Full Length Research Paper

Seroprevalence of Epstein-Barr Virus IgM Antibodies among HIV-Patients and Apparently Healthy Blood Donors Attending Ahmadu Bello University Teaching Hospital, Shika-Zaria, Nigeria

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Abstract. Epstein-Barr virus (EBV) is a ubiquitous herpesvirus latentely affecting over 95% of the world’s population. EBV infections in apparently healthy individuals are usually asymptomatic, but cause malignancies in immunosuppressed patients, especially those with HIV/AIDS. The need for blood to save dying patients requiring blood transfusion increases daily. Viral-contaminated blood from donors is unsafe for transfusion. Immunosuppressed individuals are at risk of opportunistic pathogens like EBV. This research was aimed at finding the seroprevalence of EBV-IgM antibodies among the two populations, and their associations with some demographic factors and CD4+ cell count. A total of 91 blood samples were collected from 46 HIV-seropositive patients and 45 apparently healthy blood donors attending Ahmadu Bello University Teaching Hospital, Shika-Zaria, Nigeria. Sera were serologically screened for the presence of IgM antibodies to viral capsid antigen (VCA) of EBV by ELISA technique. Overall seroprevalence of EBV-IgM antibodies was 6.6%. Out of the 46 HIV-seropositive patients, 5(10.87%) had EBV-IgM antibodies, and of which 2(4.35%) were males and 3(6.53%) were females. Among the 45 apparently healthy blood donors, 1(2.22%) had EBV-IgM antibodies. Although no statistical associations were found between EBV-IgM antibodies and the respondents’ age and gender, higher occurrence was found among the females and those within age-group 21-30 years. HIV-seropositive patients with CD4+ cells count <200cells/mm² had more occurrence of EBV-IgM antibodies, but it generally decreased with increase in CD4+ cells count. Compatible blood for transfusion must be safe from harmful agents like viruses (including HIV, EBV). Efforts in vaccines development against EBV should be intensified.

Keywords: Blood donors, CD4+ cells, EBV-IgM, Epstein-Barr Virus, HIV, Nigeria, seropositive

1. INTRODUCTION

Viral co-infections among HIV/AIDS patients are major concerns all over the world because their interactions affect the severity and natural progression of HIV/AIDS (Abdollahi et al., 2014). One of such viruses that can significantly exacerbate the HIV-related morbidity and mortality in a co-infection is EBV (Ling et al., 2003). EBV is an ancient virus (McGeoch et al., 1995) discovered from a B-lymphocyte-derived tumour (Burkitt’s lymphoma) in the year 1964 (Adjei et al., 2008) and named after Anthony M. Epstein and Yvonne Barr (Jain et al., 2011). It is a tumourigenic (Crawford et al., 2002) human herpesvirus-4 (HHV-4) (Douglas et al., 2002) that is endemic worldwide with over 90% individuals having lifelong infections of their B-lymphocytes. Two types of the virus exist: EBV type-1 and EBV type-2. The two types are both common in Africa and New Guinea (van Baarle et al., 2000). Infected people shed the virus in their body fluids like saliva, semen, blood and breast milk, and organ transplants (Sitki-Green et al. 2003; Adjei et al., 2008; Gulley and Tang, 2008). Though infection with EBV occurs early in life, it persists and spreads among family members, and primary infections or seroconversion are asymptomatic among the immunocompetent (Crawford et al., 2002; Griffiths, 2009; Ajei et al., 2010; Linde and Falk, 2007). High standard of hygiene delays primary EBV infection until adulthood, which leads to infectious mononucleosis and other lymphoproliferative disorders in the immunocompromised (Crawford et al., 2002; De Paschale et al., 2009).

Patients with AIDS that are co-infected with EBV are at risks of AIDS-defining malignancies (Kaposi sarcoma, non-hodgkin’s lymphoma) and non-AIDS-defining malignancies like Hodgkin’s disease, nasopharygeal carcinoma, oral leukoplakia of the lingual squamous epithelium (MacMahon et al., 1991;
Cheung et al., 2005; Hjalgrim et al., 2007b; Mwakigonja et al., 2010). Such patients also have 10-20 times more circulating EBV-infected B-lymphocytes than apparently healthy individuals (Abdollahi et al., 2014). Sub-Saharan Africa accounted for about 70% cases of global HIV infections (UNAIDS, 2013). Globally, Nigeria is second in HIV burden with 3.2 million (3.34%) of her population infected (Bashorun et al., 2014). The presence of EBV-IgM antibodies in sera is indicative of primary infections or reactivations from latency (Sixbey et al., 1984). Many Nigerians have responded to the call for blood donation in order to save lives as a social and civic responsibility during the 2015 World Blood Donor Day (Vanguard, 2015 July 8\textsuperscript{th}). However, donated blood requires adequate screening for any underlying diseases (like EBV infection) to avoid their transmissions to vulnerable patients in need of blood transfusion. Hence, this study was aimed at determining the seroprevalence and some demographic factors associated with EBV-IgM antibodies among HIV-patients and apparently healthy blood donors attending Ahmadu Bello University Teaching Hospital, Shika-Zaria, Nigeria.

2. MATERIALS AND METHODS

2.1. Study area and populations

The study was conducted at Ahmadu Bello University Teaching Hospital (ABUTH), Shika-Zaria in Kaduna State, Nigeria. The hospital is located on Latitude: 11\textdegree 10'26.04" and Longitude: 7\textdegree 36'18.39" (http://ng.geoview.info/ahmadu_bello_university_teaching_hospital_samaru,776296517\textalpha). HIV-seropositive and blood donating individuals constituted the two comparative study populations for this study.

2.2. Consent and Ethical Approval

Consents of patients involved in this study were obtained from them, and an ethical committee of the teaching hospital approved the study. All data collected from the patients were treated with confidentiality and used only for the aim of this study.

2.3. Collection of blood samples

Three (3) ml of blood samples were collected via venous puncture using sterile 5ml syringes and needles and transferred into EDTA K-3 bottles and labelled. A total of 91 blood samples were collected from 46 HIV-seropositive patients and 45 apparently healthy blood donors. All the samples were kept in cool containers and transferred to the Department of Microbiology, Faculty of Science in Ahmadu Bello University Zaria for laboratory analysis.

2.4. Questionnaire administration

Structured questionnaires were used to collect some demographic data of the consented study populations. For the sake for this study, CD4+ cell counts of the HIV-seropositive patients were secondarily obtained from current laboratory reports of their immune status conducted in the teaching hospital.

2.5. Processing of blood samples

The blood samples were allowed to attain a room temperature before centrifugation at 3000rpm for 5mins to obtain sera. The sera were transferred into appropriately labelled sterile plain bottles and stored at 0\textdegree C until all the samples were ready for analysis by ELISA technique.

2.6. Blood screening for HIV

To ascertain the HIV status of the apparently healthy blood donors, all their blood samples were subjected to HIV screening using the HIV test strips.

2.7. EBV-IgM ELISA Screening

The frozen sera samples were brought out to attain a room temperature and labelled according to the alphabet on the ELISA plate. Dilution of 10:200 (i.e. 10\textmu l of serum + 200\textmu l sample diluent) was used in each well; but the first five wells were used for blank, negative control, positive control, calibrator 1 and calibrator 2 respectively. The solutions were run according to the instruction of the manufacturer of the ELISA EBV-VCA IgM test kit (Cortez Diagnostics, USA). After the reaction stop time, positive samples changed from blue to yellow colour. The colour intensities were measured photometrically within 30mins in an ELISA microwell reader at 450nm and the results were taken.

2.8. Statistical analysis

Demographic data collected on structured questionnaires during collection of blood samples, together with laboratory findings were subjected to Chi Square (χ\textsuperscript{2}) analysis using IBM SPSS Statistics Version 21 at P=0.05. Results were simplified in tables and charts.
3. RESULTS AND DISCUSSIONS

3.1. Results

Ninety-one individuals comprising of 46 HIV-seropositive patients and 45 apparently healthy blood donors were included in this study. The mean age of the former group was 36.5yrs made up of 40% males and 60% females. The group had a mean age of 34.5yrs with 68.9% males and 31.1% females. All the apparently healthy blood donating individuals that were screened for HIV were non-reactive. Hence, the seroprevalence of HIV among apparently healthy blood donors at ABUTH Shika-Nigeria was 0.0% (Fig. 1).

The overall seroprevalence of Ebstein-Barr Virus IgM in this study was 6.6%. However, among the HIV-seropositive population the seroprevalence was 10.9% and among the blood donors it was 2.2% (Table 1).

The females (12.0%) among the HIV-seropositive patients had higher occurrence of EBV-IgM antibody than the males (9.5%), but this relationship was not statistically significant as \( P = 0.788 \) (Table 2).

The single case of EBV- IgM among the blood donors occurred among age-group 21-30yrs accounting for 4.2%. Also, the highest occurrence of EBV-IgM antibodies among the HIV-seropositive patients was found among 21-30yrs group (accounting for 8.3%). Those in age-group 31-40yrs and 41-50yrs had EBV-IgM occurrences of 5.7% and 4.5% respectively; all the other age-groups were negative. This distribution of EBV-IgM by age was also not statistically significant in both study populations (Table 3).

Among the HIV patients, the highest occurrence of EBV-IgM (33.3%) was found among those with CD4+ cell count <200 cells/mm\(^3\) and decreased with increasing CD4+ cell count. However, it was not a statistically significant association (\( P = 0.256 \)) as indicated in Table 4.

Table 1: Seroprevalence of EBV IgM antibody among HIV-seropositive patients and Apparently Healthy Blood Donors

<table>
<thead>
<tr>
<th>Category Of Individuals</th>
<th>No. of Samples Examined</th>
<th>EBV IgM No. (%) Positive</th>
<th>EBV IgM No. (%) Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-Seropositive Patients</td>
<td>46</td>
<td>5 (10.9)</td>
<td>41 (89.1)</td>
</tr>
<tr>
<td>Apparent Healthy Blood Donors</td>
<td>45</td>
<td>1 (2.2)</td>
<td>44 (97.8)</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>6 (6.6)</td>
<td>85 (93.4)</td>
</tr>
</tbody>
</table>

\( \chi^2 = 0.128, P = 0.751 (P>0.05) \)

Table 2: Gender Distribution of EBV IgM Antibody among HIV-seropositive patients and Apparently Healthy Blood Donors

<table>
<thead>
<tr>
<th>Category Of Individuals</th>
<th>Gender</th>
<th>No. of Samples Examined</th>
<th>EBV IgM No. (%) Positive</th>
<th>EBV IgM No. (%) Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-Seropositive Patients</td>
<td>Female</td>
<td>25</td>
<td>3 (12.0)</td>
<td>22 (88.0)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>21</td>
<td>2 (9.5)</td>
<td>19 (90.5)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>46</td>
<td>5 (10.9)</td>
<td>41 (89.1)</td>
</tr>
<tr>
<td>Apparent Healthy Blood Donors</td>
<td>Female</td>
<td>14</td>
<td>1 (7.1)</td>
<td>13 (92.9)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>31</td>
<td>0 (0.0)</td>
<td>31 (100.0)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>45</td>
<td>1 (2.2)</td>
<td>44 (91.8)</td>
</tr>
</tbody>
</table>

\( \chi^2 = 0.072, p = 0.788; \chi^2 = 2.265, p = 0.132 \)

3.2. Discussion

The presence of EBV-IgM in the study populations was indicative of current (or acute) infections with EBV or reactivation of a latent infection housed in the B-lymphocyte (Sixbey et al., 1984; De Paschale and Clerici, 2012). It also shows that the virus is actively spreading in the population. However, there is currently no registered vaccine for prevention of EBV infection, no prophylaxes and the glycoprotein gp340 vaccine is yet on clinical trials (Public Health Agency of Canada, 2010).

The seroprevalence of EBV was 6.6% which was far lower than the finding of Crawford et al. (2002), who obtained 753 seropositive cases out of 1006 university students. Seroprevalence of EBV in this study was also far less than that of Adjei et al., (2008), who obtained 20.0% and 87.2% seroprevalence of
EBV among apparently healthy blood donor and HIV/AIDS patients respectively in Ghana-Africa. Among US children (of 6-19yrs), a seroprevalence of 66.5% had been obtained (Dowd et al., 2013). Information gathering regarding the epidemiology of EBV infections and cancers due them will be helpful in the development of vaccines. Many researchers have raised calls on vaccine development initiative (Crawford et al., 2002; Dowd et al., 2013; Khan and Hashim, 2014).

**Table 3:** Age-related Distributions of EBV IgM Antibody among the Respondents

<table>
<thead>
<tr>
<th>Respondents' Age Group (Years)</th>
<th>No. of Sample Examined</th>
<th>HIV-seropositive Patients No (%) Positive</th>
<th>Apparently Healthy Blood Donors No (%) Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-10</td>
<td>3</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>11-20</td>
<td>2</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>21-30</td>
<td>24</td>
<td>2 (8.3)</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>31-40</td>
<td>35</td>
<td>2 (5.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>41-50</td>
<td>22</td>
<td>1 (4.5)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>51-60</td>
<td>4</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>61 and above</td>
<td>1</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>91</td>
<td>5 (5.5)</td>
<td>1 (1.1)</td>
</tr>
</tbody>
</table>

\( \chi^2 = 2.132, \ p = 0.907; \ \chi^2 = 2.265, \ p = 0.322 \)

**Fig. 1:** Seroprevalence of HIV among Apparent Healthy Blood Donor at ABUTH Shika-Zaria, Nigeria

**Table 4:** Effect of CD4+ Cell Count of HIV-seropositive Patients on Occurrence of EBV

<table>
<thead>
<tr>
<th>CD4+ (cells/mm(^3))</th>
<th>No. of Samples Examined</th>
<th>EBV IgM No. (%) Positive</th>
<th>EBV IgM No. (%) Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;200</td>
<td>3</td>
<td>1 (33.3)</td>
<td>2 (66.7)</td>
</tr>
<tr>
<td>( \geq 200 &lt; 500 )</td>
<td>21</td>
<td>3 (14.3)</td>
<td>19 (85.7)</td>
</tr>
<tr>
<td>( \geq 500 )</td>
<td>22</td>
<td>1 (4.5)</td>
<td>21 (95.5)</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>5 (10.9)</td>
<td>41 (89.1)</td>
</tr>
</tbody>
</table>

\( \chi^2 = 2.724, \ p = 0.256 \)

Many malignant lymphomas had been associated with viral infections in man. Such viruses include EBV (Kaaya et al., 2006), HIV (Mwakigonja et al., 2008) and Kaposi’s sarcoma associated herpes virus/human herpesvirus type-8 (Mwakigonja et al., 2010). It means therefore, that those with two or more of these implicated viruses stand at higher risk of suffering one form of malignancy or other. Of all
deaths due to cancers, EBV-related malignancies accounted for 1.8% (i.e., 143,000 deaths) in 2010 (Khan and Hashim, 2014).

There was a higher seroprevalence of EBV-IgM antibodies among the HIV-seropositive patients compared to the apparently healthy blood donors. Immunocompromised patients are usually prone to opportunistic infections. This high occurrence of EBV among the HIV/AIDS patients indicated their predisposition to development of many malignancies as well as the exacerbation/progression of HIV infection (Hjalgrim et al., 2007a).

The high occurrence of EBV IgM antibodies among 21-50years age-group was indicative of sexual trends of activities, and in particular, the possibility of sexual intercourse or close related behaviour in its transmission. This had been indicated by the findings of Nuvor et al. (2001), Crawford et al. (2002) and Higgins et al. (2007) that sexual activities are routes for spreading EBV. From the sexual secretions of infected males and females, EBV had been detected (Sixbey et al., 1986) and can be transmitted via these secretions, especially among multiply sexual partners (Crawford et al., 2002).

Females in this study had more EBV-IgM antibodies than the males; the former might be more exposed to the infection. It can be reasoned that women are often in contact with children than men. Hence, in this part of the world women can easily acquire EBV from infected children, though more research has to be done to validate this. A significantly greater prevalence of EBV had been recorded among females than among men, and those sexually active had more of the infections than those not (Crawford et al., 2002; Higgins et al., 2007).

The CD4+ cells count of HIV patients showed that 3 of the patients had less than 200 cells/mm² and 1 out of them had EBV. This very low CD4+ count was an indication of progressed AIDS, with higher chances of developing more malignancies among many opportunistic infections. But patients with HIV type-1 are at high risk of developing EBV-related lymphomas (Ling et al., 2003). In general, EBV is most frequently isolated from HIV-patients because their low immune status promotes reactivation/proliferation of the virus (Santos et al., 2014). Among the immunosuppressed (like HIV) patients, B-cell lymphoma, Burkitt's lymphoma, Hodgkin's disease and nasopharyngeal carcinoma are related to concomitant EBV infections (Figueira-Silva et al., 2004)

Before blood is accepted into the blood bank or used for transfusion, it is essential that series of tests should be carried out first to ensure its safety. Apart from the threat of blood incompatibility, other underlying diseases are transmissible via blood, such as EBV and HIV. Though there has been a call to donate blood in Nigeria due to chronic blood shortage and insufficient blood banks in hospitals across the country, the blood banking system in Nigeria is still poorly developed; as such some centers depend on paid donors whose haematological and infectious status may not be adequately determined prior to blood donations (Vanguard, 2015 July8th). Therefore, apparently healthy individual willing to donate blood to patients or the blood banks must be screened for EBV among other diseases markers. Other markers have been Human herpesvirus-8 (HHV-8), cytomegalovirus (CMV) which can be ongenic if blood containing them is transfused to immunosuppressed patients (Adjei et al., 2008).

4. CONCLUSION

Epstein-Barr virus is harmful because of its association with many malignancies. Its occurrence is preventable if effective vaccines are developed against primary infections; but this yet to be achieved. Information provided from this study will add to growing global data regarding its epidemiology. An overall seroprevalence of 6.6% was found in this study, but an occurrence of 10.9% among HIV/AIDS patients was far higher than 2.2% occurrence among apparently healthy blood donors in Zaria, Nigeria. Hence, HIV/AIDS patients should be screened for asymptomatic EBV infection to prevent development of malignancies. Though the blood donors in this study had no occurrence of HIV infection, EBV was detected amongst them. EBV infection among the blood donors signifies the possibility of its transmission via blood, together with other underlying disease conditions to patients in need of blood transfusion. Female HIV/AIDS patients had more of the EBV infections than males, and the single case among the blood donating population was among the females. This shows that females are more likely to become infected than the males during exposure, though this finding was not statistically significant and needs to be validated. Higher occurrence of EBV infection was found among the sexually active age-group of 21-50years. Globally, blood to be donated must be critically screened for HIV, EBV among myriads of infectious agents before safe-storage in blood banks or for onward transfusion. To reduce the burden of malignancies and deaths due to EBV, especially among the immunocompromised, a shift of attention towards vaccine development will serve a great prevention strategy. It is imperative, therefore, to monitor and administer treatment to HIV-seropositive patients in consideration of EBV infection, its reactivation and associated malignancies.
REFERENCES


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