ZANCO Journal of Pure and Applied Sciences
The official scientific journal of Salahaddin University-Erbil
https://zancojournals.su.edu.krd/index.php/JPAS

**ISSN (print ):**2218-0230**, ISSN (online):** 2412-3986**, DOI: http://dx.doi.org/10.21271/zjpas**

RESEARCH PAPER

# *Does the cytomegalovirus infection cause kidney transplant rejection in Erbil city patients, Kurdistan region of Iraq?*

Sardar Hussein Rasool[1], Monika Henryka Miasko[2], Heshu Jalal Ahmed[3], Shukur Wasman Smail[4,5*], Zhikal Omar Khudhur[3], Shang Ziyad Abdulqadir[4], Shatha Saadi Jumaah[3], and Abdulkarim Yasin Karim[4,3]

[1]Ministry of Education-KRG, Erbil, Kurdistan region, Iraq
[2]Department of Medical Microbiology, College of health Science, Hawler Medical University, Erbil, Kurdistan Region, Iraq
[3]Department of Medical Analysis, Faculty of Applied Science, Tishk International University, Erbil, Kurdistan Region, Iraq
[4]Department of Biology, College of Science, Salahaddin University-Erbil, Erbil, Kurdistan Region, Iraq
[5]Department of Biology, College of Science, Cihan University-Erbil, Kurdistan Region, Iraq

## A B S T R A C T:

**Abstract:**

Cytomegalovirus (CMV), the most significant infectious agent, belongs to the family of Herpesviridae. There is a high risk of severe –viral reactivation among patients with kidney transplantation, particularly in the first three months after transplantation (where patients are at the peak for immune suppression), The infection has a high morbidity rate. Hence, this study was designed to assess the association of CMV infection with kidney transplantation and recognize the symptoms that are more related to kidney transplantation (KT) in the Erbil city, Kurdistan region of Iraq. The study enrolled 72 patients who received renal allograft from March 2018 to December 2019, and this population has been characterized as Middle Eastern descent and ethnic miscegenation. Data included age and gender of the recipient, type of donor, symptomatic and asymptomatic CMV patients. Quantitative Polymerase Chain Reaction (qPCR) was used to detect and amplify the extracted virus DNA from blood samples. The CMV was found in 43 patients infected with CMV with graft rejection of about 37.21%. While, it was observed in low rate 13.79% in 20 other patients with graft rejection which had free from CMV. The graft rejection rates were significantly higher among the CMV positive group than controls (P= 0.029). In the light of the results of this study, it has been concluded that the CMV infection in patients after kidney transplantation surgery was deemed an important predisposing factor for acute allograft rejection. The study revealed that the screening of CMV among donor could decrease the possibility of kidney graft rejection among recipients.

KEY WORDS: Allograft rejection, Inflammation, Cytomegalovirus, Kidney transplantation, Quantitative polymerase chain reaction.
DOI: http://dx.doi.org/10.21271/ZJPAS.34.1.8
ZJPAS (2022) , 34(1);80-86 .

## 1. INTRODUCTION:

Cytomegalovirus (CMV), is a member of the subfamily; Betaherpesvirinae, and one of the most commonly recognized opportunistic immunomodulating virus (Clark et al., 2003). It is also potent in causing infections post organ transplantation (Brennan, 2001).

Many patients are prone to infections after organ transplantation and these infectious agents are associated with increased morbidity and mortality (Ramanan et al., 2013). Patients get CMV infection after transplantation surgery, especially after kidney transplantation (KT). CMV

* **Corresponding Author:**
Shukur Wasman Smail
E-mail: shukur.smail@su.edu.krd
**Article History:**
Received: 19/10/2021
Accepted: 06/12/2021
Published: 24/02 /2022

infection occurs in the most solid-organ transplant recipients, primarily in the first three months post-transplantation, when immunosuppression is most intense; CMV infections are shown to prevalent in as much as 44 to 85% of kidney, heart, and liver transplant recipients operation (Browne et al., 2010, Giakoustidis et al., 2012). Factors that can accelerate the progress of the virus and disease progression include serological presence in the recipient's and donor's blood, the utilization of lymphocyte-exhausting agents, immunosuppression level, and donor type (alive or deceased) (Hughes et al., 2008, Selvey et al., 2017). The events of infections in kidney transplantation are also affected by the surgical technique used and environmental factors (Fishman et al., 2019).

The load of CMV can be different from patient to patient. Some cases are asymptomatic, characterized by the presence of dynamic viral replication and might result in ending the organ, while in symptomatic CMV cases, KT patients might experience prolonged-lasting fever, diarrhea, hepatitis, colitis, or transplanted organ injury. Patients undergoing KT have increased risk for severe diseases, high chances of severe graft rejection, low chances of graft endurance, higher occurrence of persistent allograft nephropathy, and lowered patient survival rate (De Keyzer et al., 2011, Andrews et al., 2011).

Between 20 to 60 % of transfer recipients happen to have indicative CMV infections leading to critically increased mortality and morbidity rates (Brennan, 2001). CMV infections are primarily caused via infected donor organs or cellular blood products, following solid-organ transplantation. In contrast, with other organ transplantation types, KT patients are at a lower risk for CMV, primarily due to lower latent viral load in the transplanted kidney. Furthermore, in cases of infection with CMV, long-term cellular and humoral immunity usually develops in immunocompetent individuals but CMV remains latent or persistent within the host. In case of patients undergoing transplantation under immunosuppressive drugs, chances of secondary CMV infections are quite high. The incident rate is reported to range between 8 and 32% (Patel and Paya, 1997, Hartmann et al., 2006).

There is a high risk of severe –viral reactivation among patients with KT, particularly in the first three months after transplantation (where patients are at the peak for immune suppression). The CMV infection in transplanted patients can be prevented by administering antivirals, especially in early post-transplantation cycle. Also, CMV infections are shown to usually occur in KT patients when antivirals are not given to them or due to suspension of prophylactic antivirals (Weikert and Blumberg, 2008).

To this day, the correlation between CMV infections and organ rejection is still precisely deciphered. Although, some studies have emphasized on the association present between them, some, others couldn't confirm this relationship (Akposso et al., 1997). The reason for acute KT rejection following CMV infection remains unexplained. However, immune alteration after the CMV infection can be a significant factor. Some studies suggest immunity stimulation after CMV infection by up-regulation of adhesion molecules on epithelial cells inducing the inflammatory process (von Willebrand et al., 1986). The present study investigates the relationship between CMV infection and acute rejection in KT patients in Erbil City, Iraq.

## 2.0 PATIENTS AND METHODS STUDY DESIGN

The study took place between March 2018 and December 2019 on 72 patients receiving a renal allograft in the Erbil city of Kurdistan region of Iraq. This population has been characterized as Middle Eastern descent and ethnic miscegenation. An informed consent was obtained in writing from all the study participants. Data included age and gender of the recipient, type of donor, symptomatic and asymptomatic CMV patients.

Participants were divided into case and control groups. The case group consisted of patients who underwent KT and later were infected with CMV (i.e. presence of CMV-DNA levels more than 2000 copies per millilitres). On the other hand, the control group included KT patients without CMV infection. Patients who had been injected with polyclonal antibodies or experienced any acute rejection episode were excluded from the study.

Patients in both groups were evaluated for signs and symptoms such as weakness, fever and leucopenia and events of the respiratory system were used to suspect the CMV disease. If clinical symptoms pointed towards a CMV infection, blood sample was collected, and analyzed using the qPCR (Rotor-Gene 6000) (Qiagen Corbett,

Hilden, Germany). The presence of DNA higher than 2000 copies per millilitres (the used kit was) confirmed viral infection. Confirmed cases with CMV were instantly stopped with antimetabolite and started with antivirals such as ganciclovir or valganciclovir and continued for at least 21 days.

The data were analyzed statistically using the GraphPad prism software. Parametric tests (Kolmogorov-Smirnov, De-Agostino, Shapiro) were used to know the normal distribution of data. Continuous variables were analyzed by independent student t-test while the categorical variables were analyzed by chi-square test. A P-value less than 0.05 were considered statistically significant.

## 3.0 ETHICAL ISSUES

The study was performed in agreement with the principles of the Declaration of Helsinki, 1996 version and its later amendments and Good Clinical Practice standards. Each patient who took part in the study signed the informed consent form that was permitted by ethical committee of Salahaddin University-Erbil.

## 4.0 RESULTS

Patient disposition has been represented in table-1. In this study, 72 KT recipients were included, of which 43 (59.7%) developed CMV infection, while 29 (40.3%) did not develop CMV infection during the KT (Table 1). The mean age of transplant patients who developed CMV infection was 46.16 ± 1.987, but for those who did not develop CMV infection, the mean was 40.62±2.324. There was no significant difference between the age (p-value=0.076). Regarding patient gender, 76.39% were males, and 23.61% were females.

More than three-fourths of participants (i.e., 76.4%) received a kidney from a living donor, while 23.6% of them received it from deceased donors. Also, overwhelming three-quarters of symptomatic patients (i.e., 74.42%) were detected with CMV, while a quarter of asymptomatic patients (i.e., 25.58%) were diagnosed with CMV (Table 2). Nearly half of the CMV positive group showed fever as a symptom (51.62%), while 18.60%, 4.65% had diarrhea and leukopenia, respectively.

The association between development of infection among recipients from deceased donors significantly higher than recipients from living donors (88% vs. 51%, P = 0.006). The graft rejection rates were significantly more among the CMV positive group as compared to the group did not develop CMV infection (P= 0.029) (Table 2).

## 5.0 DISCUSSION

The infection with CMV has been recognized as the most common viral ailment in patients who received KT. The infection by CMV can significantly enhance morbidity and mortality rates in patients who have undergone KT. While the patients who haven't received prophylaxis against CMV, the disease could develop the infection relatively soon after KT's surgery, usually during the first month (Hartmann et al., 2006). While there are numerous examples of infection and morbidity, there is very less data available on mortality rates. In a particular study by Bradley et al, 17/62 patients who underwent solid organ transplantation died, at a median of 3.8 years. Mortality among those who relapsed was 39% (19/49) vs 36% (43/121) in those who remained relapse-free, indicating that CMV risk and mortality is high even if antivirals are administered (Gardiner et al., 2019), CMV infection occurs more in patients with other organ transplantation than in KT (Danovitch, 2017).

Many risk factors play a role in CMV infection, including the suppressant agents that deplete peripheral and lymph node lymphocytes, co-morbid illness and infection, and donor-recipient graft mismatch. Herein, CMV infection occurred in 59.72% of patients with KT (Table 2). Generally, the prevalence of infection with CMV varies from 10% to 70% at different renal transplant centres This variability refers to several factors, comprising seroprevalence of CMV in the general population, CMV serological status of the organ and donor-recipient, available procedures used for CMV diagnosis, and types of immunosuppressants (Ramanan and Razonable, 2013).

CMV could lead to clinical and subclinical diseases that might invade the body organs. The infection course starts with such signs and symptoms of fever in 51.62% of cases, 18.60% having diarrhea, 4.65% having leukopenia. Some other studies indicated that diarrhea and abnormal haematological parameters being the most apparent signs and symptoms of CMV infection (Jung et al., 2010). Our results in this study mainly support the results presented by Cordero et al, confirming that fever is the most apparent clinical sign observed in patients with CMV

infection followed by diarrhea and then leukopenia (Cordero et al., 2012). A growing body of evidence suggests that latent CMV infection reactivation is triggered by molecular mechanisms triggered by molecular mechanisms which activate CMV gene expression and lytic infection. Viral dissemination is then facilitated by immunosuppression mechanisms. The initial activation of viral gene expression may be mediated by oxidative stress, DNA damage, or inflammatory cytokines which act synergistically (Heald-Sargent et al., 2020).

On the other hand, acute rejection is the significant cause behind allograft failure and could be an important predictor for chronic rejection in the long run. Rapid reduction of kidney activity is the critical characteristic of acute allograft rejection, which is frequently observed in the early six months of the KT (Sagedal et al., 2002a).

Our study assesses the correlation between CMV infection with an acute kidney transplant. Our results showed that those recipients who received the kidney from deceased patients have a significantly lower chance of getting CMV infection than those receiving the kidney from living donors ($P<0.05$) (Table 1). Hence, it indicated that using a living organ increases the likelihood of developing CMV infection, which conflicts with most other studies (Schröeder et al., 2005, de Matos et al., 2017).

Generally, renal rejection has been occurring and is more prevalent in groups with CMV infection. Moreover, previous researches showed that CMV infection is one of the substantial risk factors leading to acute renal allograft rejection. The study carried out by Sagedal et al, 2002 on 477 patients with KT demonstrated that the CMV infection could be used as a predictor for renal rejection (Sagedal et al., 2002b). Besides, the study results were conducted by Toupance et al. confirm that CMV infection, rather than viremia, is a significant risk factor of acute renal rejection (Toupance et al., 2000). Conversely, these results do not match with the results concluded by Michael et al. who after five years of following up refuted this idea and demonstrated that CMV is not a risk factor of chronic or acute rejection as it was thought previously (Dickenmann et al., 2001). The CMV disease could trigger immune system alteration that might augment rejection probability. Several studies revealed that the CMV infection affects the immune system by increasing the immune response and accelerating the synthesis of collagen (Lautenschlager et al., 1999).

The limitation of the study was the small sample size of kidney transplant patients. We recommend using a larger sample size for future studies to improve the clinical evidence, which could lead to improving the clinical evidence, which could lead to the development of recommendations/guidelines on the management of patients undergoing KT at a risk of CMV infection. Furthermore, use of renal biopsy for the diagnosis of the acute rejection in all patients are recommended.

## 6.0 CONCLUSION

Based on the results obtained, it was observed that the CMV infection could be a risk factor in increasing the occurrence of acute rejection of kidney transplant, decreasing acute rejection episode, and controlling CMV infection after KT would increase the chance of KT success.

**Acknowledgements**

We thank all the nurse staff of the hospitals in Erbil city who cooperated in conducting this study.

**Authors' contribution**

SHR carried out the experiment with the help of MHM. HJA wrote the manuscript. ZOK and SZA contributed to data analysis and interpretation of the result. KKH revised the manuscript. SWS and AYK supervised the findings of this paper.

**Conflict of interest**

The authors declare no conflict of interest.

**Table 1**
Characteristics of Patients with CMV infection

| Characteristics | Frequency (%) |
|---|---|
| **No. of patients with CMV infection** | 43 |
| Age (yr.) | |
| Mean (SE) | 46.16 ± 1.987 |
| **Gender** | |
| Male | 31 (72.09%) |
| Female | 12 (27.91%) |
| **Signs and Symptoms/episode** | |
| Symptomatic | 32 (74.42%) |
| Asymptomatic | 11 (25.58%) |
| **Signs and Symptoms** | |
| Fever | 22 (51.62%) |
| Diarrhea | 8 (18.60%) |
| Leukopenia | 2 (4.65%) |

**Table 2**
Comparison between Cytomegalovirus (CMV) pp65-positive group and CMV pp65-negative group.

| Characteristics | CMV Positive (n = 43; 59.72%) | CMV Negative (n = 29; 40.28%) | P-value | CMV Total (n = 72; 100%) |
|---|---|---|---|---|
| Age (yr.) | | | | |
| Mean (SE) | 46.16 ± 1.987 | 40.62 ± 2.324 | 0.076[b] | 43.93 ±1.535 |
| Range | 15.00-69.00 | 8.000-65.00 | | 8.000-69.00 |
| Median | 47.00 | 41.00 | | 44.00 |
| Sex | | | | |
| Male | 31 (72.09%) | 24 (82.76%) | 0.296[a] | 55 (76.39%) |
| Female | 12 (27.91%) | 5 (17.24%) | 0.538 (0.1667 to 1.737) OR | 17 (23.61%) |
| Donor Type | | | | |
| Living | 28 (65.1%) | 27 (93.1%) | 0.006[a] | 55 (76.4%) |
| Deceased | 15 (34.9%) | 2 (6.9%) | 0.1383 "0.028 to 0.663" | 17 (23.6%) |
| Rejection | | | | |
| No | 27 (62.79%) | 25 (86.21%) | 0.029 | 52 (72.22%) |
| Yes | 16 (37.21%) | 4 (13.79%) | 0.2700 "0.079 to 0.917" | 20 (27.78%) |

P values by [a]Chi-square test. And [b]independent student t-test.
CMV, Cytomegalovirus; SE, standard error; yr, year.

**Source of funding**
Not funded.

## REFERENCES

AKPOSSO, K., RONDEAU, E., HAYMANN, J.-P., PERALDI, M.-N., MARLIN, C. & SRAER, J.-D. 1997. Long-term prognosis of renal transplantation after preemptive treatment of cytomegalovirus infection. *Transplantation,* 63**,** 974-976.

ANDREWS, P. A., EMERY, V. C. & NEWSTEAD, C. 2011. Summary of the British Transplantation Society guidelines for the prevention and management of CMV disease after solid organ transplantation. *Transplantation,* 92**,** 1181-1187.

BRENNAN, D. 2001. Cytomegalovirus in renal transplantation. *Journal of the American Society of Nephrology,* 12**,** 848-855.

BROWNE, B. J., YOUNG, J. A., DUNN, T. B. & MATAS, A. J. 2010. The impact of cytomegalovirus infection≥ 1 year after primary renal transplantation. *Clinical transplantation,* 24**,** 572-577.

CLARK, D. A., EMERY, V. C. & GRIFFITHS, P. D. 2003. Cytomegalovirus, human herpesvirus-6, and human herpesvirus-7 in hematological patients. *Semin Hematol,* 40**,** 154-62.

CORDERO, E., CASASOLA, C., ECARMA, R. & DANGUILAN, R. Cytomegalovirus disease in kidney transplant recipients: incidence, clinical profile, and risk factors. Transplantation proceedings, 2012. Elsevier, 694-700.

DANOVITCH, G. M. 2017. *Handbook of kidney transplantation*.

DE KEYZER, K., VAN LAECKE, S., PEETERS, P. & VANHOLDER, R. J. A. J. O. K. D. 2011. Human cytomegalovirus and kidney transplantation: a clinician's update. 58**,** 118-126.

DE MATOS, S. B., MEYER, R. & LIMA, F. W. D. M. 2017. Cytomegalovirus Infection after Renal Transplantation: Occurrence, Clinical Features, and the Cutoff for Antigenemia in a University Hospital in Brazil. *Infection & chemotherapy,* 49**,** 255-261.

DICKENMANN, M. J., CATHOMAS, G., STEIGER, J., MIHATSCH, M. J., THIEL, G. & TAMM, M. 2001. Cytomegalovirus infection and graft rejection in renal transplantation. *Transplantation,* 71**,** 764-767.

FISHMAN, J. A., COSTA, S. F. & ALEXANDER, B. D. 2019. Infection in Kidney Transplant Recipients. *Kidney Transplantation - Principles and Practice***,** 517-538.

GARDINER, B. J., CHOW, J. K., BRILLEMAN, S. L., PELEG, A. Y. & SNYDMAN, D. R. 2019. The impact of recurrent cytomegalovirus infection on long-term survival in solid organ transplant recipients. *Transpl Infect Dis,* 21**,** e13189.

GIAKOUSTIDIS, D., ANTONIADIS, A., FOUZAS, I., SKLAVOS, A., GIAKOUSTIDIS, A., OUZOUNIDIS, N., GAKIS, D., KOUBANAGITI, K., MYSERLIS, G. & TSITLAKIDIS, A. Prevalence and clinical impact of cytomegalovirus infection and disease in renal transplantation: ten years of experience in a single center. Transplantation proceedings, 2012. Elsevier, 2715-2717.

HARTMANN, A., SAGEDAL, S. & HJELMESÆTH, J. 2006. The natural course of cytomegalovirus infection and disease in renal transplant recipients. *Transplantation,* 82**,** S15-S17.

HEALD-SARGENT, T. A., FORTE, E., LIU, X., THORP, E. B., ABECASSIS, M. M., ZHANG, Z. J. & HUMMEL, M. A. 2020. New Insights Into the Molecular Mechanisms and Immune Control of Cytomegalovirus Reactivation. *Transplantation,* 104**,** e118-e124.

HUGHES, D., HAFFERTY, J., FULTON, L., FRIEND, P., DEVANEY, A., LOKE, J., WELSH, K. I., HANDA, A. & KLENERMAN, P. 2008. Donor and recipient CMV serostatus and antigenemia after renal transplantation: an analysis of 486 patients. *Journal of clinical virology,* 41**,** 92-95.

JUNG, G., KIM, S.-J., CHOI, G.-S., MOON, J., KIM, J., SIN, M., KIM, E., KWON, C., JOH, J. & LEE, S.-K. The effect of cytomegalovirus antigenemia titer on the efficacy of preemptive therapy for the prevention of cytomegalovirus disease after kidney transplantation. Transplantation proceedings, 2010. Elsevier, 804-810.

LAUTENSCHLAGER, I., SOOTS, A., KROGERUS, L., INKINEN, K., KLOOVER, J., LOGINOV, R., HOLMA, K., KAUPPINEN, H., BRUGGEMAN, C. & AHONEN, J. 1999. Time-related effects of cytomegalovirus infection on the development of chronic renal allograft rejection in a rat model. *Intervirology,* 42**,** 279-284.

PATEL, R. & PAYA, C. V. 1997. Infections in solid-organ transplant recipients. *Clinical microbiology reviews,* 10**,** 86-124.

RAMANAN, P. & RAZONABLE, R. R. 2013. Cytomegalovirus infections in solid organ transplantation: a review. *Infection chemotherapy,* 45**,** 260.

RAMANAN, P., RAZONABLE, R. R. J. I. & CHEMOTHERAPY 2013. Cytomegalovirus infections in solid organ transplantation: a review. 45**,** 260.

SAGEDAL, S., NORDAL, K. P., HARTMANN, A., SUND, S., SCOTT, H., DEGRÉ, M., FOSS, A., LEIVESTAD, T., OSNES, K. & FAUCHALD, P. 2002a. The impact of cytomegalovirus infection and disease on rejection episodes in renal allograft recipients. *American Journal of Transplantation,* 2**,** 850-856.

SAGEDAL, S., NORDAL, K. P., HARTMANN, A., SUND, S., SCOTT, H., DEGRÉ, M., FOSS, A., LEIVESTAD, T., OSNES, K. & FAUCHALD, P. J. A. J. O. T. 2002b. The impact of cytomegalovirus infection and disease on rejection episodes in renal allograft recipients. 2**,** 850-856.

SCHRÖEDER, R., MICHELON, T., FAGUNDES, I., BORTOLOTTO, A., LAMMERHIRT, E., OLIVEIRA, J., SANTOS, A., BITTAR, A., KEITEL, E., GARCIA, V., NEUMANN, J. & SAITOVITCH, D. 2005. Antigenemia for cytomegalovirus in renal transplantation: choosing a cutoff for the diagnosis criteria in cytomegalovirus disease. *Transplant Proc,* 37**,** 2781-3.

SELVEY, L. A., LIM, W. H., BOAN, P., SWAMINATHAN, R., SLIMINGS, C.,

HARRISON, A. E. & CHAKERA, A. 2017. Cytomegalovirus viraemia and mortality in renal transplant recipients in the era of antiviral prophylaxis. Lessons from the western Australian experience. *BMC infectious diseases,* 17**,** 1-10.

TOUPANCE, O., BOUEDJORO-CAMUS, M. C., CARQUIN, J., NOVELLA, J. L., LAVAUD, S., WYNCKEL, A., JOLLY, D. & CHANARD, J. 2000. Cytomegalovirus-related disease and risk of acute rejection in renal transplant recipients: a cohort study with case-control analyses. *Transplant international,* 13**,** 413-419.

VON WILLEBRAND, E., PETTERSSON, E., AHONEN, J. & HÄYRY, P. 1986. CMV infection, class II antigen expression, and human kidney allograft rejection. *Transplantation,* 42**,** 364-367.

WEIKERT, B. C. & BLUMBERG, E. A. 2008. Viral infection after renal transplantation: surveillance and management. *Clinical Journal of the American Society of Nephrology,* 3**,** S76-S86.