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ORIGINAL ARTICLE

Pre-Transplant Patterns of Cytomegalovirus and Epstein–Barr Virus Serostatus Among Renal Transplant Donor–Recipient Pairs in Iraq

Nadia Hekmat Mohammed [1], Arwa Mujahid Al-Shuwaikh [1,*], Mustafa Rasool Hussein [2]

[1] *Department of Microbiology, College of Medicine, Al-Nahrain University, Baghdad, Iraq*
[2] *Center of Kidney Diseases and Transplantation, Medical City, Baghdad, Iraq*

[*] *Corresponding author email: arwa.mujahid@nahrainuniv.edu.iq*

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ABSTRACT

Background/Aim: Cytomegalovirus (CMV) and Epstein–Barr virus (EBV) are major public health concerns because of their high worldwide prevalence and potential for serious clinical outcomes after transplantation. This study assessed CMV and EBV seroprevalence among renal transplant donors and recipients in Iraq and provided baseline epidemiological data to inform future strategies to reduce high-risk donor–recipient viral mismatches.

Methods: This retrospective cross-sectional serostatus study included 200 renal transplant donor–recipient pairs at Medical City Teaching Hospital and Al-Karama Teaching Hospital between January 2025 and July 2025, with post-transplant CMV DNA assessment in recipients during the first post-transplant year.

Results: Among 200 recipients, 126 (63.0%) were male and 74 (37.0%) were female, with a mean age of 34.5 ± 12.4 years. CMV IgG seroprevalence was high in donors (87.0%) and recipients (83.5%). EBV IgG seroprevalence was approximately 51.0% in both groups. High-risk D+/R- serostatus was observed in 6.0% of CMV pairs and 3.0% of EBV pairs. Among recipients, CMV IgG prevalence was highest in the 41–50-year age group (90.4%), whereas EBV IgG prevalence was highest in the 21–30-year age group (61.7%). No significant association was found with age, sex, smoking, or hemodialysis duration. IgM detection was rare (2.5%) for both viruses. CMV DNA was detected in 21/200 recipients (10.5%) and was significantly associated with diabetes mellitus (p = 0.001).

Conclusion: CMV IgG seroprevalence was high, and EBV IgG seroprevalence was moderate among renal transplant donor–recipient pairs. Post-transplant CMV DNAemia was significantly associated with diabetes mellitus. These findings support routine pre-transplant serological screening, although further studies with standardized PCR monitoring are required.

Key words: Kidney transplantation; Cytomegalovirus; Epstein–Barr virus; Donor–recipient serostatus; Seroprevalence.

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INTRODUCTION

Iraq performed its first successful kidney transplant in 1973, making it a regional pioneer. However, because of the absence of a formal brain-death protocol and the effects of conflict, no deceased donor program currently exists. Transplantation services have since been restored and expanded [1].

Cytomegalovirus (CMV) and Epstein–Barr virus (EBV) are ubiquitous human herpesviruses that establish lifelong latency after primary infection [2, 3]. Global seroprevalence ranges from 60% to 100%, varying by country. Both viruses can reactivate during immunosuppression and resume productive replication [4]. In renal transplantation, CMV is a common opportunistic infection associated with adverse outcomes [5], including a 1.6-fold higher risk of acute graft rejection [6]. EBV is associated with post-transplant lymphoproliferative disorder (PTLD) and chronic effects such as accelerated vasculopathy [7].

Infection may result from latent reactivation during immunosuppression or from donor transmission. To stratify viral risk in transplantation, donor–recipient (D/R) serostatus is used. For high-risk patients (donor positive, recipient negative; D+/R-), prophylactic medication is recommended to prevent infection. In contrast, a preemptive approach may be used for patients at moderate risk (D+/R+ or D-/R+) or low risk (D-/R-) [8, 9].

Given the lack of local epidemiological data, this study aimed to determine the pre-transplant seroprevalence of CMV and EBV among renal transplant donors and recipients in Iraq and to quantify the frequency of potential high-risk serological mismatches. As a secondary aim, post-transplant CMV DNAemia was assessed during the first year after transplantation.

MATERIALS AND METHODS

Sample size calculation

Based on an estimated global CMV IgG seroprevalence of 83% [3], with a 95% confidence level and a 5% margin of error, the minimum required sample size was calculated as 196 recipients using a single-population formula. A total of 200 donor–recipient pairs were enrolled.

Study design and sample collection

This was a retrospective cross-sectional serostatus study of renal transplant donor–recipient pairs, with post-transplant CMV DNA assessment in recipients during the first post-transplant year. From January 2025 to July 2025, 200 renal transplant donor–recipient pairs were enrolled from Medical City Teaching Hospital and Al-Karama Teaching Hospital. Inclusion criteria comprised renal transplant recipients within the first year after transplantation who consented to enrollment, regardless of clinical presentation. Exclusion criteria included incomplete laboratory records, duplicate records, and declined participation.

Recipient data included age, sex, hemodialysis duration, smoking status, diabetes mellitus, immunosuppression regimen, and CMV/EBV serostatus. Diabetes mellitus was defined according to documented medical history and/or recorded use of antidiabetic medication in the medical files. For donors, only CMV and EBV IgG/IgM serostatus data were available; donor demographic data were not available.

Serological data

Pre-transplant CMV and EBV IgG/IgM serostatus for all 200 recipients and 200 donors was obtained from hospital records. These tests had been performed as part of the routine pre-transplant clinical workup using ELISA (BioCheck, Inc., USA, for CMV; Diagnostic GmbH, Germany, for EBV).

Polymerase chain reaction

Blood samples were collected from all 200 recipients for CMV DNA assessment. All recipients underwent CMV PCR during the first post-transplant year without a fixed schedule. Ten milliliters of whole blood were collected in EDTA tubes. Plasma was separated by centrifugation at 13,000 rpm for 5 minutes and stored at -20°C. DNA was extracted using Viral Nucleic Acid Extraction Kit III (Geneaid, Taiwan), according to the manufacturer's instructions. Quantitative real-time PCR was performed using the HCMV qRT-PCR kit (HEALGEN, USA), which targets the CMV UL83 gene with a FAM-labeled probe and includes a β-globin internal control. Master mix preparation, amplification, and result interpretation were carried out according to the manufacturer's protocol. A sample was considered CMV-positive if the FAM channel showed a typical amplification curve with Ct $\leq$ 40. EBV DNA testing was not performed because of technical and financial constraints.

Immunosuppression and antiviral prophylaxis

All transplant recipients received anti-thymocyte globulin (ATG) as induction therapy. Maintenance immunosuppression consisted of tacrolimus and mycophenolate mofetil (MMF), while a minority received tacrolimus alone. After transplantation, recipients received valganciclovir prophylaxis (450 mg orally) according to the routine protocol; however, the exact duration could not be determined because of incomplete medical records and patients' lack of awareness.

Ethical approval was obtained from the Institutional Review Board, College of Medicine, Al-Nahrain University (IRB No.

20241129, dated 21 January 2025). Informed consent was obtained from patients before sample collection.

Data were analyzed using Statistical Package for the Social Sciences version 25 (SPSS Inc., Chicago, IL, USA). Categorical variables were presented as frequencies and percentages. Because donor and recipient observations were paired within each transplant pair, no independent chi-square comparison was performed between donor and recipient seropositivity. The donor–recipient serostatus analysis was intended for descriptive risk stratification rather than formal comparison of marginal donor and recipient seroprevalence. Therefore, donor–recipient serostatus categories were summarized descriptively as n (%) with 95% confidence intervals. Group comparisons for recipient-level analyses were performed using the chi-square (χ^2) test or Fisher's exact test when more than 25% of cells had expected frequencies below five. Statistical significance was defined as $p \leq 0.05$.

RESULTS

Baseline demographic data

This study included 200 renal transplant recipients: 126 males (63.0%) and 74 females (37.0%), with a mean age of 34.5 ± 12.4 years (range: 7–65 years). Most patients were adults (150/200, 75.0%). The majority were from Baghdad (101 patients, 50.5%), followed by Al-Anbar (24, 12.0%), Nineveh (15, 7.5%), Najaf (12, 6.0%), Wasit (11, 5.5%), Karbala (10, 5.0%), Diyala (8, 4.0%), Babil and DhiQar (6 each, 3.0%), Saladin (3, 1.5%), Basra (2, 1.0%), and Maysan (2, 1.0%). All patients underwent hemodialysis before transplantation. Dialysis duration was less than three months in 101 cases (50.5%) and three months or longer in 99 cases (49.5%). Donors and recipients were related in 86 cases (43.0%) and unrelated in 114 cases (57.0%).

CMV and EBV serostatus in donor–recipient pairs

In the overall study population (200 donors and 200 recipients), CMV IgG seroprevalence was 341/400 (85.3%), including 87.0% of donors and 83.5% of recipients. EBV IgG seroprevalence was 203/400 (50.8%), including 51.0% of donors and 50.5% of recipients. IgM positivity was low (2.5% for both viruses in donors and recipients). Donor–recipient pairs were categorized into four groups (D+/R+, D-/R-, D+/R-, and D-/R+), as shown in Table 1. The moderate-risk D+/R+ group constituted the majority for CMV IgG (81.0%; 95% CI: 74.9–86.2) and approximately half for EBV IgG (48.0%; 95% CI: 40.9–55.2), consistent with the background seroprevalence of these latent viruses. The high-risk D+/R- group, in which a seronegative recipient received a graft from a seropositive donor, represented a small proportion of both CMV IgG pairs (6.0%; 95% CI: 3.1–10.2) and EBV IgG pairs (3.0%; 95% CI: 1.1–6.4).

Post-transplant CMV DNAemia in renal recipients

During the first year after transplantation, CMV DNA was detected in 21/200 patients (10.5%). No statistically significant association was observed between donor/recipient CMV serostatus and CMV DNA detection when classified by IgG ($p = 0.780$) or IgM ($p = 0.480$), as shown in Table 2. For CMV IgG serostatus, CMV DNA positivity was observed in 2/12 D+/R- pairs (16.7%), 17/162 D+/R+ pairs (10.5%), and 2/21 D-/R- pairs (9.5%); no DNA was detected in the D-/R+ group. For CMV IgM serostatus, subgroup estimates were based on very small numbers and were interpreted descriptively only. EBV DNA testing was not performed because of technical and financial constraints.

Risk factors associated with CMV/EBV serostatus and CMV DNAemia

As shown in Table 3, no significant associations were found between recipient demographic or clinical variables and CMV or EBV seropositivity, except for CMV DNA detection. Age showed no significant association with CMV IgG ($p = 0.084$), CMV DNA ($p = 0.052$), or EBV IgG ($p = 0.129$). Nevertheless, CMV IgG positivity was highest in the 41–50-year age group (90.4%), while EBV IgG positivity peaked in the 21–30-year age group (61.7%). The lowest seroprevalence for both viruses was observed in patients older than 50 years. Similarly, sex was not associated with CMV IgG ($p = 0.930$), CMV DNA ($p = 0.546$), or EBV IgG ($p = 0.350$).

Diabetes mellitus was significantly associated with CMV DNA positivity: 21/62 patients with diabetes mellitus (33.9%) tested positive compared with 0/138 patients without diabetes mellitus (0.0%; $p = 0.001$). Diabetes mellitus was not associated with CMV IgG ($p = 0.610$) or EBV IgG ($p = 0.480$). All patients underwent hemodialysis, with duration ranging from 1 day to 3 years. Hemodialysis duration was categorized as less than 3 months (n = 101, 50.5%) and 3 months or longer (n = 99, 49.5%) to allow balanced group sizes while maintaining clinical interpretability. Hemodialysis duration showed no association with CMV IgG ($p = 0.902$), CMV DNA ($p = 0.855$), or EBV IgG ($p = 0.572$). Smoking status also showed no statistically significant differences, although smokers had numerically higher CMV IgG (94.7% vs. 82.3%; $p = 0.207$) and EBV IgG (63.2% vs. 49.2%; $p = 0.336$) seroprevalence than non-smokers.

Table 1. Pre-transplant CMV and EBV serostatus according to donor–recipient pairs (n = 200).

Serostatus category	CMV, n (%)	95% CI for CMV	EBV, n (%)	95% CI for EBV
IgG				
D+/R+	162 (81.0)	74.9–86.2	96 (48.0)	40.9–55.2
D+/R–	12 (6.0)	3.1–10.2	6 (3.0)	1.1–6.4
D–/R–	21 (10.5)	6.6–15.6	93 (46.5)	39.4–53.7
D–/R+	5 (2.5)	0.8–5.7	5 (2.5)	0.8–5.7
IgM				
D+/R+	1 (0.5)	0.0–2.8	0 (0.0)	0.0–1.8
D+/R–	4 (2.0)	0.5–5.0	5 (2.5)	0.8–5.7
D–/R–	191 (95.5)	91.6–97.9	190 (95.0)	91.0–97.6
D–/R+	4 (2.0)	0.5–5.0	5 (2.5)	0.8–5.7

Data are presented as n (%). Confidence intervals were calculated using the Wald method with continuity correction. CI, confidence interval; CMV, cytomegalovirus; EBV, Epstein–Barr virus.

Table 2. Association between CMV serostatus and CMV DNA detection.

Serostatus	Total (n = 200)	CMV DNA positive (n = 21)	CMV DNA negative (n = 179)	*p*-value
CMV IgG				
D+/R+	162	17 (10.5)	145 (89.5)	0.780
D+/R–	12	2 (16.7)	10 (83.3)	
D–/R–	21	2 (9.5)	19 (90.5)	
D–/R+	5	0 (0.0)	5 (100.0)	
CMV IgM				
D+/R+	1	0 (0.0)	1 (100.0)	0.480
D+/R–	4	0 (0.0)	4 (100.0)	
D–/R–	191	20 (10.5)	171 (89.5)	
D–/R+	4	1 (25.0)	3 (75.0)	

Data are presented as n (%). *p*-values were calculated using Fisher's exact test. IgM subgroup results should be interpreted cautiously because of small cell counts. CMV, cytomegalovirus.

Table 3. Distribution of CMV IgG, CMV DNA, and EBV IgG by risk factors among renal transplant recipients (n = 200).

Variable	Patients (n = 200)	CMV IgG positive (n = 167)	CMV DNA positive (n = 21)	EBV IgG positive (n = 101)
Age group (years)				
≤ 20	31	25 (80.6)	4 (12.9)	18 (58.1)
21–30	47	41 (87.2)	10 (21.3)	29 (61.7)
31–40	51	42 (82.4)	5 (9.8)	20 (39.2)
41–50	52	47 (90.4)	2 (3.8)	27 (51.9)
> 50	19	12 (63.2)	0 (0.0)	7 (36.8)
p-value	–	0.084[a]	0.052[a]	0.129[a]
Sex				
Male	126	105 (83.3)	12 (9.5)	60 (47.6)
Female	74	62 (83.8)	9 (12.2)	41 (55.4)
p-value	–	0.930[a]	0.546[a]	0.350[a]
Diabetes mellitus				
Yes	62	54 (87.1)	21 (33.9)	28 (45.2)
No	138	113 (81.9)	0 (0.0)	73 (52.9)
p-value	–	0.610[a]	**0.001**[b]	0.480[a]
Hemodialysis duration (months)				
< 3	101	84 (83.2)	11 (10.9)	53 (52.5)
≥ 3	99	83 (83.8)	10 (10.1)	48 (48.5)
p-value	–	0.902[a]	0.855[a]	0.572[a]
Smoking status				
Smokers	19	18 (94.7)	1 (5.3)	12 (63.2)
Non-smokers	181	149 (82.3)	20 (11.0)	89 (49.2)
p-value	–	0.207[b]	0.697[b]	0.336[b]

Data are presented as n (%). [a]*p*-values were calculated using the χ^2 test. [b]*p*-values were calculated using Fisher's exact test. CMV, cytomegalovirus; EBV, Epstein–Barr virus.

DISCUSSION

CMV and EBV infections increase morbidity and mortality in renal transplant recipients, with the highest risk in seronegative recipients who receive a kidney from a seropositive donor (D+/R-) because of the absence of pre-existing virus-specific immunity. Although D+/R- transplantation is not absolutely contraindicated, appropriate prophylaxis and monitoring can mitigate the increased risk of primary infection [10]. In the present study, the high-risk D+/R- group represented only 6.0% of CMV IgG pairs and 3.0% of EBV IgG pairs, consistent with the high seropositivity reported in the Iraqi population [8]. CMV DNA was detected in 2/12 patients (16.7%) in the CMV IgG D+/R- group, although this association was not statistically significant.

The D+/R+ serostatus constituted the majority of CMV IgG pairs (81.0%), with CMV DNA detected in 17/162 patients (10.5%), indicating a continuing possibility of reinfection or reactivation. Pre-existing immunity may not fully protect against different donor-derived strains, and D+/R+ pairs may show higher antigenemia, CMV disease, and acute rejection risk [11], possibly reflecting the effect of immunosuppressive therapy on T-cell immunity [12]. Overall CMV seropositivity among donors and recipients was 85.3% (341/400), with comparable seropositivity between recipients (83.5%) and donors (87.0%), consistent with global averages [3]. Donor CMV seroprevalence in this study (87.0%) also aligns with regional rates reported in Iran [13], Turkey [14], and Jordan [15].

The D-/R- serostatus constituted 10.5% (21/200) of CMV IgG pairs, yet CMV DNA was detected in 2/21 patients (9.5%). This finding may be explained by false-negative serology due to recent infection, transfusion-related exposure, immunosuppression [16], or persistence of specific memory B cells without detectable circulating antibodies [12]. Similarly, Sester et al. [17] reported that CMV-negative recipients of CMV-negative grafts may remain at risk for CMV infection.

For EBV, the D+/R+ category represented nearly half of the cohort (48.0%), while the high-risk D+/R- group was small (3.0%). EBV IgG seropositivity among recipients and donors was 50.5% and 51.0%, respectively, similar to the 57.6% prevalence reported in a study of Iraqi patients with chronic renal disease [18]. Higher rates have been reported in Iraqi blood donors (79.8%) and Iranian populations (85.0%) [19], as well as in Taiwan (88.5%) [20]. These differences may reflect socioeconomic, age-related, or methodological variation. EBV DNA testing was not performed because of technical and financial constraints. Although serology is a straightforward screening tool, EBV DNA monitoring is essential for PTLD surveillance, as EBV infection precedes PTLD in many patients [21].

IgM positivity was sporadic (2.5% for both viruses in donors and recipients), aligning with global estimates [3, 22]. IgM is uncommon in pre-transplant populations, reinforcing the value of IgG-based risk stratification. IgM positivity alone does not confirm active viral replication because persistent IgM, cross-reactivity, and false-positive results may occur. In addition, IgM may be detected in primary infection, reinfection, or reactivation [23].

Age was not significantly associated with CMV or EBV IgG seropositivity ($p = 0.084$ and $p = 0.129$, respectively), although the highest prevalence was observed at 41–50 years for CMV (90.4%) and 21–30 years for EBV (61.7%), consistent with cumulative viral exposure [2, 24, 25]. The decline among patients older than 50 years may reflect survivor bias or the small sample size in this subgroup (n = 19). Sex showed no significant association with CMV IgG or EBV IgG, aligning with some reports [8, 24, 26] but differing from other studies that showed higher rates among females [2, 25].

A strong association was found between diabetes mellitus and CMV DNA positivity: 21/62 patients with diabetes mellitus (33.9%) tested positive compared with none of the 138 patients without diabetes mellitus ($p = 0.001$). Diabetes mellitus induces oxidative stress, endothelial dysfunction, and a pro-inflammatory state that may impair immune surveillance of latent CMV [27]. It has also been identified as an independent risk factor for CMV DNAemia after kidney transplantation [28]. The absence of an association between CMV IgG and diabetes mellitus ($p = 0.610$) suggests comparable prior exposure between groups. No significant associations were observed with hemodialysis duration, consistent with Betjes et al. [29], who reported that CMV seroprevalence among hemodialysis patients mirrors that of the general population. Smoking history also showed no significant association, consistent with another study [8].

The Fourth International Consensus Guidelines on the Management of Cytomegalovirus in Solid Organ Transplantation recommend routine pre-transplant CMV IgG serologic testing for all donors and recipients to establish risk stratification [30]. These recommendations underscore the clinical importance of the present findings and confirm that systematic pre-transplant serological screening is essential even in regions with high background seroprevalence, such as Iraq. These findings may support closer post-transplant monitoring in selected higher-risk groups, but standardized prospective studies are needed before specific monitoring or prophylaxis protocols can be recommended.

This study has several limitations, including its two-center design and possible selection bias. Donor demographic data were not available from hospital records; only donor CMV and EBV serostatus were recorded. EBV DNA testing was not performed because of technical and financial constraints, precluding PTLD risk assessment. CMV PCR testing was performed at unspecified and variable time points within the

first post-transplant year. Incomplete documentation of valganciclovir prophylaxis duration and the absence of standardized CMV PCR monitoring limited the interpretation of post-transplant CMV DNAemia. Small subgroup sizes, particularly for D+/R- pairs, and the absence of long-term outcomes, including renal function, rejection, graft loss, and mortality, also limited the interpretation of the findings. Future multicenter prospective studies should address these limitations through standardized PCR monitoring, complete prophylaxis documentation, systematic collection of donor demographic data, serial EBV DNA testing, and extended longitudinal follow-up.

CONCLUSION

This study found high CMV IgG seroprevalence and moderate EBV IgG seroprevalence among renal transplant donor–recipient pairs. D+/R+ serostatus constituted the majority for CMV and nearly half for EBV, while high-risk D+/R- mismatches were observed in only a small proportion. Post-transplant CMV DNAemia was detected in 10.5% of recipients and was significantly associated with diabetes mellitus. These findings support routine pre-transplant CMV and EBV serological screening for risk stratification. Further prospective studies with standardized CMV and EBV PCR monitoring are required to assess post-transplant viral reactivation and clinical outcomes.

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ETHICAL DECLARATIONS

- **Ethics Approval and Consent to Participate**

  Ethical approval was obtained from the Institutional Review Board, College of Medicine, Al-Nahrain University (IRB No. 20241129, dated 21 January 2025). Informed consent was obtained from patients before sample collection.

- **Consent for Publication**

  Not applicable.

- **Availability of Data and Material**

  The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

- **Competing Interests**

  The authors declare that there is no conflict of interest.

- **Funding**

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- **Use of Generative Artificial Intelligence**

  The authors declare that Paperpal, an AI-assisted academic writing and language-editing tool, was used only to improve English language, grammar, clarity, and academic style, and to improve the manuscript's publication-quality presentation. The tool was not used to generate scientific content, collect data, perform statistical analysis, interpret results, create figures or tables, or draw conclusions. All scientific content, data accuracy, interpretation, and final manuscript approval remain the full responsibility of the authors.

- **Authors' Contributions**

  Nadia Hekmat Mohammed contributed to sample and data collection, laboratory work, and statistical analysis as part of her PhD thesis. Arwa Mujahid Al-Shuwaikh contributed to supervision, study design, and manuscript writing. Mustafa Rasool Hussein contributed to supervision and clinical aspects of sample collection.

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