The Effect of Human Cytomegalovirus on IL-18 level in Iraqi Kidney Failure Patients

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ABSTRACT
Kidney failure disease (CKD) represents an international major public anxiety, and its prevalence continues to rise. In order to study the effect of human cytomegalovirus (HCMV) on IL-18 levels in CKF patients and compared with healthy control, the current study was designed. It was conducted from first December 2019 to the end of September 2020. Detection of HCMV (IgG/IgM) and IL-18 levels were assayed (by ELISA technique) in the sera of 76 chronic kidney failure patients and 70 healthy control. The patients’ samples 44(62.86%) males and 26(37.14%) females were collected from (dialysis units) of Baghdad Teaching Hospital, AL-Kindy Teaching Hospital, House nurses Hospital, AL-Emam Ali Hospital, and AL-Kadhimiya Teaching Hospitals. Seventy (70) healthy individuals were selected randomly (without kidney disease and HCMV infection), that include 53(75.71%) males and 17(24.29%) females. Age ranges were between (30-75 years) to each group (patient and control). All patients’ samples were showed seropositive of HCMV-IgG but only 7 patients with anti HCMV-IgM positive. Serum level of IL-18 was increased in CK patients (0.109 ±0.009 pg/ml) as compared with control (0.071 ±0.003 pg/ml). The present result revealed that IL-18 was up-regulated in kidney failure infected with Anti-HCMV patients, an observation that suggests their involvement in the pathogenesis of IL-18.

Keywords: HCMV, Kidney failure, IL-18

INTRODUCTION
Dialysis is the first procedure to partially replace renal function in end-stage of renal diseases, despite several adverse side effects, such as infections (Vidal-Castilleira et al., 2019). The incidence of Cytomegalovirus disease after solid organ transplant may be reduced by antiviral prophylaxis or preemptive therapy. However, antiviral prophylaxis may lead to delayed-onset CMV infection or disease, particularly in CMV-seronegative recipients of organs from CMV-seropositive donors (CMV D+ /R−). After kidney transplantation, both early-onset and delayed-onset tissue invasive CMV disease have been significantly associated with allograft loss and mortality (Boudreault et al., 2011). Some authors report a reduction in delayed-onset CMV disease when antiviral prophylaxis is extended to 6 months post-transplant (Humar et al., 2010).

The innate immune response represents one of the first and most rapid host defense barriers against infecting microbes. Pattern recognition receptors (PRRs) are initially responsible for detecting pathogen- and danger-associated molecular patterns (PAMPs and DAMPs, respectively) and thereby activating innate signaling processes that culminate in the expression of antiviral effectors and secretion of immunologically active factors (Botto et al., 2019). IL-18 is mainly produced during the acute immune response by monocytes, macrophages, and immature dendritic cells and participates in cellular and humoral responses. Depending on the immunological context, IL-18 is involved in both T helper (Th)1 and Th2 immune responses. Because of these multiple functions, IL-18 is thought to play a major role in host defense against viral infection while, in parallel, the cytokine also induces autoimmune diseases and propagates inflammatory processes (Gracie et al., 2003). In fact, serum levels of this cytokine have been found elevated during primary CMV infection (van de Berg et al., 2010). Human cytomegalovirus (HCMV) is a ubiquitous and species-specific beta herpes virus that invariably infects with lifelong persistence. This is accomplished largely through multifaceted and sophisticated phenotypic mechanisms of immune evasion. While the virus is largely asymptomatic in healthy individuals, it can cause severe disease in immunocompromised and immune-deficient populations (Botto et al., 2019). So, this study was aimed to estimate the levels of serum IL-18 in chronic kidney failure (CKF) patients and compared with healthy control individuals and studies the effect of HCMV infection on these levels.

MATERIALS AND METHODS
Age Distribution of the Study groups
The total number of the study groups (patients and controls) was 140 individuals (males and females) with ages ranging from 30 to 75 years. This prospective study was carried out after obtaining the requisite ethics committee permission at the department of biology (University of Baghdad) a proved the study protocol (BEC/1020/0034 On 19th October 2019) and informed consents from patients. The patient’s group was consisted of 70 individuals have CKF that collected from (dialysis units) of Baghdad Teaching Hospital, AL-Kindy Teaching Hospital, House nurses Hospital, AL-Emam Ali Hospital, and AL-Kadhimiya Teaching Hospitals. The other 70 healthy individuals (without kidney disease and showed seronegative HCMV IgG and IgM), were enrolled as a control group. However, the mean ages of both groups were 58.18 and 48.93 years respectively.
Specimen Collection
All blood samples were collected from first December 2019 to end of September 2020 from each patient and control via vein-puncture method. Blood was transferred to 10 ml sterile serum separator tubes (gel tube), these tubes were centrifuged for 5min. The sera were distributed into several 0.5 ml aliquots and immediately frozen at -20°C until used for detection of HCMV (IgG-IgM) and Interleukin-18 level.

Detection of HCMV (IgG/IgM) by Enzyme Linked Immunosorbent Assay
Serum samples from the entire study group (70 chronic kidney failure patients and 70 for control group), were tested for CMV by ELISA technique as recommended by manufacture (CMV-IgG and IgM Human, Germany).

Detection of IL-18 by Enzyme Linked Immunosorbent Assay
Serum samples from the entire study group (70 kidney failure patients and 70 control group), were tested for the presence of IL-18 level by using ELISA technique as recommended by manufacture (My Biosource, USA).

The Human IL-18 ELISA kit is a sandwich enzyme-linked immune sorbent assay designed for the quantitative detection of Human IL-18 concentration in serum. This assay uses an antibody specific for Human IL-18 coated on a 96-well plate. Standards and specimens are pipettes into the wells and IL-18 found in a sample is bound to the wells by the immobilized antibody. Then the wells are washed, and biotinylated anti-human IL-18 antibody is added. After washing away unbound biotinylated antibody, HRP- conjugated streptavidin is added to the wells. The wells are washed again, and then a TMB substrate solution is pipetted to the wells and color change according to the amount of IL-18 bound. The Stop Solution alters the color from blue to yellow, and the intensity of the color is measured at 450 nm.

The statistical analysis of this study was performed using the statistical analysis system (SAS-2012) program to study the effect of different factors in study parameters. Least significant difference-LSD test was used to significant comparison between means.

RESULTS AND DISCUSSION

![Figure 1: Standard curve of IL-18.](Image)

The statistical analysis of this study was performed using the statistical analysis system (SAS-2012) program to study the effect of different factors in study parameters. Least significant difference-LSD test was used to significant comparison between means.

Detection of Immune Response against HCMV by ELISA
Enzyme Linked Immunosorbent Assay was employed to assess the presence of anti-HCMV antibodies (IgM/IgG). All patients' samples were showed seropositive of HCMV-IgG and only 7 patients with anti HCMV-IgM positive table (1).

Table 1: Distribution of anti-CMV IgG and IgM antibodies in patient group

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Positive mean%</th>
<th>Negative mean %</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-CMV IgG Ab</td>
<td>70 (100%)</td>
<td>zero (0%)</td>
<td>70</td>
</tr>
<tr>
<td>Anti-CMV IgM Ab</td>
<td>7 (10%)</td>
<td>63(90%)</td>
<td>70</td>
</tr>
</tbody>
</table>

Patients in end-stage renal diseases experience disturbances of the immune system and are highly susceptible to infections arising from dialysis. The frequency of mortality of patients in dialysis is higher than in the general population, especially in patients in hemodialysis compared with those in peritoneal dialysis (McDonald SP et al, 2009; Lukowsky et al., 2013). The risk of infections in these patients increases following kidney transplantation, especially during the first 12 months, because of the initial immunosuppression, which makes the recipient susceptible to serious infections such as human cytomegalovirus (CMV) (Humar et al, 2006).
Primary infection or reactivation with CMV may cause a viremia and can lead to severe CMV disease with organ involvement (Crough and Khanha, 2009). The obtained results appear highly sera prevalence of HCMV-IgG between kidney failure patient and only 7 patients show positive HCMV-IgM result. The frequency of Anti-HCMV IgG in Iraqi population is highly prevalent. The obtained results of this study reflect the ubiquitous nature of HCMV through different human populations across the world and are supported by global, regional and local studies. HCMV in developing countries is an important pathogen with 60-70 percent seroprevalence and up to 100 percent seroprevalence in developed countries (Beam & Razonable, 2012). Previous local studies (Abdalhussien and Al-azzawi, 2015; Al-nuimi et al, 2018; Khudhair and Al-azzawi, 2018; Salman and Al-azzawi, 2020) showed similar result that the percentage of HCMV- IgG were (63.63%; 90%; 55%; and 95%) respectively. By other studies (Saadoon., 2015), in Tikrit it was evaluated the frequency of CMV-IgG and IgM antibodies among hemodialysis patients which were 87.9% for IgG and 8.6% for IgM agree with this study. Additionally other study illustrate high risk factor in Seropositivity of anti-HCMV IgG which was 100% in renal failure, while (18.66%) were positive for anti-HCMV IgM antibodies by ELISA test in Al- Najaf governorate (Al-Khaweledy et al, 2014).

However, detection of IgM was frequently low and not relied on due to lack of specificity for primary infection, the possibility of false positive results and its uselessness in diagnosis of immune compromised patients (Dollard et al, 2011; Pass, 2018). While Aljumaili et al, 2014 obtained higher seroprevalence for CMV IgG (98.3%) and IgM (8.3%).

The other studies appeared that HCMV anti-IgG in Iran was72.1% (Bagheri et al., 2012), and in Nigeria was 87% (Delfan-Beiranvand et al., 2011). The results from the present study were higher to those obtained in developed countries for example in France 46.8% and in Australia 56.9% (Picone et al., 2009). According to an American study, the prevalence of HCMV infection is higher in females (64%) as compared to males (54%) (Bate et al, 2010).

**Detection of Immune Response against IL-18 level by ELISA**

Serum level of IL-18 is significantly increased (p ≤ 0.01) in chronic kidney failure patients infected with Anti-HCMV (0.109 ±0.009) as compared to control (0.071 ±0.003) as shown in table 2.

<table>
<thead>
<tr>
<th>Table 2: Comparison between patients and control in IL-18 levels</th>
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<tbody>
<tr>
<td><strong>Group</strong></td>
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<tr>
<td>-------------------</td>
</tr>
<tr>
<td>Patients</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>T-test</td>
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<tr>
<td>P-value</td>
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</table>

**[P<0.01].**

**Figure 2:** The present result revealed that IL-18 was up-regulated in kidney failure patients infected with Anti-HCMV patients, an observation that suggests their involvement in the pathogenesis of it.

In renal disease, IL-18 plays a crucial role in renal interstitial inflammation, infiltration of neutrophils and macrophages, and tubular cell apoptosis (Miao et al., 2019). Within the past years, several new potential biomarker molecules that are measurable in urine or plasma samples of patients with AKI have been discovered including neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), interleukin-18 (IL-18), liver-type fatty acid-binding protein (L-FABP), tissue inhibitor of metalloproteinase-2...
(TIMP-2), insulin-like growth factor-binding protein 7 (IGFBP7) and calprotectin (Zhou et al. 2016). IL-18 possess pivotal role in it’s used as a marker in diagnosis of kidney disease. IL-18 produce when damage occurs in renal tubular, therefore, it can provide the ability to define diagnosis of loss of renal tubular functions (Orluwene et al., 2015). Study in 2017 showed elevated (IL-18) levels in urine and serum of patients with chronic kidney damage (Lipiec et al., 2017). Other study in 2015, showed that an increase in serum IL-18 concentrations above the cutoff point (1584.5 pg/mL) (Fornamowi et al., 2015).

The result of this study agree with result of (Izz Al-Din and Salih, 2020) in Tikrit the study showed that IL-18 was increased in renal failure patients comparing with the control group (66.5 ± 49.6 vs 49.5 ± 27). The present study showed the highly significant relation (P<0.01) between IL-18 level and Corneic kidney failure. And agreement with study (Saleh and Al-Bayati, 2016) in Diyala the results showed further a highly significant increased serum level of interleulin-18 in patients compared to controls (p ≤ 0.001).

CONCLUSION

The infection of HCMV-IgG among the Iraqi population is very highly prevalent with kidney failure patients and low prevalent observed of HCMV-IgM. Additionally, kidney failure patients showed an observable elevation in the titers of IL-18 levels as compared with group of normal controls with a high statistical significance at (P<0.01). Thus, the results obtained from this study support the suggestion that IL-18 may be involved at some point in the process of development of kidney failure.

REFERENCES


