Sys Rev Pharm 2020;11(11):1145-1149
A multifaceted review journal in the field of pharmacy

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

Dheyaa Mohammad Ahmed Karalos*[1] and Raghad H. Al-azzawi[2]

[1]Health directorate Baghdad/Al-Rusafa, Iraqi ministry of health, Baghdad, Iraq
[2]Department of Biology, Collage of Science, University of Baghdad, Baghdad, Iraq

Corresponding Author: Dheyaa Mohammad Ahmed Karalos
Email: dhiaamohamed1@yahoo.com

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 KF 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 70 chronic kidney failure patients and 70 healthy control. The patients' samples 44(62.86%0) 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 and 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 it.

Keywords: HCMV, Kidney failure, IL-18

Correspondence:
Dheyaa Mohammad Ahmed Karalos
Health directorate Baghdad/Al-Rusafa, Iraqi ministry of health, Baghdad, Iraq
Email: dhiaamohamed1@yahoo.com

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-Castiñeira, *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, IL18 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.

Interpolation of the serum sample finding was determined as a regular curve that was done in the equivalent assay. For estimation the relative O.D. 450 nm) = (every well the O.D. 450 nm) – (zero wells O.D. 450 nm). It is possible to map the normal curve as relative O.D. 450 nm of each standard solution (Y) vs. the corresponding standard solution (X). Concentrations by using normal curve fitting the human IL-18 levels of the sample as shown in figure 1.

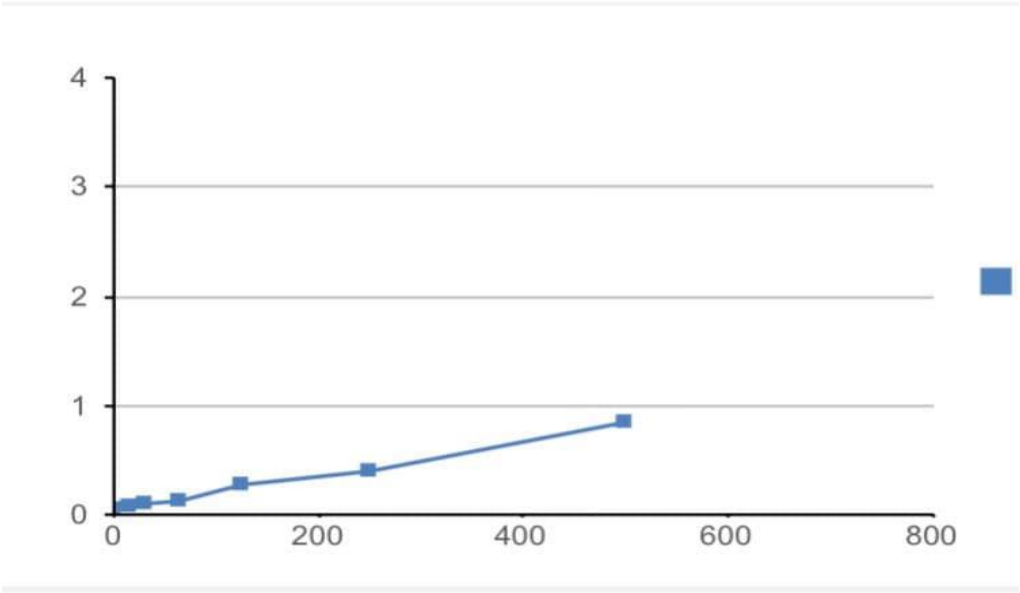


Figure 1: Standard curve of IL-18.

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

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

Patient group	Positive mean%	Negative mean %	Total
Anti-CMV IgG Ab	70 (100 %)	zero (0%)	70
Anti-CMV IgM Ab	7 (10%)	63(90%)	70

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 Khanna, 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 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).

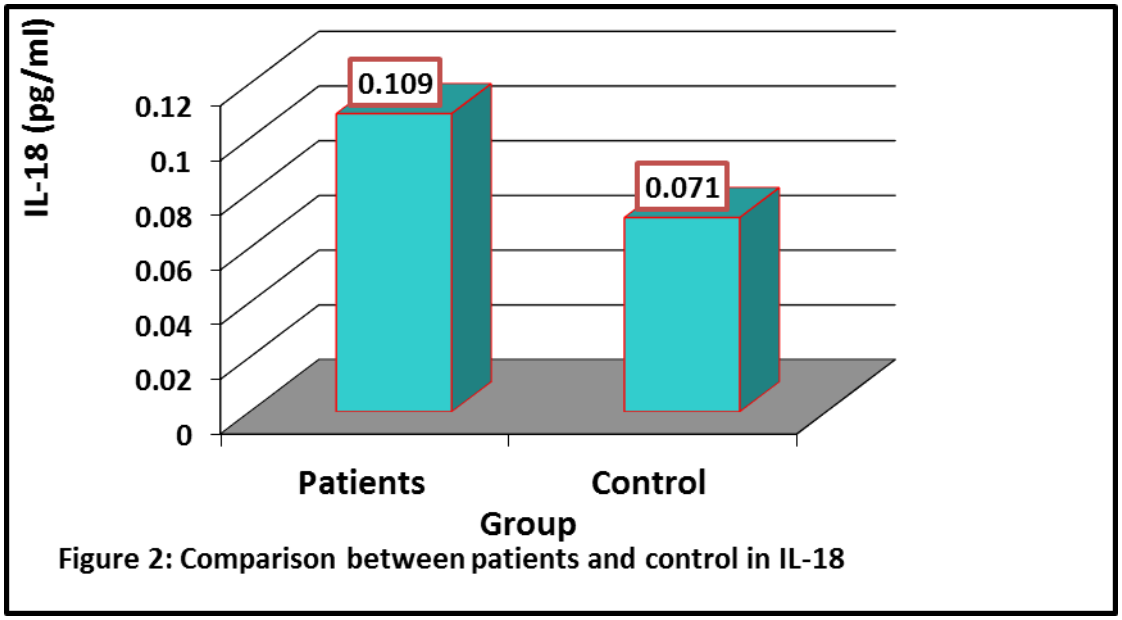
Detection of Immune Response against IL-18 level by ELISA

Serum level of IL-18 is significantly increased ($p \leq 0.01$) 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 2: Comparison between patients and control in IL-18 levels

Group	Mean ± SE of IL-18 (pg/ml)
Patients	0.109 ±0.009
Control	0.071 ±0.003
T-test	0.0199 **
P-value	0.0002
** (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 measureable 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) (Formanowi *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 Cornice 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

1. Abdulhussein TA, Al-azzawi RH (2015) Genotyping of Human Cytomegalovirus envelop glycoprotein B in Iraqi renal transplant and malignancy patients by multiplex nested Pcr. World Journal of Experimental Biosciences, 3(2): 113-117.
2. Aljumaili, Z. K. M., Alsamarai, A. M., & Najem, W. S. (2014). Cytomegalovirus seroprevalence in women with bad obstetric history in Kirkuk, Iraq. Journal of Infection and Public Health, 7(4), 277–288.
3. Al- Khaweledy A.J., Al- Ammar M.H., Ziad A.M., 2014. Cytomegalovirus infections are the most common infection among patients with renal failure at Al-Najaf province. Photon Journal of Microbiology. Photon 107, 200-206.
4. Al-Nuimi, B.N.; Al-Azzawi, R.H. and Naji, R.Z. (2018). Serodiagnosis of Human Cytomegalovirus in Iraqi Breast cancer and fibroadenoma patients. Curr Res Microbiol Biotechnol. 6(1): 1466-1469.
5. Bagheri, L., Mokhtarian, H., Sarshar, N., Ghahramani,M. (2012) Seroprevalence of cytomegalovirus infection among pregnant women in Eastern Iran. The brazilin journal of infectious disease, 16(4): 402-403.
6. Bate, S. L., Dollard, S. C., and Cannon, M. J. (2010). Cytomegalovirus seroprevalence in the United States: the national health and nutrition examination surveys, 1988–2004. Clin. Infect. Dis. 50: 1439-1447.
7. Beam, E. & Razonable, R.R., 2012. Cytomegalovirus in solid organ transplantation: epidemiology, prevention, and treatment. Current Infectious Disease Reports, 14(6), pp.633–641.
8. Botto, S., Abraham, J., Mizuno, N., Pryke, K., Gall, B., Landais, I., DeFilippis, V. R. (2019). *Human Cytomegalovirus Immediate Early 86-kDa Protein Blocks Transcription and Induces Degradation of the Immature Interleukin-1β Protein during Virion-Mediated Activation of the AIM2 Inflammasome. mBio, 10(1)*
9. Boudreault AA, Xie H, Rakita RM, *et al*. Risk factors for late-onset cytomegalovirus disease in donor seropositive/recipient seronegative kidney transplant recipients who receive antiviral prophylaxis. Transpl Infect Dis 2011; 13:244–9. 11.
10. Crough T, Khanna R. Immunobiology of human cytomegalovirus: from bench to bedside. Clin Microbiol Rev. (2009) 22:76–98.
11. DeFreitas, M. J., Katsoufis, C. P., & Abitbol, C. L. 2016. Cardio-renal consequences of low birth weight and preterm birth. *Progress in Pediatric Cardiology*, *41*: 83-88.
12. Delfan-Beiranvand, M., Sheikhian, A., Birjandi, M., Fazeli, M. (2011). Seroprevalence of cytomegalovirus Infection in Pregnant Women Referred to Health Care Center of Khorramabad. Iranian Journal of Virology, 5(4):11-16.
13. Dollard,S.C.,S taras, S.A.S., Amine, M.M., Schmid., and Cannon, M .J. (2011). National prevalence estimation for cytomegalovirus IgM and IgG avidity and associated between high IgM antibody titer and low IgG avidity. Clin. vaccine Immuno.,81:1895-1899.
14. Formanowi, Z. D.; Kossowska, M.; Pawlic Zak, E.; *et al*. (2015). Usefulness of Serum interleukin-18 in predicting cardiovascular mortality in patients with chronic kidney disease. System and Clinical approach.
15. Fraser VJ, Olsen MA. Delayed-onset cytomegalovirus disease coded during hospital readmission after kidney transplantation. Transplantation 2014; 98:187–94.
16. Gibson, W. (2008). Structure and formation of the cytomegalovirus virion. CURR TOP MICROBIOL, 325, 187–204.
17. Gracie JA, Robertson SE, McInnes IB. Interleukin-18. J Leukoc Biol 2003; 73:213–24.
18. Humar A, Lebranchu Y, Vincenti F, *et al*. The efficacy and safety of 200 days valganciclovir cytomegalovirus prophylaxis in high-risk kidney transplant recipients. Am J Transplant 2010; 10:1228–37.
19. Humar A, Michaels M, AST ID Working Group on Infectious Disease Monitoring. American Society of Transplantation recommendations for screening, monitoring and reporting of infectious complications in immunosuppression trials in recipients of organ transplantation. Am J Transplant. (2006) 6:262–74.
20. Izz al-Din, W., A 2020. Using Serum Interleukin-18 (IL-18) as a Biomarker of Chronic Kidney Failure and its relationship with Prolactin Level, Tikrit Journal of Pure Science, pages 14-18.
21. Khudhair, S. A; and Al-azzawi,RH.(2018).Estimation of Anti CMV Antibodies in Iraqi Pregnant Women Infected with Chronic Cytomegalovirus . Journal of Global Pharma Technology. 10 (11): 52-56.
22. Liabeuf, S., Okazaki, H., Desjardins, L., Fliser, D., Goldsmith, D., Covic, A., Wiecek, A., Ortiz, A., Martinez-Castelao, A., Lindholm, B. and Suleymanlar, G., 2014. Vascular calcification in chronic kidney disease: are biomarkers useful for probing the pathobiology and the health risks of this process in

the clinical scenario? *Nephrology Dialysis Transplantation*, *29*(7):1275-1284.
23. Lipiec, L.; Adam czyk, P.; Swietochowska, E.; *et al*. (2017). Angiotensin gene and Interleukin-18 as Markers of Chronic Kidney Damage in Children with a history of Hemolytic uremic syndrome. Physiol. Res. 66: 251-261).
24. Louten, J. (2016). Herpesviruses. Essential Human Virology, 235–256.
25. Mischak, H., Delles, C., Vlahou, A. and Vanholder, R., 2015. Proteomic biomarkers in kidney disease: issues in development and implementation. Nature Reviews Nephrology, 11(4):221.
26. Orluwene, C. G.; Deebii, N. and Odum, E. P. (2015). Urinary Interleukin (IL-18) as an Early Predictive Biomarker of Subclinical Proximal Tubular Dysfunction in HIV-Infected Patients Exposed to Tenofovir. J. AIDS Clin. Res. 6(9):1-4.
27. Pass, R. F. (2018). Cytomegalovirus Principles and practice of pediatric infectious diseases. P.1073-1081
28. Picone, O., Vauloup-Fellous, C., Cordier, AG. (2009). A 2-year study on cytomegalovirus infection during pregnancy in a French hospital. Journal of Gynecology, 116:818.
29. Saadoon, I., H,2015. Frequency of CMV- Infection among Hemodialysis Patients in Tikrit City, *Iraqi Journal of Science*, Pages 2523-2528.
30. Saleh,M., A,2017. Inflammatory role of some cytokines, immunoglobulins and complement proteins in immunopathogenesis of renal failure in a sample of Diyala province patients, Baghdad Science Journal, Pages 247-253.
31. Salman, O. H.; and Al-azzawi RH. (2020). SEROPREVALENCE OF HUMAN CYTOMEGALOVIRUS IN IRAQI BREAST CANCER PATIENTS. Plant Archives. 20 (2): 729-731.
32. SAS. 2012. Statistical Analysis System, User's Guide. Statistical. Version 9.1st ed. SAS. Inst. Inc. Cary. N.C. USA.
33. Van de Berg PJ, Heutinck KM, Raabe R, *et al*. Human cytomegalovirus induces systemic immune activation characterized by a type 1 cytokine signature. J Infect Dis 2010; 202: 690–9.
34. Vidal-Castiñeira, J. R., Corte-Iglesias, V., Sobrino-Diaz, L., Pérez-Fernández, S., Melón, S., López-Larrea, C., & Díaz-Corte, C. (2019). Effect of Type of Dialysis on CMV-Specific CD8+ T Cells in Kidney Transplant Candidates. Frontiers in Immunology, 10. doi:10.3389/fimmu.2019.01680
35. Zhou, F., Luo, Q., Wang, L. & Han, L. 2016. Diagnostic value of neutrophil gelatinase-associated lipocalin for early diagnosis of cardiac surgery-associated acute kidney injury: a meta-analysis. Eur J Cardiothorac Surg 49, 746–755.
36. McDonald SP, Marshall MR, Johnson DW, Polkinghorne KR. Relationship between dialysis modality and mortality. J Am Soc Nephrol. (2009) 20:155–63.
37. Lukowsky LR, Mehrotra R, Kheifets L, Arah OA, Nissenson AR, KalantarZadeh K. Comparing mortality of peritoneal and hemodialysis patients in the first 2 years of dialysis therapy: a marginal structural model analysis. Clin J Am Soc Nephrol. (2013) 8:619–28.