Sero frequency of Epstein–Barr Virus among Sudanese Blood Donors Attending Omdurman Teaching Hospital

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Abstract:

Background: In most sub-Saharan countries screening of blood-transmitted infections includes mainly HIV, HBV, HCV and syphilis. Many viruses such as Epstein-Barr virus (EBV) which also carry a risk of transmission by blood transfusion raise the question of the extent of screening for these pathogen. This study was carried out to detect of EBV in first-time blood donors in Khartoum Sudan.

Methods: This is across-sectional study, serum specimens were collected from blood donors and analyzed by ELISA for Epstein-Barr virus (EBV) viral capsid antigen (VCA) - IgM antibodies, and the result were correlated with Age.

Results: Out of the 90 blood donors. All of them were negative for EBV, the blood donors age range from 18 – 59 years group.

Conclusion: This study revealed that all of blood donors were seronegative. Further studies with large sample size is recommended.

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Introduction

Epstein-Barr virus (EBV), also called human herpes virus 4 (HHV-4), was the first human virus to be directly implicated in carcinogenesis. EBV is a part of the Herpesviridae family [1]. It was discovered by Michael Epstein and Yvonne Barr in 1964 during their research on Burkitt’s lymphoma (BL) [2]. Since its discovery, EBV has been found in a variety of other tumor types. The evidence for an association with EBV is the strongest for BL, NK/T-cell lymphoma, nasopharyngeal carcinoma (NPC), Hodgkin’s lymphoma (HL) and for malignant lymphomas in immune incompetent patients [3]. Additionally, certain epithelial cell tumors, such as gastric carcinoma [4] and breast carcinoma [5], have been found to be EBV related [6]. However, the virus may be encountered in other types of malignancies.

EBV infects more than 90% of the world’s adult population. Infection usually occurs early in childhood. EBV infections are most prevalent in developing countries, in populations of low socioeconomic status [7-8]. In countries with stringent hygiene practices, EBV seroprevalence tends to increase gradually with age, showing two seroconversion peaks: at 2 to 4 years and at 14 to 18 years [9]. The mean seroprevalence in children is approximately 50% and increases steadily to a value of 90% to 99% in adults [10].

Burkitt’s lymphoma (B cell) endemic in central part of Africa and New Guinea, with an annual incidence of 6-7 cases per 100,000 with peak incidence at 6-7 years of age

Nasopharyngeal carcinoma incidence rates are less than 1 per 100,000 except in southern China where an annual incidence of more than 2 per 100,000 is reported [11].
There are two major strains of EBV (type 1 and type 2), differing in organization of the genes that encode the EBV nuclear antigen (EBNA) [12]. Both types are detected all over the world, with type 1 being the most frequent. In spite of frequent virus detection in latently infected blood donors, transmission of EBV infection by transfusion is thought to be relatively infrequent. This phenomenon could be explained by the fact that most adult recipients of blood and blood products are already immune to EBV. Moreover, blood from seropositive donors contains EBV-neutralizing antibodies and specific memory cells, which may protect the recipient from infection. Although we do not know exact EBV infectivity by blood and its components, it seems that the viral load in blood from healthy seropositive donors, which is normally low, is rather below an infection dose. In addition, it was established that the viability of B lymphocytes carrying the EBV genome may decline during blood storage. The risk of EBV transmission from red blood cell (RBC) and/or platelet (PLT) transfusions is also significantly reduced by leukoreduction. Thus, in most instances, EBV genomes contained in blood products should not cause severe disease when the transfused recipient is immune competent. However, whole blood and blood components as a potential source of infection should be kept in mind as an association between transfusion and EBV infection, especially in immunosuppressed young patients [13].

Most primary EBV infections in normal individuals are unapparent, but occasionally EBV can cause acute infectious mononucleosis, which is a self-limited disease. Following primary EBV infection, individuals remain lifelong carriers of the virus [14]. This study aimed to estimate serofrequency of EBV –VCA IgM antibodies, and to determine the relationship between the presence of antibodies and certain factors such as age.
Material and Methods:

This was descriptive- cross sectional study which had been conducted in Omdurman Teaching Hospital, Khartoum, Sudan during period from March to April 2015, ninety blood donors were enrolled, Data was collected by using direct interviewing questionnaire; ethical clearance was obtained from research ethical committee of Faculty of Graduate Studies, written consent also was obtained from blood donors.

Specimen collection:

blood specimens were collected from 90 blood donors, under direct medical supervision by medial vein puncture using 5 ml syringe into plain tube to obtain serum by centrifugation at 5000 rpm for 10 min. serums was kept in -20°C till serological study was performed. Specimens were processed by Enzyme linked immune sorbent assay (ELISA) (3rd generation ELISA) (CALBIOTECH- USA) for detection VCA- IgM.

Enzyme linked immune sorbent assay for detection (EBV)VCA IgM.

All reagents and samples were allowed to reach room temperature (18-26 c) for 15 minutes before use. Washing buffer was prepared 1:20 from buffer concentrate with distilled water. 200μl of sample diluents was added into appropriate wells except the blank well, calibrator well, positive and negative well, 10μl from each sample was added to the appropriate wells and mixed by pipette repeatedly until liquids turn from green to blue. 100μl from calibrator, negative and positive control was dispensed and added to the negative and positive wells separately without dispensing liquid into the blank control well. and mixed well, then plate was incubated for 20 minutes at 37°C. Plate was taken out and 300μl of wash buffer was added to each well (Washing 1) and aspirated off after 20 seconds. This step was repeated for 3 times until each well become dry, 100μl of Conjugate Reagent was added
in to each well except the blank, the plate was mixed well and incubated for 20 min at 37°C. The plate cover was removed and discarded. The liquid was aspirated and each well was rinsed in wash buffer (Washing 2). This step was repeated for 3 times until each well became dry. 100μl of substrate solution was added in to each well including the Blank and mixed by tapping the plate gently. The plate was incubated at 37°C for 10 m 100 μl Stop solutions was added into each well and mixed gently.

**Measuring the absorbance:**
The plate reader was calibrated with blank well and the absorbance was read at 450 nm within 15min. Adual wavelength is recommended with reference filter of 600-650nm. The results were calculated by relating each sample optical density (OD) value to the Cut off value of plate. Calculation of Cut off (C.O) value:

\[
C.O = Cm \ OD \times CF \\
\text{(Cm= result optical density)} \\
\text{(CF=calibration factor)}
\]

Calculate the Ab(antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

**Interpretation of Results:**
*Negative results:* Samples show less than 0.9 is nonreactive for this assay.
*Positive result:* Sample show greater than 1.1 considered initially reactive.
*Borderline:* Sample within 0.9-1.1 are considered borderline and retesting of these samples in duplicate is recommended.

**Data analysis:**
Data was analyzed by SPSS (Statistical Package of Social Science)
Result:

A total of 90 blood donors were enrolled in the study, their age ranged from 18 to 59 years, All of them were males.

Analysis of the results showed a serofrequency of 0% for EBV-VCA IgM antibodies, table 1 shows the age distribution of blood donors under study most of them were belong to 18 – 48 years age.

Table 1: distribution of study population according to their age ( n = 90 )

<table>
<thead>
<tr>
<th>Age group</th>
<th>Result</th>
<th>Total</th>
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<tbody>
<tr>
<td></td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>18 – 28 years</td>
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<td>40</td>
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<tr>
<td>%</td>
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<td>44.4%</td>
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<tr>
<td>29 – 38 years</td>
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<tr>
<td>0</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>%</td>
<td>0.0%</td>
<td>33.3%</td>
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<tr>
<td>39 – 48 years</td>
<td>count</td>
<td></td>
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<tr>
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<tr>
<td>%</td>
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<td>22.3%</td>
</tr>
<tr>
<td>Total</td>
<td>count</td>
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</tr>
<tr>
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<tr>
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</table>

Discussion:

The EBV is consistently detected in blood donors to determine whether infection is an early initiating infection. This work was carried out at Omdurman hospital in Epstein-Barr virus (EBV), many viruses such as Hepatitis G (HGV) and Epstein-Barr virus (EBV) which also carry a risk of transmission by blood transfusion raise the question of the extent of screening for this pathogen.

The present study results revealed that all tested blood donors were seronegative for EBV, when compared with other studies reports regarding prevalence of EBV in blood donors, they reported higher results among blood donors of < 20 years age group In most sub-Saharan countries in Burkina Faso, in
which it was 5.4% [15], however many researchers [16, 17], mentioned in previous studies EBV prevalence was higher in the young age groups. This is in accordance to previous studies which have shown early EBV infections among young children in Africa. However, EBV prevalence was higher in blood donors from rural areas (9.4%) compared to those from urban areas (5.2%) but the difference observed is not statistically significant.

Differences in results may be due to small sample size or technique used.

**Conclusion:**

This study reports for EBV prevalence in Sudan among blood donors were found to be seronegative. Further studies with large sample size will help to define their impact on infected humans.

**Acknowledgment:**

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