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Cytomegalovirus End Organ Disease in Iranian HIV-1 Infected Patients with CD4+ Cell Counts Less Than 100 Cells/mm^3

Mohammad Reza Jabbari [1], Hoorieh Soleimanjahi [1,*], Mahboubeh Hajiabdolbaghi [2], Mohammad Sarraf-Shirazi [2] and Somayeh Shatizadeh Malekshahi [1]

[1]Department of Virology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[2]Iranian Research Center for HIV/AIDS, Tehran University of Medical Sciences, Tehran, Iran

[*]*Corresponding author*: Department of Virology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran. Email: soleim_h@modares.ac.ir

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Abstract

Background: Cytomegalovirus End-Organ Disease (CMV-EOD) is a seriously debilitating illness in patients with advanced HIV-1 infection, typically occurring with CD4+ cell counts of < 100 cells/mm^3.
Objectives: This study aimed to evaluate the prevalence of CMV-EOD in adult patients with advanced HIV-1 infection (CD4+ count < 100 cells/mm^3).
Methods: Using a convenience sampling method, a cross-sectional study was conducted on 82 patients with advanced HIV-1 infection in Iran between April 2016 and April 2018. We collected baseline characteristics (age, sex, route of HIV-1 transmission, Hepatitis C Virus (HCV) infection, Hepatitis B Virus (HBV) infection, CMV IgG, and treatment status for HIV-1 infection) and CD4 counts. The entire patients underwent clinical examinations for the diagnosis of CMV-EOD by experienced clinicians. Statistical analysis was used to measure the differences between categorical variables and the outcome of CMV-EOD diagnosis.
Results: Fourteen (17.07%) out of 82 HIV-1-infected patients were diagnosed with opportunistic infection due to CMV. Among 14 patients with CMV-EOD, retinitis occurred in the majority of patients (64.28%), followed by colitis (21.42%) and encephalitis (14.28%). No significant correlation was found between the outcome of CMV-EOD and HBV infection (P = 1.00), HCV infection (P = 0.55), and treatment status for HIV-1 infection (P = 0.53). We detected CMV-EOD more frequently among injecting drug users and patients with positive CMV-IgG (P = 0.12 and P = 0.41, respectively). The ophthalmic examination had clinical usefulness for HIV-1 positive patients with CD4 counts of < 100/mm^3.
Conclusions: It is assumed that the CD4+ cell count is not the sole predictor of the risk of developing CMV-EOD. Further large-scale studies are required for a better understanding of risk factors involved in the occurrence of CMV-EOD in HIV-1 positive patients.

Keywords: AIDS-related Opportunistic Infections, CD4+ Lymphocyte Count, Epidemiology

1. Background

In the state of immunodeficiency, CMV contributes to accelerated HIV-1 progression with more occurrence of AIDS-related events and an array of serious End-organ Diseases (EOD) (1). It is known that CMV-EOD is a seriously debilitating illness in patients with advanced HIV-1 infection, which typically occurs with CD4+ cell counts of < 100 cells/mm^3 (2). It is responsible for many different clinical outcomes including encephalitis, retinitis, esophagitis, pneumonitis, colitis, and hepatitis (3). As the most frequent manifestation in HIV infected subjects, CMV retinitis occurs in 85% of all CMV-EOD cases. In individuals with advanced HIV-1 infection, CMV retinitis can lead to visual loss. In addition, extra-ocular CMV disease contributes to AIDS-related morbidity and mortality. Gastrointestinal tract involvement represents 10% of CMV cases in AIDS patients. The remaining manifestations include hepatitis, pneumonitis, neurological disorders and adrenalitis (4). In addition, various reports showed that CMV implicated as a cofactor for rapid HIV-1 disease progression (5-7). Because of interactions between these two viruses, CMV could activate latent HIV proviral DNA either by presenting its transactivator proteins into the same cell or by stimulating the production of inflammatory cytokines, driving viral propagation (8). Potent Antiretroviral Therapy (ART) has led to a substantial decline in the incidence of CMV-EOD among HIV-infected subjects compared to those in the pre-ART era (9-11). Nonetheless, CMV still occurs in the selected groups of HIV-infected patients because of drug failure, antiviral resistance, treatment non-adherence, or in-

tolerance to prescribed regimens (12). The monitoring of CMV infection is important in specific populations, e.g., hematopoietic stem cell transplant recipients, newborns with congenital infections and CMV-EOD patients, which can affect public health (13). Representative epidemiological data on patients with HIV-1 infection and CMV are lacking in our local area.

2. Objectives

Since the prevalence of CMV-EOD remains to be elucidated in Iranian HIV-1 infected patients, herein we explored the prevalence and clinical features of CMV-EOD in adult patients with advanced HIV-1 infection (CD4+ count of $<$ 100 cells/mm^3).

3. Methods

3.1. Study Design, Setting, and Participants

Using a convenience sampling method, a cross-sectional study was conducted on 82 patients with advanced HIV-1 infection who referred to the AIDS research center in Tehran between April 2016 and April 2018. The AIDS research center is located at Imam Khomeini hospital in Iran, affiliated to the Tehran University of Medical Sciences (TUMS). This center is the largest national referral center for HIV-1 infection in Iran. One observer as the main researcher directed the study and there were no inter-observer differences. We collected 3 ml of whole blood samples from eligible patients and mixed promptly with EDTA. The CD4+ count estimation was carried out using a flow cytometer instrument (Sysmex Partec, Germany). The SPHERO™ calibration particles were used for the calibration of the flow cytometer instrument. Any color or light contamination can be detected using these particles. Indeed, the calibration process was performed once a week, three times a day at the HIV/AIDS referral center. The inclusion criteria for the study were as follows: 1) HIV-1 infected patients with CD4+ counts of less than 100 cells/mm^3 defined flow cytometry, 2) HIV-1 infected patients aged $\geq$ 18-years-old, and 3) ART-naive patients (either being medication poor adherence or being new patients). Patients were excluded if they had a previous history of diagnosis or treatment for CMV-EOD (ganciclovir therapy). On average, 200 HIV-infected patients attend the AIDS research center every month for CD4+ cell count evaluation. Of these, approximately four patients were included and the rest was excluded from the study each month. The study was approved by the Human Research Ethics Committee of Tarbiat Modares University (IR.TMU.REC.1394.308) on 2-3-2016. All patients included in this study provided written informed consent for their clinical and laboratory data after the full description of the study. We obtained the baseline characteristics (age, sex, route of HIV-1 transmission, Hepatitis C Virus (HCV) infection, Hepatitis B Virus (HBV) infection, CMV IgG, and treatment status for HIV-1 infection) and CD4+ counts via the manual examination of medical records. The entire patients underwent clinical examinations for the diagnosis of CMV-EOD by experienced clinicians. Ophthalmologic examination including dilated retinal examination using indirect ophthalmoscopy was done by experienced ophthalmologists for the diagnosis of CMV retinitis. Under suspicion of CMV encephalitis, CMV conventional PCR was performed on the Cerebrospinal Fluid (CSF) of patients as previously described (14). The diagnosis of CMV esophagitis/colitis was made with the demonstration of owl's eye intranuclear inclusions in stained biopsy tissues.

3.2. Statistical Analysis

Statistical analysis was performed using SPSS version 24 (IBM, Armonk, NY, USA). The data were analyzed by the bootstrap method as an exact method for reporting p-values. A P-value less than 0.05 was regarded as statistically significant. Descriptive analysis was performed to determine mean values and ranges for continuous variables that were reported numerically. Qualitative variables were reported through frequencies (percentages). The Chi-square test was used to measure the differences between categorical variables (HBV, HCV, CMV-IgG, transmission group, and treatment status) and the outcome of CMV-EOD diagnosis. Logistic regression analysis was performed to explain the relationship between CMV-EOD (dependent variable) and independent variables (sex, age, CMV-IgG, HBV, HCV, CD4+ count, and IDU). No statistical analysis was performed for quantitative variables (age, CD4+ count); thus, there was no need to assess the normal distribution of these variables.

4. Results

4.1. Demographic Characteristics

The study sample consisted of 82 HIV-1 infected patients. There were 67 (81.7%) male and 15 (18.29%) female patients. The mean age of the patients was 38 $\pm$ 7 years and the most frequent age group was 31 - 40 years. All of them had CD4+ cell counts of $\leq$ 100/mm^3. The mean CD4+ cell count was 46.84 cells/mm^3. The highest CD4+ cell count was 94 cells/mm^3 and the lowest was 5 cells/mm^3. Other characteristics of the patients are shown in Table 1.

Table 1. Demographics and Laboratory Data of 82 Patients Infected with HIV-1

Characteristics	Frequency (%)
Sex	
Male	67 (81.7)
Female	15 (18.29)
Mean age, y	38 ± 5
HIV transmission route	
IDU (Injecting drug use)	40 (48.7)
Sexual	3 (3.6)
Other	39 (47.5)
Tuberculosis history	
Yes	15 (18.29)
No	67 (81.7)
ART (anti-retroviral therapy)	
Poor ART adherence	55 (67.07)
Not on ART	27 (32.92)
HBV (Hepatitis B Virus)	
Positive	10 (12.2)
Negative	72 (87.8)
HCV (Hepatitis C Virus)	
Positive	43 (52.43)
Negative	39 (47.56)
Mean CD4 count, cells/μL	46.84
CMV IgG	
Positive	71 (86.5)
Negative	2 (2.4)
Undetermined	9 (10.9)

4.2. Clinical Characteristic

Among 82 HIV-1 infected patients, 14 (17.07%) patients were diagnosed with opportunistic infections due to CMV. Among 14 patients with CMV-EOD, nine (64.28%) patients developed CMV retinitis, three (21.42%) developed colitis, and two developed encephalitis (14.28%). Table 2 illustrates the details of HIV-1 infected subjects with CMV-EOD. No significant correlation was observed between the outcome of CMV-EOD and HBV infection (P = 1.00), HCV infection (P = 0.55), and treatment status for HIV-1 infection (P = 0.53). We detected CMV-EOD more frequently among injecting drug users (IDUs) (11 out of 14) and patients with positive CMV-IgG (11 out of 14); however, the differences were not statistically significant (P = 0.12 and P = 0.41, respectively). By performing logistic regression analysis, no statistically significant relationship was found between the independent variables (sex, age, CMV-IgG, HBV, HCV, CD4+ count, and injecting drug user status) and CMV-EOD.

5. Discussion

Cytomegalovirus infection in patients with compromised immune function may result in the reactivation of the virus, leading to CMV-EOD (2). It is known that CMV-EOD is associated with specific organ involvement such as the retina, gastrointestinal tract, lung, liver, adrenal glands and the nervous system (15). Our study is the first to illustrate the prevalence and significance of CMV-EOD in Iranian HIV-1 infected patients with CD4+ counts less than 100 cells/mm3. Herein, CMV-EOD was developed in 14 (17.07%) patients and retinitis was the most common manifestation presented in nine (64.28%) cases. Hence, consistent with some other studies (16, 17), CMV retinitis accounted for the largest proportion of CMV-EOD in patients with HIV-1 infection. A few cases developed colitis and encephalitis. In Mizushima et al. (2013) study of Japanese CMV-EOD patients with CD4+ counts of less than 100/μL, CMV retinitis was developed in 66.7%, esophagitis in 12.1%, gastroduodenitis in 9.1%, colitis in 18.2%, and pneumonitis in 3.0% of the cases (16). In 2015, Mizushima et al. study of CMV-EOD cases with CD4+ cell counts of < 200, CMV retinitis was developed in 23 patients, colitis in eight patients, encephalitis in five patients and esophagitis in four patients (17).

Nishijima et al. (2015) pointed out the positive effect of routine eye assessment for HIV-1 infected individuals with CD4+ counts of < 200 /μL in resource-rich settings (18). In this regard, rigorous eye checkups and ophthalmic examinations have clinical usefulness for HIV/AIDS patients with CD4+ counts < 100/mm^3. The prevalence of CMV retinitis has been addressed in some published studies in Iranian HIV-1 infected patients. In Abdollahi et al. (2009) study (19), the prevalence of CMV retinitis was 2.4% (1/41 cases) and all of the patients were on ART treatment with CD4+ counts of more than 200 cell/mm^3. In Abdollahi et al. study in 2010 (20), the prevalence of CMV retinitis was 1.4% (2/141 cases) and 50% of HIV-positive patients were taking ART at the time of ocular examination with the mean CD4+ count of 204.7 ± 123.8. In Abdollahi et al. study in 2013 (21), the prevalence of CMV retinitis was reported as 1.88% (2/106 cases) and all of the patients were on ART treatment with CD4+ counts of 110 and 105 cell/ml. Taken together, the prevalence of CMV retinitis has been addressed in three published studies in Iranian HIV-1 infected patients with CD4+ cell counts of more than 100 cell/mm^3. In other words, our study is the first to illustrate the prevalence and significance of retinitis and extraocular complication of CMV (CMV-EOD) in Iranian HIV-1-infected patients with CD4+ counts of less than 100 cells/mm^3. In addition, we excluded patients who received anti-CMV treatment. The ex-

Table 2. Details of HIV-Infected Subjects with CMV End-Organ Disease (CMV-EOD)

Patient Number	Sex	Age	CD4 Count	HIV Transmission Route	ART (Anti-Retroviral Therapy)	HBV History	HCV History	CMV-EOD
1	Male	41	53	IDU (Injecting drug use)	Poor ART adherence	-	+	Retinitis
2	Male	33	72	IDU	Poor ART adherence	-	+	Encephalitis
3	Male	30	38	IDU	Poor ART adherence	-	-	Retinitis
4	Male	52	47	IDU	Not on ART	+	+	Retinitis
5	Male	36	51	IDU	Poor ART adherence	-	-	Retinitis
6	Female	26	18	Sexual	Poor ART adherence	-	-	Colitis
7	Male	34	46	IDU	Not on ART	-	+	Retinitis
8	Female	47	25	Sexual	Poor ART adherence	+	+	Colitis
9	Male	39	10	IDU	Poor ART adherence	-	-	Retinitis
10	Male	35	48	IDU	Not on ART	-	+	Encephalitis
11	Female	40	33	IDU	Poor ART adherence	-	+	Retinitis
12	Male	29	62	Sexual	Poor ART adherence	-	-	Colitis
13	Male	31	54	IDU	Not on ART	-	+	Retinitis
14	Male	44	49	IDU	Poor ART adherence	-	+	Retinitis

tensive uptake of ART has changed drastically the epidemiology of CMV-EOD among subjects with advanced HIV infection (22). Nonetheless, studies indicate that even in the era of ART, advanced AIDS-associated immunosuppression remains a high-risk factor for developing CMV-EOD. One patient with a CD4+ count of 72 cells/mm^3 had active CMV encephalitis (Table 2). Notably, the patient with the lowest CD4+ cell count (5 cell/mm^3) had no organ involvement. In line with the results of the current study, a relatively low rate of CMV-EOD (4 out of 338 patients) in HIV-infected patients with low CD4+ cell count and CMV viremia was reported by Wohl et al. (2009) (23). The reason for this discrepancy might be related to the suboptimal immunological response. Based on our local experience, it can be concluded that ophthalmic examination has clinical usefulness for HIV/AIDS patients with a CD4+ count of <100/mm^3.

The limitation of this study was the low number of clinical cases. However, it should be noted that patients such as individuals included in this study may be uncommon given the availability of ART.

5.1. Conclusions

It is assumed that CD4+ cell count is not the sole predictor of the risk of developing CMV-EOD. Further large-scale studies are required for a better understanding of risk factors involved in the occurrence of CMV-EOD in HIV-positive patients.

Footnotes

Authors' Contribution: Hoorieh Soleimanjahi contributed to the design of the study as the corresponding author; Mahboubeh Hajiabdolbaghi and Mohammad Sarraf-Shirazi carried out patient recruitment and made diagnostic evaluations; Mohammad Reza Jabbari participated in sample collection and performed the tests and statistical analysis; Somayeh Shatizadeh Malekshahi contributed to writing the paper. All authors read and approved the final manuscript.

Conflict of Interests: The authors declare that they have no competing interests. All authors of this manuscript have not any relevant financial interests or financial conflicts.

Ethical Approval: The study was approved by the Human Research Ethics Committee of Tarbiat Modares University (IR.TMU.REC.1394.308) on 2-3-2016.

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