

Seroprevalence of Epstein-Barr virus among HIV Positive Patients Moreover and its Association with CD4 Positive Lymphocyte Count

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Abstract- Opportunistic infections are the leading cause of hospitalization and morbidity in human immunodeficiency virus (HIV) positive patients and are the most common cause of death between them. We aimed to measure IgG antibody against EBV viral capsid antigen (EBV-VCA IgG) to determine the seroprevalence of this infection in HIV-positive population. A case-control study between September 2011 and October 2012 was conducted in a teaching hospital enrolling 114 HIV-positive patients as case group and 114 healthy individuals as control with similar age and sex. Enzyme-linked immunosorbent assay (ELISA) technique was used for determination of EBV-VCA IgG in obtained samples. Of 114 serum samples obtained from HIV-positive patients, 103 (90.4%) samples were found positive for EBV-VCA IgG antibody. There was no significant difference in seroprevalence of EBV VCA IgG antibody between patients received antiretroviral therapy and naive patients (91.5% vs. 87.5%, $P>0.05$). There was no statistically significant difference in EBV-VCA IgG seroprevalence between three groups of CD4+ in HIV positive group. In conclusion current study showed that seroprevalence of EBV in HIV-positive patients is higher than HIV-negative healthy participants; however, administration of HAART and CD4+ lymphocyte count did not reveal a significant effect in seroprevalence of EBV. Due to the significance of this virus in mortality and morbidity and causing certain malignancies in patients with AIDS, these patients are strongly recommended to be tested for this virus.

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Introduction

Viral coinfections in HIV positive patients and acquired immunodeficiency syndrome (AIDS) are a major health concerns all over the world. As a result of the interaction between HIV status and other viral infections, changes in severity or the natural progress of both infections may occur. However, opportunistic infections are the leading cause of hospitalization and morbidity among HIV-positive patients leading to significant mortality in this population (1-2). Cytomegalovirus (CMV), herpes simplex virus type 1 and 2 (HSV1 and HSV2), varicella zoster virus (VZV) and Epstein Barr virus (EBV) are relatively prevalent coinfections in HIV-positive individuals (1-5).

One of the important coinfections that may

significantly decline HIV-related morbidity and mortality is Epstein-Barr virus (6). EBV or the human herpes virus 4 (HHV-4) is a gamma herpesvirus that is potentially endemic and highly prevalent around the world with over 90% of individuals establishing a lifelong infection (7). Primary infections may result in variable manifestations including asymptomatic seroconversion (mostly in younger children), infectious mononucleosis (IM), and lymphoproliferative disorders (especially in immunosuppressed individuals) (7-8). Oral hairy Leukoplakia (OHL) is also a permissive lesion observed in immunocompromised patients infected with EBV. There are two categories of malignancies that are seen more frequently among patients with AIDS both caused by EBV, NHL (being among AIDS-defining category of malignancies) caused

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by EBV and non-AIDS-defining category of malignancies (including Hodgkin's disease and nasopharyngeal cancer) both caused by EBV (1-2,4,6,9-10). Owing to T-cell impairment in suppressing EBV-infected cells, HIV positive patients have 10 to 20 times more circulating EBV-infected B cells than healthy individuals (11).

Prior to the development of EBV-related NHL, EBV-specific cytotoxic T cells were decreased with a concomitant increase in EBV viral load (10-13). There are variations in the relative frequencies of specific opportunistic infections according to epidemiological difference (14). Given the significant contribution of this virus to certain malignancies, mortality and morbidity in patients with AIDS under study were defined.

Furthermore, available information about opportunistic infections among HIV-seropositive individuals in Iran is scanty. Hence, the aim of this study was to determine the seroprevalence of EBV in an Iranian HIV positive population.

Materials and Methods

This case-control study was conducted at Imam Khomeini Hospital affiliated with the Tehran University of Medical Sciences (TUMS), in Tehran, Iran. Between September 2011 and October 2012, 114 HIV-positive patients with confirmed diagnosis with serology, PCR or western-blot test [We followed the national AIDS control organization recommendation (NACO 2007) for diagnosis of HIV infection] were enrolled and compared with 114 healthy HIV-negative participants in a control group attending our laboratory for routine checkup. Patients with pregnancy, autoimmune disease, malignancy or hematological disorders were excluded from the study. Five to six millilitres (ml) of the blood sample were obtained from each patient for CBC, CD4+ lymphocyte count and defining serum concentration of EBV antibody. Complete blood count was done with Sysmex-K21, Japan instrument on blood samples with anticoagulated with EDTA. CD4+ and CD8+ T lymphocytes were counted by flow cytometry device (FCM) (PARTEC, Japan).

The clotted blood was centrifuged in 3000 g for 15 minutes and extracted serum was then stored in a -70 centigrade Celsius freezer. EBV-VAC IgG antibody titer was measured by ELISA (enzyme-linked immunosorbent assay) techniques in room temperature using Mono Bind Inc, Lake Forest, CA, USA kit. Following the instruction provided by the manufacturer, A serum level of VCA IgG antibody >22 U/ml, between

18-22 U/ml and < 18 U/ml were interpreted positive, borderline, and negative, respectively. Seropositivity rate was compared between HIV-positive group and healthy control group and additionally among different subgroups of HIV-positive patients according to the age, gender, CD4+ cell count, transmission route, and antiretroviral therapy.

Patients gave an informed consent before entering to the study, and the institutional review board of the Tehran University of Medical Sciences approved the study protocol.

Data were analyzed using Statistical Package for Social Sciences (SPSS version 18, Chicago, Inc). Chi-square test was employed to compare the EBV antibodies between HIV positive and HIV negative groups. T-test was also used to analyze the association between hematologic parameters and CD4+ cell count, and IgG titre and values were deemed significant at $P < 0.05$.

Results

A total of 228 patients (case and control) were included in this study, 114 HIV-positive patients (case group) and 114 healthy sex and age matched individuals (control group). The mean age of patients was 37.48 ± 1.01 years in the case group compared with 36.92 ± 2.03 years in the control group with no significant difference ($P > 0.05$). One hundred three samples in the case (90.4%) were positive for EBV-VCA IgG antibody compared with 37 samples (32.5%) in the control group which revealed that EBV-VCA IgG is significantly higher in HIV-positive patients ($P < 0.0001$).

Table 1 shows EBV seroprevalence among HIV-positive patients' subgroups according to the age, gender, CD4+ cell counts, transmission route, and antiretroviral therapy. As the table shows, 80 (70.2%) male patients were in HIV-positive group. There was no statistically significant difference in EBV-VCA IgG antibody between male and female patients in the case group ($P > 0.05$). Patients were classified according to the age into four groups. Among HIV-positive patients, 60 (55%) patients were between 30-40 years old. The seroprevalence of EBV in HIV-positive patients did not differ significantly between age groups ($P > 0.05$). The majority of transmissions (52.6%) was through intravenous drug use (IDU) followed by heterosexual contact. HIV transmission route was not significantly associated with EBV-VCA IgG antibody seropositivity ($P > 0.05$).

In HIV positive EBV coinfecting patients, mean level

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of platelet count was significantly lower compared with EBV negative patients (195.70±7.42 vs. 246.27±31.06; $P=0.04$); these patients also had lower mean±SD eosinophil count (179.90±14.78 vs. 222.73±54.32) and

higher mean±SD neutrophil count (3001.2±126.67 vs. 2580.9±289.34); however, there was no statistically significant difference between HIV+ and HIV- groups in terms of red blood cells.

Table1. EBV seroprevalence among HIV positive subgroups according to age, gender, CD4⁺ cell counts, transmission route, and antiretroviral therapy characteristics

Groups		Prevalence	EBV VCA IgG (+)	P-value
Age (yrs)	≤ 30	18 (16.5%)	88.9%	0.996
	30<≤40	60 (55%)	90%	
	40<≤50	19(17.5%)	89.5%	
	>50	12 (11%)	91.7%	
Gender	Male	80 (70.2%)	90%	0.84
	female	34 (29.8%)	91.2%	
CD4 ⁺ cell counts	<200	34 (30.6%)	91.2%	0.94
	200≤<500	59 (53.2%)	91.5%	
	≥500	18 (16.2%)	88.9%	
Transmission route	Injecting drug use	60(52.6%)	88.3%	0.52
	heterosexual	40(35%)	90%	
	Blood trans	3 (2.6%)	100%	
	Maternal	1 (0.9%)	100%	
Antiretroviral Therapy	unknown	10 (8.8%)	100%	0.04
	Yes	82 (71.9%)	91.5%	
PI (Kaletra)	No	32(28.1%)	87.5%	0.04
	Yes	13 (15.9%)	76.9%	
Total		114	90.4%	

(PI=Protease Inhibitor)

There was no significant difference in seroprevalence of EBV-VCA IgG antibody between HIV-positive patients receiving antiretroviral therapy and naive patients (91.5% vs. 87.5%; $P>0.05$). Moreover, considering the type of HAART, the EBV coinfection was significantly less prevalent in patients treated with Kaletra (a protease inhibitor) (76.9% vs. 94.2%, $P=0.04$); however no significant difference in EBV coinfection was observed in patients treated with different antiretroviral regimens including Zidovudine (66 patients, 80.5%), Lamivudine (78 patients, 95.12%), Stavudine (4 patients, 4.9 %), Tenofovir (5 patients, 6.1%), Didanosine (3 patients, 3.65%), and Efavirenz (63 patients, 76.83%) compared with naive patients.

Based on CD4⁺ cell counts, HIV positive patients were grouped in three categories according to the CDC criteria as group 1 with CD4⁺<200 cells/μl; group 2 with 200≤CD4⁺<500 cells/μl and group 3 with CD4⁺≥500 cells/μl. There was, however, no significant difference in seroprevalence of EBV-VCA IgG antibody between three different subgroups.

Discussion

Being the leading cause of death, opportunistic infections play an important role in HIV-positive

patients. Of these, EBV is contributing to the pathogenesis of most of lymphoproliferative disease in HIV-positive patients such as OHL; however, there is not sufficient evidence in this regard. The majority of patients in current study (70.2%) were male which is consistent with the study by Gonzalez *et al.*, in which male patients constituted the most proportion of the study population but in contrast to other reports showing that prevalence of EBV-related OHL is higher in females (15-17). The higher seroprevalence of EBV in males could be due to the homosexual relationship. In a large study on 120 HIV-positive children, only 22 patients (18%) showed OHL indicating that seroprevalence of EBV is low in HIV-positive children (18). As some cross-sectional studies showed, EBV-VCA IgG titers and oropharyngeal EBV excretion has increased in HIV-positive patients and other studies on homosexual men indicated that EBV-VCA IgG titers is elevated in HIV infection increasing with progression of the disease. Also Rahman *et al.*, in agreement with the results of previous studies stated that oropharyngeal EBV secretion is increasing in HIV-positive homosexual men; these findings confirm results of present study showing that EBV-VCA IgG level is higher in HIV-positive patients (19-26).

In a study on oral lesion of immunocompromised

patients especially HIV positive ones, prevalence of OHL was higher than HIV-negative patients (27-28). However, some other studies found EBV viruses in oropharynx of HIV-positive patients without OHL and concluded that EBV infection might occur in immunosuppressed patients and coincide with OHL (29).

The incidence of CNS lymphoma is increased in HIV-positive population due to immunosuppression and because of the potential role of EBV (30-32). Some other studies have reported increased incidence of EBV infection in HIV disease confirming the results of the current study in which EBV seroprevalence was higher in HIV-positive patients compared to HIV-negative ones (33-36).

OHL is present in mild to a moderate immunodeficiency as well as in pregnant women and diabetes mellitus patients with normal immunity. In addition, in this study, there was a positive correlation between decreased CD4+ cell count and OHL prevalence in a way that the prevalence of EBV increased with decreasing the CD4+ cell count (37). However, this was not statistically significant which is confirmed by the study of Dehee *et al.*, and Stevance *et al.*, (38-44). Herein *et al.*, showed that seroprevalence of EBV is higher among HIV/AIDS patients compared to HIV-negative subjects showing the role of sexual transmission for EBV infection (45). In this study, IV drug use was the most common route of EBV infection followed by heterosexual intercourse. This could be due to a higher prevalence of addiction in our country.

Steven *et al.*, declared no association between HAART and EBV viral load. In Dias *et al.*, study on HIV-positive children, HAART did not affect the presence of OHL. According to a study on HIV positive patients with OHL, lesions did not disappear in patients who received HAART confirming that the antiretroviral therapy may not influence the presence of EBV-related lesions (46). In the agreement with this, this study showed no significant difference in seroprevalence of EBV antibody in patients receiving HAART (39).

This study bears some limitations that need to be considered before interpretation of its results. The cross-sectional design and small sample size prohibit extrapolation of our findings to other settings. Moreover, application of quantified methods clarifies association of study findings with the presence of EBV. Future multicenter investigations may answer these debates.

Current study showed that seroprevalence of EBV is higher in HIV-positive patients than in healthy HIV-negative participants. Due to the significance of this

virus in mortality and morbidity and causing certain malignancies in AIDS patients, these patients are strongly recommended to undergo a test for this virus.

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