations was the availability of certain laboratory equipments. The ability to obtain sample from certain hospitals was a limitation to this project. The availability of certain buffers purchasing and delivery of certain laboratory equipments.

Recommendations
The prevalence of HCMV infection in renal failure patients indicates the require for application of prevention programs and control measures in Al Najaf city.

References
Iran. American Journal of Infectious Diseases. 6(1), 8-12.


