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Sero-Prevalence and Plasma Viral Load of Epstein Barr Virus among Iraqi Blood Donors

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Abstract

Background Epstein-Barr virus (EBV) is one of the most common latent viruses inside the humans' B-lymphocytes and it has been documented as a causative agent of many cancers. The virus may be transmitted when infected blood transfused to immunocompromised as well as immunocompetent individuals.

Objective To estimate the prevalence of EBV among apparently healthy blood donors by enzyme-linked immunosorbent assay (ELISA) and by quantitative real time polymerase chain reaction (RT-PCR).

Methods Four hundred fifty (450) blood donors were enrolled in this study. Plasma samples were screened by ELISA technique for detection of EBV viral capsid antigen (VCA-IgG). DNA extracted from 50 representative samples of these 450, and plasma EBV viral load was investigated by RT-PCR.

Results The overall sero-prevalence of EBV IgG was 79.8%, with a significantly higher prevalence among females than males. RT-PCR results were negative for all of the 50 representative samples.

Conclusion The high EBV sero-prevalence rates among Iraqi subjects raise the possibility of increasing the risk of EBV-associated malignant diseases.

Keywords Epstein-Barr virus, seroprevalence, VCA-IgG, real-time PCR, blood donors

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List of abbreviation: EBV = Epstein-Barr virus, ELISA = Enzyme-linked immunosorbent assay, LMP = Latent membrane protein, PTLD = Post-transplant lymphoproliferative disorder, RT-PCR = Real time polymerase chain reaction, TPHA = Treponema pallidum heamagglutination, VCA = Viral Capsid antigen

Introduction

Blood transfusion is still a significant mode of transmission of transfusion-transmissible infectious pathogens, given the need to determine sero-prevalence in the blood donors and to evaluate the residual risk in the blood recipients [(1,2)]. Epstein-Barr virus (EBV) infection is extremely common worldwide and approximately 90% of adults become antibody-positive before the age of 30 years [(3,4)]. Viral transmission is generally via saliva by kissing [(5)]. However, transmission via blood products, transplantation, and sexual transmission could also occur [(7-9)] and the risk of transfusion-transmitted infections is still considerable [(9,10)].

Previous studies reported that EBV infections might lead to severe morbidities and mortalities in healthy individuals [(12,13)]. Furthermore, the virus is causally linked to several malignancies, including Burkitt's lymphoma, Hodgkin lymphoma, nasopharyngeal carcinomas and leukemia [(13-19)].

Post-transfusion EBV infection is of concern in certain groups of immunocompromised individuals such as neonates, pregnant women, recipients of bone marrow and solid organ transplants and individuals with immuno-deficiency diseases especially in EBV sero-negative (susceptible) recipients [20-22].

In Iraq, to the best of our knowledge, there is no previous sero-prevalence study on EBV among healthy individuals. Thus, this study aimed to determine the sero-positivity of EBV in blood donors to establish basic knowledge for future studies.

Methods

This cross-sectional study was conducted from September 2015 to January 2016. Four hundred fifty (450) blood donors enrolled in the study including 400 males and 50 females who attended The Blood Donation Center in Al Imamein Al Kadhimein Medical City, and The National Blood Center. This study was approved by the Ethical Committee of the College of Medicine / Al-Nahrain University. Informed consent was obtained from all donors before taking samples.

Blood donors were all apparently healthy subjects, selected after responding to a panel of questions comprising a medical history. Healthy individuals aged between 18-63 years were eligible for blood donation. Donor selection was under the World Health Organization (WHO) guidelines to assessing donor suitability for blood donation. All samples were screened by enzyme-linked immunosorbent assay (ELISA) for hepatitis B virus surface antigen (HBsAg), and antibodies to HBc (core), (HIV1,2 Ab and HIV Ag), HCV and Treponema pallidum heamagglutination (TPHA) in the Blood Donation Centers as part of routine screening of donated blood units.

Three mL whole blood collected in EDTA-blood tubes and then plasma obtained by centrifugation of whole blood at 3,000 rpm for 10 min. The supernatant (plasma) was aspirated and stored at -40 °C until be used.

Measurement of the EBV IgG Viral Capsid antigen (VCA) Ab titer by ELISA

The anti-EBV VCA IgG Antibody ELISA Test Kit (DEMEDITEC/Germany) designed for the detection and the quantitative determination of specific IgG antibodies against EBV VCA in serum and plasma was used in this study. EBV VCA antigen bound on the surface of the microtiter strips. Diluted patient plasma or ready-to-use standards were pipetted into the wells of the microtiter plate. If the specimens contain antibodies to EBV VCA, a binding between the IgG antibodies of the plasma and the immobilized EBV antigen takes place. Then ready-to-use anti-human-IgG peroxidase conjugate was added, which will bind to the anti-human-IgG antibodies. After that the substrate (TMB) solution was added and then incubated at room temperature for 30 minutes. The development of a blue color in the wells indicates the presence of EBV IgG antibodies present in the specimens. The resulting color was measured spectrophotometrically at the wavelength of 450 nm. The concentration of the IgG antibodies is directly proportional to the intensity of the color.

Detection of EBV DNA using quantitative real time polymerase chain reaction (RT-PCR)

Fifty samples were subjected to viral DNA extraction and then RT-PCR for detection of EBV active viremia. These 50 samples randomly selected to be representative to all samples.

DNA was extracted from 200 µl of plasma using DNA-sorb-B (Sacace, Italy). DNA extraction steps included disruption/lysis of plasma sample, removal of the contaminants and recovery of the nucleic acid. The concentration and purity of the DNA were measured using the nucleic acid measuring instrument nanoDrop (England).

EBV Real-TM Quant Kit (Sacace, Italy) was used for the detection of LMP-gene in EBV genome. EBV LMP DNA amplification was detected on JOE (Yellow) channel, while the IC glob gene DNA amplification was detected on FAM

(Green) channel and exogenous Internal Control IC was detected on Rox (Orange) channel. The quantity of reactants for one reaction was 10 µL of PCR-mix -1 and 1.5 µL of PCR-mix-2 buffer and 0.5 µL of hot Start taq polymerase. DNA from sample/ standard/ positive or negative control was added to the mix. The final volume per reaction tube was 25 µL.

The RT-PCR instrument used in the study was STRATAGENE MxPro QPCR (Agilent Technologies, USA). The thermal protocol for Sacace Quantification Kit is composed of an initial denaturation for activation of the HotStarTaq DNA Polymerase at 95 °C for 15 min, followed by five cycles of thermal cycling 95 °C for 15 sec, and 60 °C for 20 sec, and 72 °C for 15 sec, and finally 40 cycles of 95 °C for 10 sec, and 60 °C for 40 sec, and 72 °C for 15 sec.

Statistical analysis

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), version 20 was used for statistical analysis. EBV sero-positivity rates were calculated and compared according to different dependent variables. Differences were evaluated using the Chi-square test or Fisher exact test if there is 25% of cells less than expected count. P value of ≤ 0.05 was considered statistically significant.

Results

The results of EBV IgG anti-VCA titer were recorded as: negative, borderline, weak positive and positive according to the levels of Calibrator A, Calibrator B, Calibrator C and Calibrator D, respectively, according to kit instructions.

Results were also interpreted according to those instructions. Calibrator B with its concentration of 10 U/mL serves as cut-off value. If the value of the sample is higher than the cut-off +20% it was considered positive result, which represented 359/450 (79.8%) (Table 1).

The value below the cut-off– 20% was considered negative result which represented 18/450 (4%). Values with a range of +/-20% the cut-off were reported as borderline. As in relation to cut-off value equivocal samples which represented 73/450 (16.2%) were excluded from the study because they need further follow-up after 2-4 weeks to determine whether there are primary EBV infection or non-specific antibodies causing false positive.

Table 1. Frequency of EBV anti-VCA IgG antibody titer among blood donors

		Frequency	Percent
EBV IgG antibody titer	Positive	326	72.4
	Weak positive	33	7.3
	Border line	73	16.2
	Negative	18	4
Total		450	100%
EBV IgG antibody titer	Total positive	359/450	79.8%

Blood donors enrolled in this study included (88.9%) 400 males and (11.1%) 50 females. The results of this study showed higher rate of EBV positivity in females than in males with percentage 90% and 78.5%, respectively, which was statistically significant (P=0.036), as shown in table (2).

A significantly higher EBV sero-prevalence rate in those living in Baghdad than in those from other governorates, with percentage of 81.5% and 65.3%, respectively, (P=0.009), as shown in

table (2). This study showed that only one sample was positive for HBs-Ag, 14 for HBc-Ab, and three for TPHA. All samples were negative for anti HCV-Ab and HIV-Ab. There was no statistically significant association of EBV IgG, either with HBs-Ag, HBc-Ab or TPHA, P values were: 0.2, 0.6, and 0.5 respectively, none of the subjects had co-infections with any of these screened pathogens.

Quantitative real time PCR was conducted on 50 representative samples out of the 450 cases, according to the results of EBV VCA-IgG titers, to detect EBV viral load, using primers for EBV LMP-gene. The results of this study showed that all the 50 samples were negative for EBV LMP-gene.

Table 2. The association between EBV serology results and blood donors' descriptive data

Variable		IgG anti-VCA Negative N=91	IgG anti-VCA Positive N=359	Total	P value
Age groups	≤20 years	7 (26.9%)	19 (73.1%)	26	0.553 NS
	21-30 years	39 (22.7%)	133 (77.3%)	172	
	31-40 years	29 (19.1%)	123 (80.9%)	152	
	41-50 years	14 (17.3%)	67 (82.7%)	81	
	>50 years	2 (10.5%)	17 (89.5%)	19	
Gender type	Female	5 (10.0%)	45 (90.0%)	50	0.036 S
	Male	86 (21.5%)	314 (78.5%)	400	
Residence	Rural	12 (16.2%)	62 (83.8%)	74	0.22 NS
	Urban	79 (21.0%)	297 (79.0%)	376	
	Baghdad	74 (18.5%)	327 (81.5%)	401	0.009 S
	Governorates	17 (34.70%)	32 (65.30%)	49	
Blood groups	A	22 (17.46%)	104 (82.54%)	126	0.339 NS
	B	27 (19.57%)	111 (80.43%)	138	
	AB	5 (13.89%)	31 (86.11%)	36	
	O	37 (24.67%)	113 (75.33%)	150	
Occupation	Governmental employee	27 (20.0%)	108 (80.0%)	135	0.523 NS
	Private sector	54 (21.3%)	200 (78.7%)	254	
	Housewife	4 (11.1%)	32 (88.9%)	36	
	Student	6 (24%)	19 (76.0%)	25	
Cupping	No	67 (21.3%)	247 (78.7%)	314	0.223 NS
	Yes	24 (17.6%)	112 (82.4%)	136	
Travel	No	70 (21.7%)	252 (78.3%)	322	0.126 NS
	Yes	21 (16.4%)	107 (83.6%)	128	

NS: No statistical significance (p>0.05), S: Statistical significance (p<0.05).

Discussion

This study enrolled four hundred fifty (450) blood donors from the two main blood donation centers in Baghdad, and EBV sero-positivity among these blood donors was 79.8%, which is comparable to other results in the surrounding countries. For instance, sero-prevalence was reported among blood donors in Iran was 85% [24].

On the other hand, in Taiwan, Chen et al, 2015 reported that overall seropositive rate of EBV was (88.5%) [25]. In USA Balfour et al, 2013

reported that (90%) of healthy subjects had IgG antibodies against EBV viral capsid (VCA) antigen [26].

One explanation of the lower sero-prevalence reported in this study in comparison to other studies, whether in the surrounding countries or worldwide, is that the majority of studies showed that sero-positivity increases with age [3,25,27]. In this study, 350 out of 450 (78%) of subjects aged below 40 years old, i.e. in young age group, which could justify this slightly lower sero-prevalence rate.

Antibodies to EBV-Viral capsid Ag are the most commonly investigated Abs in sero-prevalence studies [28-31]. In the current study, survey of EBV was based on the presence of EBV IgG anti-VCA. Since anti-VCA IgG response has an important role in the detection of past infection, and some individuals might be anti-VCA IgM non-responders after acute primary infection [32], therefore, the current study was designed to detect anti-VCA IgG, and this has also been applied in many EBV sero-prevalence studies worldwide [5,30,33,34].

EBV causes a mild, self-limited infection in immunocompetent subjects. It is latent in B lymphocytes and can be transmitted through blood transfusion and usually presents as a clinical health hazard in high-risk recipients, such as immunosuppressed individuals [35-36].

Several studies suggested an association between blood transfusions and post-transplant EBV infection. The greatest risk is seen in EBV sero-negative patients receiving allografts from EBV-seropositive donors. Therefore, testing of donors will be helpful in the appropriate transfusion decision [37-38].

None of the blood donors enrolled in this study gave recent history of even minor flu-like illness or fatigue. In order for PCR to be effective in detecting the viral load it must be performed early during active infection before the immune system of the host eliminates the virus [39]. On the other hand, absence of viremia may be due to the very low viral load in these asymptomatic infected individuals (i.e. viral load could be below the detection limit) [40]. Although most individuals might be expected to carry EBV DNA in their lymphocytes, viral nucleic acid is not usually found in plasma in the absence of active EBV disease, since EBV is primarily B cell-associated, and plasma viremia is rare [41,42].

However, a study in Iraq on renal transplant subjects conducted by Shams-Aldin et al, 2015 in the same center of study and used the same EBV real time PCR kit on plasma samples, EBV viremia was detected in 19/57 (33%) of renal transplant recipients [43].

Gender type distribution among EBV sero-positive donors was significantly high in females than in males (P=0.036), a result that agrees with the findings of other studies. Crawford et al, 2002 and Chen et al, 2015 found that prevalence of EBV sero-positivity was significantly greater among women than men [6,25]. This difference is in accordance with the concept that women in general mount more vigorous antibody and cell mediated immune responses following infection or vaccination than men [37,44]. Thus, these antibodies could be detected more easily in women than in men.

Furthermore, the most common mode of EBV transmission for adults is via exposure to infected children [3]. Infected children actively excrete the virus in their saliva. In Iraq as this study observed, that women are at high risk of getting such childhood infectious diseases because of their higher contact rate with children than men, mainly mothers and teachers in kindergartens, daycares, and primary schools in which the vast majority are women teachers.

Most of previous studies showed that sero-positivity for EBV increases with age [3,25,27]. However, the current study revealed non-significant difference. Although similar result has been reported by some researches [45,46], this non-significant association of age could be explained by the fact that 40% of the participants are within 21-30 years old.

The study revealed significantly higher EBV sero-positivity among those living in Baghdad

as compared to those coming from other governorates (P=0.009). A finding that is mainly explained by the overcrowding conditions in Baghdad, in which there is higher crowding index as compared to other governorates. Such condition was frequently reported to be associated with high EBV sero-positivity [24,47]. On the other hand, the sample size from Baghdad was much more than other governorates (401 and 49 respectively) which, no doubt, influence the result of the investigation.

Collectively, these data suggest the high prevalence of anti-EBV IgG antibodies among Iraqi blood donors. This will raise the possibility of increasing the risk of EBV-associated malignancy. However, a further study to detect the prevalence of anti-EBV IgM antibodies among those donors before sounding the alarm.

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Author contributions:

Redha: Collection of specimens, ELISA testing, DNA extraction, and real -time PCR, writing of the manuscript. Dr. Al-Obaidi: Supervision and performance of viral DNA extraction and real-time PCR run, writing of the manuscript. Dr. Ghazi: Supervision and performance of ELISA testing, and performance of all statistical analysis. Dr. Kadhim: Supervision of ELISA testing and final editing of the manuscript.

Conflict of interest

Authors declare no conflict of interest.

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