Frequency of Cytomegalovirus Viral Load in Iranian Human Immunodeficiency Virus-1-Infected Patients with CD4+ Counts <100 Cells/mm³

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Keywords

Cytomegalovirus infection · Human immunodeficiency virus-1 · Retinitis · Viral load · Epidemiology · Virology

Abstract

Objectives: The aim of present work was to assess cytomegalovirus (CMV) viremia in Iranian human immunodeficiency virus (HIV)-1-infected patients with a CD4+ count <100 cells/mm³ and to explore whether CMV DNA loads correlate with CD4+ cell counts or associated retinitis. **Methods:** This study was conducted at the AIDS research center in Iran on HIV-1-infected patients with CD4+ count <100 cells/mm³, anti-retroviral therapy-naïve, aged ≥18 years with no previous history of CMV end-organ disease (CMV-EOD). **Results:** Thirty-nine of 82 patients (47.56%) had detectable CMV viral load ranging from 66 to 485,500 IU/mL. CMV viral load in patients with retinitis ranges from 352 to 2,720 IU/mL, and it was undetectable in 2 patients. No significant associations between CMV viremia and CD4+ cell count was found (p value = 0.31), whereas significant association of CMV viremia in HIV-infected patients with retinitis was found (p < 0.02). **Conclusions:** We estimated the frequency of CMV viral load infection in Iranian HIV-1-infected patients with a CD4+ cell count <100

mm³/mL in the largest national referral center for HIV-1 infection in Iran. Further research is required on the relevance of CMV viral load in diagnostic and prognostic value of CMV-EOD.

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Introduction

Roughly 37 million people are infected with human immunodeficiency virus (HIV) globally [1]. Cytomegalovirus (CMV) is a ubiquitous human herpes virus with a prevalence rate of 60 and 95% in adult individuals in developed and developing countries, respectively [2, 3]. Primary CMV infection in immunocompetent people could be mild or subclinical, although primary CMV infection in immunocompromised individuals can produce diverse life-threatening end-organ diseases (EODs) [4]. The clinical presentations of CMV-EOD include CMV retinitis, pneumonitis, nephritis, encephalitis, esophagitis, hepatitis, colitis, myocarditis, and others [5]. Although antiretroviral therapy has dramatically reduced its incidence among HIV-infected patients [6, 7], CMV disease remains a threat in patients with immunodeficiency.

Table 1. Characteristics of 39 patients with detectable CMV viral load

Patient, n	Sex	Age, years	CD4 ⁺ cell count, cells/mm ³	CMV-EOD	CMV viral load, IU/mL
1	Male	34	48	–	1,240
2	Male	41	53	–	580
3	Male	33	20	–	940
4	Male	33	72	Encephalitis	72
5	Male	35	32	–	2,480
6	Male	43	23	–	190
7	Male	30	38	Retinitis	1,480
8	Male	57	60	–	720
9	Male	28	66	–	110
10	Male	52	47	Retinitis	2,720
11	Male	44	40	–	875
12	Male	35	18	Colitis	590
13	Female	30	39	–	654
14	Male	44	43	–	350
15	Male	36	51	Retinitis	740
16	Male	34	38	–	556
17	Male	38	5	–	820
18	Female	47	25	Colitis	485,500
19	Male	41	74	–	235
20	Male	34	81	–	1,140
21	Male	34	46	Retinitis	352
22	Male	48	19	–	565
23	Male	45	37	–	573
24	Male	39	41	–	430
25	Male	39	10	Retinitis	872
26	Male	36	54	–	680
27	Male	47	68	–	920
28	Female	40	33	Retinitis	1,052
29	Male	48	46	–	85
30	Male	44	54	–	235
31	Male	42	16	–	560
32	Male	31	54	Retinitis	650
33	Male	45	47	–	724
34	Male	29	62	Colitis	66
35	Male	41	77	–	475
36	Male	38	8	–	126
37	Male	44	48	Encephalitis	530
38	Male	40	73	–	615
39	Male	35	64	–	374

CMV, cytomegalovirus; EOD, end-organ disease.

ciency [8]. CMV disease typically occurs when the CD4⁺ cell count falls below 100 cells/mm³ in HIV-positive patients [9]. Thus, earlier diagnosis and pre-emptive treatment may help reduce the morbidity and mortality in these patients as seen in transplantation [10]. The goal of the present investigation was to assess CMV viremia in

Iranian HIV-1-infected patients with CD4⁺ counts <100 cells/mm³ and to explore whether CMV DNA loads correlate with CD4⁺ cell counts or associated retinitis. To the best of our knowledge, this is the first demonstration of the frequency of CMV viral load in Iranian HIV-1-infected patients with CD4⁺ counts <100 cells/mm³.

Materials and Methods

Study Design

This study was conducted at the AIDS research center in Imam Khomeini Hospital in Iran, affiliated to the Tehran University of Medical Sciences (TUMS) between April 2016 and April 2018. The study population comprised 82 HIV-1-infected patients with CD4⁺ counts <100 cells/mm³, antiretroviral therapy-naïve (either being poor medication adherence or being new patients) aged ≥18 years, and patients with no previous history or evidence of CMV-EOD. The current study was approved by the Ethical Committee of Tarbiat Modares University (IR.TMU.REC.1394.308), and all patients who met the entry criteria signed an informed consent prior to the enrollment.

CMV Viremia Assay

Three milliliters of whole blood samples were mixed promptly with the EDTA from the eligible patients, and plasma DNA extraction was carried out using a DNA extraction kit (Invisorb® Blood Universal Kit, strateg) according to manufacturers' instruction. Quantitative plasma CMV DNA PCR was measured using a GeneProof RT-PCR kit and run on the StepOne Real-Time PCR System. The assay had a lower detection limit of 0.907 copies DNA per µL. The PCR conditions were as follows: 10 min at 95°C, followed by 45 cycles of 5 s at 95°C, 40 s at 60°C, and 40 s at 60°C.

Statistical Analysis

Statistical analysis was performed using SPSS version 24 (IBM, Armonk, NY, USA). A *p* value <0.05 was considered significant. The Kolmogorov-Smirnov and independent *t* tests were used to assess the association of CMV viremia with the CD4⁺ cell count and retinitis, respectively.

Results

Demographic Characteristics

The details of the basic demographic and laboratory data of the HIV-1-infected patients are shown in our previously published article [11]. In brief, among 82 HIV-1-infected patients, there were 67 (81.7%) male and 15 (18.29%) female, with a mean age of 38 ± 7 years. All of them had CD4⁺ cell counts of <100/mm³, with a mean CD4⁺ cell count of 46.84 cells/mm³. The status of CMV IgG was as follows: 71 (86.5%) patients were positive, 2 (2.4%) patients were negative, and 9 (10.9%) patients were undetermined.

Table 2. Characteristics of HIV-1-infected patients with retinitis and the quantity of CMV DNA along with their HLA type

Patient, <i>n</i>	Age, years	Sex	CD4 ⁺ cell count, cells/mm ³	CMV-EOD	CMV viral load, IU/mL
1	52	Male	47	Retinitis	2,720
2	30	Male	38	Retinitis	1,480
3	41	Male	53	Retinitis	–
4	36	Male	51	Retinitis	740
5	34	Male	46	Retinitis	352
6	39	Male	10	Retinitis	872
7	40	Female	33	Retinitis	1,052
8	31	Male	54	Retinitis	650
9	44	Male	49	Retinitis	–

CMV, cytomegalovirus; EOD, end-organ disease; HIV, human immunodeficiency virus.

CMV Viral Load, CD4⁺ Cell Count, and CMV-EOD

Table 1 shows the quantities of CMV viral load, age, sex, CD4⁺ cell count, and CMV-EOD in 39 patients with detectable CMV viral load.

CMV Viremia Status

Of the 82 plasma samples tested for CMV viremia, 39 patients (47.56%) had detectable viral load ranging from 66 to 485,500 IU/mL. Table 2 demonstrates the characteristics of patients with retinitis and their quantity of CMV DNA. CMV viral load in patients with retinitis ranges from 352 to 2,720 IU/mL, and it was undetectable in 2 patients. No significant associations between CMV viremia and CD4⁺ cell count were found (p value = 0.31), whereas a significant association of CMV viremia in HIV-infected patients with retinitis was found ($p < 0.02$).

Discussion

Our study of 82 HIV-infected patients with CD4⁺ counts <100 cells/mm³ who were screened concomitantly for the presence of CMV viremia showed that the overall prevalence of retinitis in this population was 10.9% at 1 year. CMV retinitis is a common and relatively late manifestation of HIV-associated ocular disease [12]. A systematic review of HIV-related CMV retinitis in resource-limited settings found that the prevalence of CMV retinitis remains high, especially in Asian countries with a pooled prevalence of 14% [13]. In fact, compared to sur-

veys conducted in South Africa (2.6%) [14], Tanzania (1.3%) [15], Nigeria (1.2%) [16], and Malawi (4.9%) [17], much higher levels of HIV-related CMV retinitis in Asian populations such as Shanghai, China (7.6%) [18], South Korea (11%) [19], Thailand (19.8%) [20], and Myanmar (24%) [21] were reported. Early screening of CMV retinitis among patients with CD4⁺ counts <100 cells/mm³ is an important issue for preventing vision loss, but it is rarely done in practice [22]. In Abdollahi et al.'s [23] study, the prevalence of CMV retinitis in Iranian HIV-1-infected patients with CD4⁺ counts 143 ± 84 cells/ μ L was reported 1.88%. It could be assumed that the main difference between the latter and the current study is considered to be in the CD4⁺ cell count. This is in line with the findings that a CD4⁺ cell count less than 100 cells/ μ L is associated with ophthalmic manifestations, as shown by Lai et al. [24] with a mean CD4⁺ count of 85.9 cells/ μ L and Gharai et al. [25] with a median CD4⁺ count of 75 cells/mm.

The current study is the first demonstration of the frequency of CMV viral load in Iranian HIV-1-infected patients with CD4⁺ counts <100 cells/mm³. It helps increase the data on the association between CMV viremia and CD4⁺ cell count. In the present study, CMV viremia was found in the approximately half of the patients (47.56%) over a period of 1 year with a range of 66–485,500 IU/mL. From a limited number of published data in developing countries, the prevalence of CMV viremia in HIV-infected patients was reported to be 42.4% in Cambodia [26], 5.2% in South Africa [27], and 26.3% in Thailand [5]. A number of studies have shown that patients with detectable levels of CMV DNA in the blood and CD4⁺ T-cell counts <100 cells/ μ L may be at increased risk of developing CMV diseases [28–30]. For example, the results of a recent study conducted by Tang et al. [30] showed that HIV-infected patients with CD4 cell counts $<50/\mu$ L along with a CMV DNA level $>2,000$ copies/mL are at higher risk of developing CMV retinitis. Accordingly, in our previous study, 14 patients were diagnosed with CMV-EOD, in whom retinitis occurred in the greatest number of patients (64.28%), followed by colitis (21.42%) and encephalitis (14.28%) [11]. However, there was an unusual finding in our study that CMV DNA was undetectable in 2 patients with retinitis. Likewise, in El Amari et al.'s [31] study, CMV DNA was undetectable in 17% of the patients who developed CMV-EOD. In this regard, they argued about the delay between CMV DNA measurement and the occurrence of the disease (median 141 days). Another study pointed out about the transient occurrence of CMV viremia and missing its detection due to intermittent

sampling [32]. Further studies observing more cases are needed to explore the reason of this issue. Moreover, our results did not support the association between CMV viremia and the CD4+ cell count (p -value = 0.31). It means that the presence of CMV in the blood is independent of the CD4+ cell count. However, there was a significant association between CMV viremia and retinitis. The results of studies by Erice et al. [33] and Wohl et al. [34] showed that the predictors of CMV-EOD disease include detectable CMV DNA with a viral load greater than 200 copies/ μ L. In contrast, Wiselka et al. [35] reported that CMV viremia had a poor predictive value for subsequent CMV disease in a cohort of patients with advanced HIV infection. Other than the predictive value, the high diagnostic value of CMV viremia for the diagnosis of CMV-EOD has been demonstrated in some studies [36, 37]. However, further research is required on the relevance of CMV viral load in diagnostic and prognostic value of CMV-EOD [33].

In this study, we tried to investigate the effect and importance of CMV existence as an important cofactor in HIV disease, with an influence on clinical outcome. We estimated the frequency of CMV viral load infection in Iranian HIV-1-infected patients with CD4+ cell counts less than 100 mm³/mL in the largest national referral center for HIV-1 infection in Iran. Our data contribute to the significant association between CMV viremia and retinitis. But, the presence of CMV in the blood was independent of the CD4+ cell count. Based on the present finding, one can say that when the CMV viral load is >352 IU/mL in HIV-1-infected patients, ophthalmic examination is

endorsed, which can be helpful for patients' management. Due to the relatively small sample size, further research in larger multicenter clinics is required on the relevance of CMV viral load in diagnostic and prognostic value of CMV-EOD in order to verify these findings.

Statement of Ethics

The current study was approved by the Ethical Committee of Tarbiat Modares University (IR.TMU.REC.1394.308).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Hoorieh Soleimanjahi designed the study and is the corresponding author. Mohammad Reza Jabbari participated in sample collection and carried out the laboratory experiments. Somayeh Shatizadeh Malekshahi wrote the manuscript. Mohammad Gholami and Leila Sadeghi coworked on RT-PCR experiments. Minoo Mohraz carried out patients' recruitment and made diagnostic evaluations. All authors read and approved the final manuscript.

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