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Comparison between cytomegalovirus and HIV viral load among HIV patient who underwent antiretroviral therapy

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ABSTRACT

Introduction: Cytomegalovirus (CMV) is one of the most prevalent viral infections in humans. The prevalence is approximately 20 percent among children and nearly 100 percent among adults. Among immunocompetent individuals, CMV infection remains predominantly asymptomatic. However, in immunocompromised patients such as people with HIV (PWH), it can cause end-organ diseases that may be life-threatening. With the increasing administration of antiretroviral therapy (ART), HIV infection can be controlled, and complications from CMV infection have been decreasing. One critical method for monitoring CMV infection is identifying CMV viremia. This study aims to investigate whether CMV viremia persists in PWH undergoing ART and to explore its relationship with HIV viral load. The research seeks to provide insights to guide clinical management of CMV reactivation in this patient population

Methods: This cross-sectional study analysed archived plasma samples to detect and compare CMV viral load and HIV viral load among PWH undergoing ART. The samples were archived biological materials collected for HIV viral load detection. All samples were previously tested by PCR to detect CMV. Data were analysed using the Mann-Whitney test and Spearman correlation test.

Result: Among 67 total samples, seven were identified as CMV positive, displaying various viral load concentrations. Analysis using the Mann-Whitney test demonstrated a statistically significant difference between variables, while the Spearman correlation test showed no correlation between them. This indicated that CMV viremia may not be directly influenced by HIV infection.

Conclusion: Despite the significant difference between variables, the CMV viral load among PWH undergoing ART was not correlated with HIV viral load status. These findings suggest that CMV monitoring should be considered independently of HIV viral load status. However, the limited sample size suggests caution in generalizing these findings.

Keywords: Cytomegalovirus, Cytomegalovirus viral load, HIV, HIV viral load, antiretroviral therapy, Q-PCR.
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INTRODUCTION

Cytomegalovirus (CMV) is a prevalent human viral infection characterized by a lifelong latent infection with intermittent viral shedding.[1–3] CMV infections manifest with varying clinical presentations depending on the host's immunological status. Among immunocompetent individuals, CMV infections typically remain latent, presenting with no symptoms or mild, nonspecific febrile illness. In contrast, immunocompromised patients may experience severe infections leading to significant end-organ diseases with potential poor outcomes.[1,2,4]

Human immunodeficiency virus (HIV) is a pathogen that fundamentally alters the human immune system. Its primary target is CD4+ T lymphocyte cells, a critical component of immune defence. HIV predominantly replicates in activated T cells, while remaining latent in inactivated cells. Infection of CD4+ T lymphocytes can lead to a significant reduction in cell count, potentially falling below 200 cells/µl.[2,5] Consequently, people with HIV (PWH) become vulnerable to various clinical complications, including opportunistic infections, malignancies, neurological disorders, and constitutional syndromes.[2] Among these, CMV infection represents one of the most frequent opportunistic infections.[6]

Opportunistic CMV and HIV co-infections are prevalent among PWH.[7] Moreover, CMV viremia may significantly increase mortality rates in this population. HIV replication is facilitated by activated CD4+ T lymphocytes, and the presence of CMV triggers T cell activation.[4,6] Consequently, PWH with CMV co-infection demonstrate accelerated disease progression and a higher risk of developing acquired immunodeficiency syndrome (AIDS). Conversely, HIV infection reduces CD4+ T cell counts, which can precipitate CMV reactivation. CMV reactivation among immunosuppressed

patients manifests with more severe clinical manifestations and may result in mortality, particularly when CD4+ cell counts fall below 100 cells/µl.[8–10]

Antiretroviral combination therapy (ART) has demonstrated substantial benefits in mitigating CMV infection complications. Since its introduction between 1995 and 1996, ART has resulted in a significant reduction in CMV end-organ disease incidence among HIV patients.[4] This phenomenon is likely attributed to immune system recovery, which effectively inhibits CMV reactivation. CMV replication is additionally influenced by HIV DNA levels, with research indicating that patients with elevated HIV DNA levels exhibit subclinical CMV replication.[4,6] Consequently, suppression of HIV viral load correlates with decreased CMV replication. This study aims to investigate whether CMV viremia persists in PWH undergoing ART and to explore its relationship with HIV viral load. The research seeks to provide insights to guide clinical management of CMV reactivation in this patient population.

METHODS

Study Design and Sample Selection

This cross-sectional study analysed stored plasma samples from PWH receiving antiretroviral therapy. Samples were collected between June and July 2023. The study period was based on the sample availability with sample size calculation based on the expected CMV prevalence of 10-15% in HIV patients on ART. All samples had previously undergone HIV viral load testing.

Sample selection criteria included samples with HIV viral load results ≥40 copies/mL, which is the lower limit of detection for the HIV viral load assay used in routine monitoring. The samples should have a minimum plasma volume of 200 µL, which is the required volume for DNA extraction procedures using the GeneProof DNA extraction kit. This extraction kit is the recommended kit to be used in conjunction with the GeneProof Cytomegalovirus PCR kit.

Control samples consisted of plasma from healthy individuals, confirmed HIV-negative using the Abbot Bioline™ HIV 1/2 3.0 rapid diagnostic kit

Laboratory Procedures

All samples underwent quantitative PCR (Q-PCR) testing following a standardized protocol such as DNA extraction, PCR testing, and viral load quantification. DNA extraction was performed using the GeneProof PathogenFree DNA Isolation Kit, strictly adhering to manufacturer protocols. PCR testing was conducted using the GeneProof Cytomegalovirus (CMV) PCR kit (CMV/ISEX/100) with manufacturer-specified amplification parameters. This PCR kit is equipped with a positive control set. Testing was performed on the CFX Opus 96 Real-Time PCR Detection System. CMV viral load quantification was calculated using the following standardized formula: Copies per millilitre (cp/ml) = (Sample Concentration × Elution Volume) ÷ Isolation Volume (GeneProof CMV PCR Kit manual)

Statistical Analysis

Statistical analysis employed two primary tests, based on data distribution characteristics. The Mann-Whitney test was chosen for comparing the CMV viral

Table 1. Patients Characteristics of CMV-Positive Samples

Sample ID	Age (Years)	Sex	ART Duration
CMV-21	34	M	4 years
CMV-23	34	M	5 years
CMV-54	26	F	No data
CMV-55	36	M	No data
CMV-57	30	M	6 months
CMV-58	39	F	6 months
CMV-61	56	M	1 year

Table 2. CMV Q-PCR Results and HIV Viral Load of the CMV Positive Samples

Sample ID	CMV DNA Ct	CMV Viral Load (copies/mL)	HIV Viral Load (copies/mL)
CMV-21	39.95	36	60,433
CMV-23	35.69	698	283
CMV-54	39.35	55	263,674
CMV-55	39.41	53	267,451
CMV-57	31.73	10,950	892
CMV-58	32.05	8,785	40
CMV-61	40.17	31	161,432

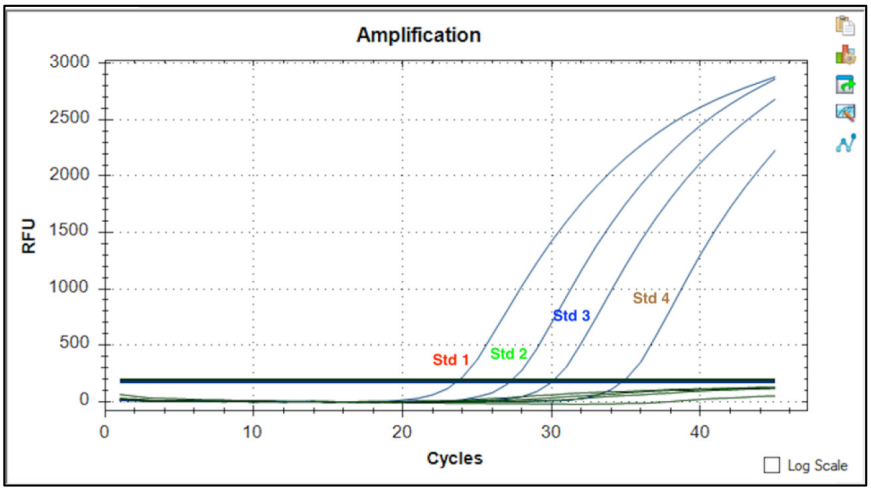

Figure 1. Amplification Curve of PCR kit Calibrator

loads between samples with different HIV viral load categories, since the data did not follow a normal distribution. The Spearman correlation test was selected to assess the relationship between HIV and CMV viral loads, as it is appropriate for non-parametric data.

All statistical analyses were conducted using IBM SPSS statistic version 25.0 for Windows. A p-value <0.05 was considered statistically significant.

RESULTS

Of the 72 initial samples with HIV viral load ≥40 copies/mL, 67 met the study inclusion criteria. The samples were categorised based on HIV viral load levels: 24 samples (36%) showed 40-75 copies/mL (classified as "not detected"), 26 samples (39%) showed 75-10,000 copies/mL (classified as low viral load), and 17 samples 25% showed >10,000 copies/mL (classified as high viral load).

Q-PCR testing identified seven CMV-positive samples (**Fig. 3**) with varying characteristics (**Table 1**). The Cycle Threshold (Ct) values ranged from 31.73 to 40.17 (**Table 2**). Meanwhile, the CMV viral load concentrations varied from 31 to 10,950 copies/ml. The positivity was determined based on the amplification curve according to the PCR kit manufacturer's manual. Notably, the sample with the highest CMV viral load (10,950 copies/ml) corresponded to a low HIV viral load (892 copies/ml). Conversely, the sample with the highest HIV viral load (267,451 copies/ml) demonstrated a relatively low CMV viral load of 53 copies/ml (**Fig. 1**).

Mann-Whitney test revealed statistically significant differences between HIV and CMV viral loads ($p<0.05$). Spearman correlation test found no significant correlation between the two viral loads ($p>0.05$). Statistical analysis revealed a significant difference in viral loads, but no correlation between CMV and HIV viral loads.

DISCUSSION

Our findings diverge from previous research by Gianella and Letendre (2016), which suggested that suppressed HIV would lead to immune system recovery

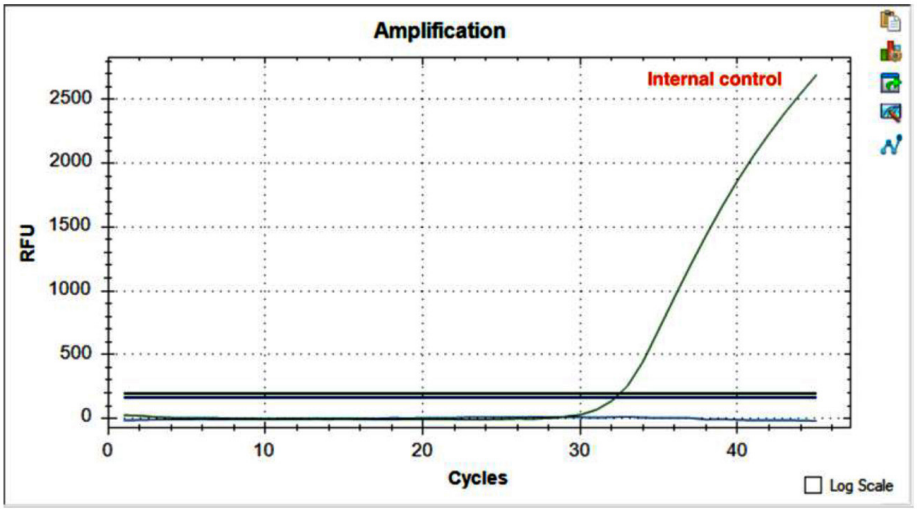

Figure 2. Amplification Curve of the Negative Control

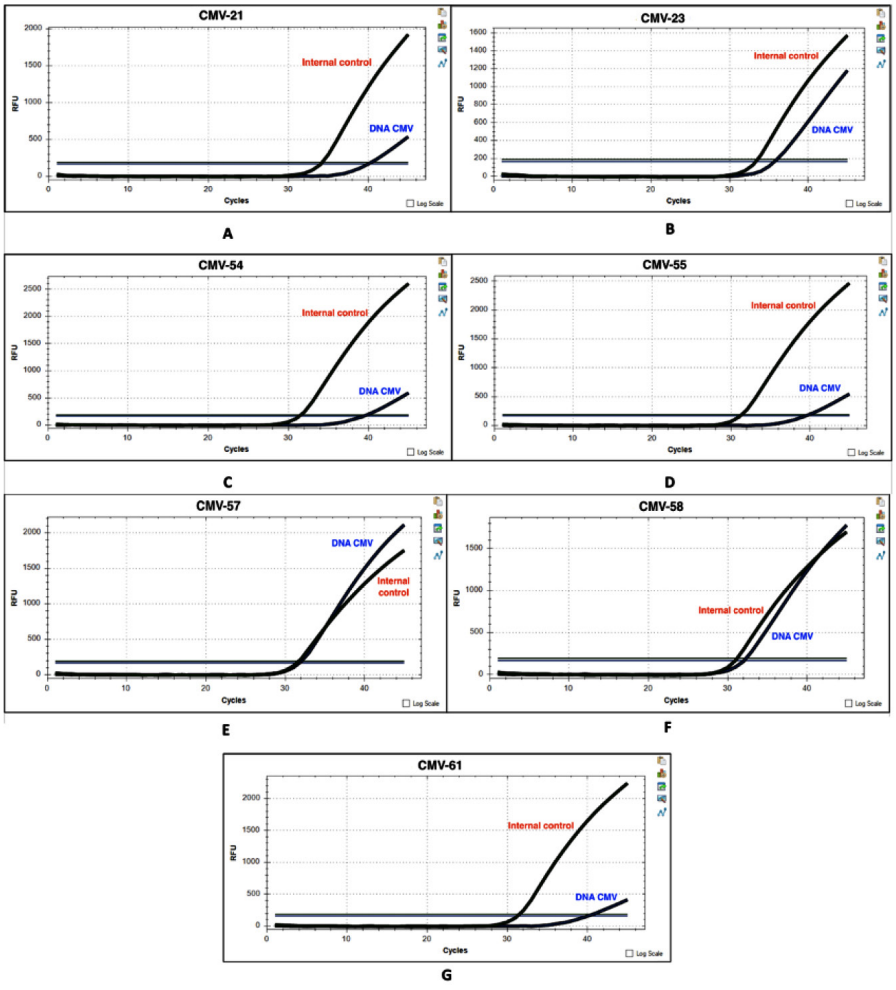

Figure 3. Amplification Curve of CMV Positive Samples
Legends: A: CMV-21 sample; B: CMV-23 sample; C: CMV-54 sample; D: CMV-55 sample; E: CMV-57 sample; F: CMV-58 sample; G: CMV-61 sample

and subsequent CMV suppression.[4] However, this study evaluated the CMV levels and HIV RNA in peripheral blood mononuclear cells of 107 HIV-infected patients who started ART within the first month of HIV infection. Instead, our results align more closely with Levi et al. (2022),[11] who demonstrated that PWH receiving ART may experience persistent CMV replication. This study identified CMV DNA in plasma in the PWH with ART. The CMV reactivation among most of the patients happened during the first six months of the ART, prior to recovery of the anti-CMV immunity. CMV already infected most patients in the study before starting the HIV treatment. After beginning the antiretroviral therapy, the blood test showed that their CMV infection levels were higher.[11] Although the study by Levi et al seems to support the result of this study, the CMV viremia data were not available in this study due to the CMV-positive samples were referred samples from other health facilities. Therefore, the clinical data, including the opportunistic infection screening data were incomplete.

The complex relationship between CMV and HIV infections involves several critical mechanisms. Both viruses are associated with inflammation and immune aging where the CMV infection will bring more severe effect than HIV. CMV infection can trigger immune senescence that will lead to the slower immune recovery in individuals with HIV and CMV coinfection. Furthermore, CMV presence can activate CD4+ T cells, potentially accelerating HIV progression, activated CD4+ T cells facilitate HIV replication.[4] Overall, the processes. will induce the progressivity of the HIV infection with the infected individuals have a higher risk to develop AIDS state.[1]

This study utilized plasma samples to detect CMV positivity and viral load. Quantitative PCR (Q-PCR) serves as a surrogate marker for detecting the presence of CMV end-organ diseases.[12,13] Mizushima et al. (2015)[13] demonstrated that plasma samples possess high diagnostic value in identifying CMV retinitis and other CMV end-organ diseases among patients with advanced HIV-1 infections. Their research revealed that CMV DNA plasma levels exceeding 10.086 IU/mL and 2.946 IU/mL exhibit high specificity (94,1-95,3 %) as markers of CMV end-organ diseases. Conversely, undetectable CMV DNA loads indicate the absence of CMV end-organ diseases with 91.3-91.9% sensitivity.[13]

In addition to plasma samples, whole blood can be used to detect CMV DNA. Rzepka et al. (2022)[14] conducted a real-time PCR test on 156 whole blood samples from 53 patients with latent CMV. The samples were subsequently processed to obtain plasma and tested using real-time PCR. Of the samples, 59 (37.8%) plasma samples showed undetectable CMV DNA, while 77 (79.4%) of the positive samples demonstrated higher DNA levels in whole blood. However, their analysis found no correlation between CMV viral load and sample type, suggesting that patient clinical conditions may have influenced the results.[14]

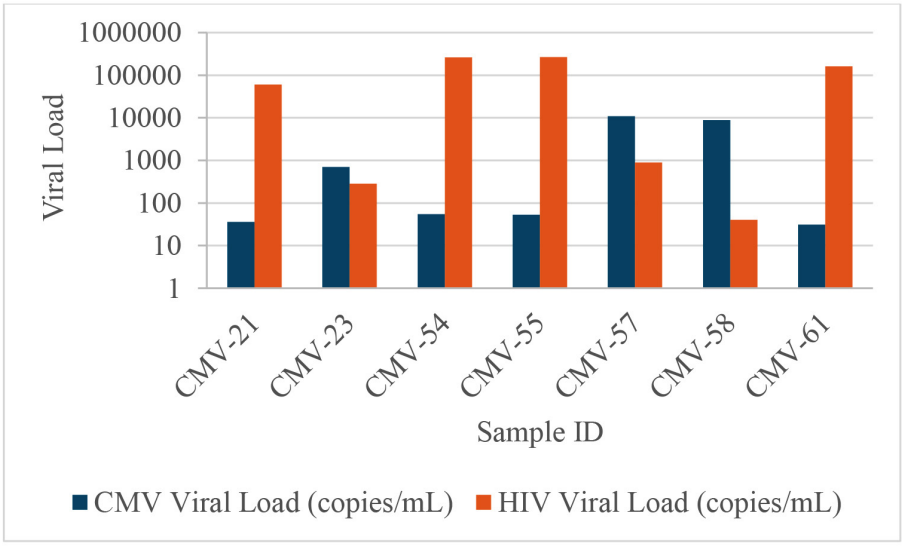

Figure 4. Comparative Viral Load Analysis of CMV and HIV Across Clinical Samples

Table 3. Mann-Whitney Test Result

Independent Variable	Dependent Variable	p-value
HIV viral load	CMV viral load	<0.001

Table 4. Spearman Correlation test Results

Variables	r	p-value
HIV viral load CMV viral load	0.176	0.127

Study limitations include the small number of CMV-positive samples and the cross-sectional study design. Future research should employ longitudinal studies with larger cohorts to provide more comprehensive insights into CMV-HIV interactions.

CONCLUSION

This investigation underscores the importance of comprehensive CMV monitoring among PWH undergoing antiretroviral therapy. The findings reveal that CMV viral load may occur independently of HIV viral load. Key findings include the identification of CMV viremia in 10.4% of HIV patients on ART with viral loads ranging from 31 to 10,950 copies/mL.

Future research should prioritize longitudinal studies with larger cohorts to further elucidate the intricate immunological interactions between CMV and HIV.

DISCLOSURES

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Conflict of Interest
None of the author has any conflict of interest to disclose.

Author Contribution
All authors prepare the manuscript and agree for this final version of manuscript for submission to this journal.

Ethic Approval
This study was approved by the Health Research Ethics Committee of Medical Faculty of Universitas Brawijaya, Approval number: No. 377/EC/KEPK-PSPDS/11/2023, Date: 29 November 2023

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