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Epstein Barr Virus IgM among HIV patients in Calabar, Nigeria

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Abstract

The magnitude of the Epstein Barr Virus (EBV) infection and its associated diseases are underestimated and there are no well-established prevention and control strategies in many developing countries, including Nigeria. However, its coinfection with Human Immunodeficiency Virus (HIV) is associated with several complications and eventually lead to death. The aim of this study was to determine the burden of acute EBV among HIV patients in Calabar, Nigeria. A prospective cross-sectional survey was conducted among 91 HIV patients attending University of Calabar Teaching Hospital (UCTH) in Calabar, Nigeria. Laboratory analysis of EBNA-specific IgM antibodies was done using an enzyme-linked immunoassay method on venous blood samples. Chi-square was used to determine the association of the infection with socio-demographic factors. Of the 91 subjects, 1(1.1%) were seropositive for EBNA IgM antibody while 90(98.9%) were observed to be seronegative for EBNA IgM

antibody. Higher prevalence of EBNA IgM antibody was found in age group 31-45 years (4.7%), in singles (3.8%) and among students (6.0%). Though the prevalence of EBNA IgM antibodies in this study is low, its presence underscores the need for awareness, more specific tests and screening of HIV patients in order to reduce the devastating effects of the virus in HIV patients.

Keywords: Antibodies, EBNA, IgM, HIV, Incidence, Calabar

Introduction

Epstein Barr virus is an important human pathogen that causes an acute and contagious disease. It is an enveloped DNA virus belonging to the family, Herpesviridae (Young et al., 2016). The route of transmission is oral via saliva and genital secretions (Amon et al., 2004). EBV is also known as "kissing disease" and presents in over 95% of adult population, most of which primarily occurred in childhood, resulting in asymptomatic and/or unrecognized infection (Nicholas, 2011). Most people, known to have been infected with EBV, gain adaptive immunity (Chen et al.,1991). Infants become susceptible to EBV as soon as maternal antibody protection disappears (Balfour et al., 2005). Many children infected with EBV usually show no clinical symptoms distinct from the other mild, brief childhood illnesses. However, when EBV infections occur amongst the adolescent, it usually causes infectious mononucleosis (CDC, 2012).

EBV is self-limiting, and like other gamma herpes viruses, establishes latent infection in lymphocytes and can induce proliferation of the latently infected cells (Jarmai et al., 2020). The virus first infects the oropharyngeal epithelial cells, replicates in the cells of the oral mucus, thus releasing its progeny into the saliva and spreads during mouth-to-mouth contact or sharing of oral utilities, like tooth brush and drinking cups (Wadowsky et al., 2003), no wonder the name kissing disease. During latency, EBV infects oropharyngeal epithelial cells, enters the blood, binds to CD21 receptors on B lymphocytes, and persists for life (Shi et al., 2021). EBV is a etiologically linked to multiple malignancies including nasopharyngeal carcinoma, Hodgkin's lymphomas, Burkitt's lymphoma and infectious mononucleosis (Kafita et al., 2018).

The prevalence of EBV in Hodgkin lymphoma is elevated in HIV-positive individuals compared to the general population (Shindiapina et al., 2020). In most cases, EBV has been associated with tumours in HIV infected individuals (Kafita et al., 2018) with the AIDS patients having a greater risk of developing aggressive lymphomas. Co-infections of EBV in HIV patients pose a serious health challenge leading to a high rate of morbidity and mortality among the HIV positive patients (Jarmai et al., 2020).

Several factors and analysis of risk factors such as; age, gender, country or region of residence, household educational level, kissing, smoking habit and sexual activity have been associated with the EBV seropositivity (Anejo-Okopi et al., 2019). There is paucity of information concerning the prevalence of EBV infection amongst HIV patients in the state. Therefore, this study was carried out to determine the burden of recent EBV infection among HIV-infected individuals in Calabar, Nigeria.

Materials and methods

Study Area

The study was conducted in the University of Calabar Teaching Hospital situated in Calabar, Cross River State, Nigeria. Calabar Municipality and Calabar South has a

combined population of 371,000 in 2006 census. The population of Cross River State has been growing at the rate of about 3% annually since 1991. Growth rates are considerably higher in Calabar city. The region has a rainy season from April until October, during which 80% of the annual rain falls, with peaks in June and September. Annual rainfall averages 1,830 millimeters (72 in). Average temperatures range from 24 °C (75 °F) in August to 30 °C (86 °F) in February. Relative humidity is high, between 80% and 100%. There is also little variance between daytime and night-time temperature, as temperatures at night are typically only a few degrees lower than the daytime high temperature.

Study design

A hospital based cross-sectional design was adopted and used to determine the prevalence of EBV antibodies among HIV positive patients in Calabar, Nigeria. Ethical approval was obtained from the University of Calabar teaching hospital (UCTH). Informed consent was obtained from all participants. Each study participant completed a clinical epidemiologic questionnaire and donated blood for laboratory testing.

Scope of the Study

The study was conducted from March through November, 2019 in HIV positive patients attending University of Calabar Teaching Hospital (UCTH), Calabar, Cross Rivers State, Nigeria The occurrence of EBV IgM antibody among the HIV patients were investigated using ELISA. The influence of age, marital status, occupation, educational status, on the prevalence of EBV antibody was also considered.

Study population

The study population comprised of 91 HIV-positive patients, which includes 24 males and 67 females attending the antiretroviral clinic of University of Calabar Teaching Hospital (UCTH). The sera of individuals were subjected to serological assay for IgM using enzyme-linked immunosorbent assay. Individuals that were included in the study were males and females confirmed and documented as HIV – positive. Individuals selected in the study were either ART-exposed or naive. Individuals who decline involvement in the study were not included in the study.

Sample Collection

Three milliliters of blood was aseptically collected by venipuncture from 91 consenting HIV patients and dispensed into appropriately labeled sample tube, screwed-capped and left at room temperature for about 40 minutes, after which it was spun at 3,000rpm for 10 minutes to separate serum from blood. Samples were clearly identified with codes in order to avoid misinterpretation of results. The sera were carefully aspirated into plain bottles and stored at -20^{o}C until analyzed.

Serological Analysis

The sera were analyzed for EBNA IgM using the ELISA kit manufactured by DIA.PRO Diagnostic Bioprobes (Milano) – Italy. ELISA tests were performed and results interpreted according to the manufacturer's instructions. Test results were interpreted as a ratio of the sample OD450nm and the Cut-Off value (or S/Co) following the manufacturer's instructions. S/Co<1.0 as Negative, >1.2 as Positive and 1.0 – 1.2 as Equivocal.

Data Analysis

The data obtained from questionnaires and laboratory analysis were entered into Microsoft Excel, analyzed using Statistical Package for Social Sciences version 21. Pearson Chi-square was calculated at 95% confidence interval and *P*-value < 0.05 was considered significant to

determine the association between the presence of the antibodies to the virus and other parameters.

Results

Socio demographics of the Study Population

A total number of 91 HIV patients were enrolled in this study. The socio-demographic data for the participants were stratified and shown in Table 1. The age ranges from 4-62 years. Age group 31 – 45 years constituted the largest populations making up 53.8%, followed by age groups 46-60 years (23.1%), and ≥ 61 years which was the least (3%). More females (70.3%) took part in the study compared to males (26.4%). For educational background, 4.4%, 28.6% and 67.0% of the study subjects had primary, secondary and tertiary education respectively. A greater number of those enlisted for this study were business people (29.7%), 14.3% were traders, 7.8% artisans, 17.6% students, 14.3% civil servants, 1.1% drivers and 2.2% Teachers, farmers, and public servants each. Married individuals constituted 70.3% while the singles were 29.7%.

Prevalence of EBV IgM antibodies

Of the 91 sera tested, 1 (1.1%) was positive for EBV IgM antibodies and 98.9% were negative. Therefore, the prevalence of EBV infection among HIV infected patients in Calabar was 1.1% as summarized in Table 1. This means that 1.1% of the HIV patients have a real or an actual susceptibility of Epstein Barr viral infection. Two percent of the overall seropositivity of anti-EBV IgM was obtained in participants in age group 31-45 years, 1.5% in females, 3.8% in singles, 3.8% in those with secondary education and 6.3% in students.

Table 1: Prevalence of EBV IgM antibody in the Population

Socio-Demographic Characteristics	Groups	No. Tested (%)	No. Positive (%)
Age (years)	<30	18(19.8)	0(0.0)
	31-45	49(53.9)	1(2.0)
	46-60	21(23.1)	0(0.0)
	61>	3(3.3)	0(0.0)
Gender	Male	24(26.4)	0(0.0)
	Female	67(70.3)	1(1.5)
Marital Status	Married	64(70.3)	0(0)
	Single	27(29.7)	1(3.8)
Educational Status	Primary	4(4.4)	0(0.0)
	Secondary	26(28.6)	1(3.8)
	Tertiary	61(67.0)	0(0.0)
Occupational Status	Student	16(17.6)	1(6.3)
	Unemployed	7(7.8)	0(0.0)
	Civil servants	13(14.3)	0(0.0)
	Trading	13(14.3)	0(0.0)
	Artisans	7(7.8)	0(0.0)

	Business Executive	25(27.7)	0(0.0)
	Farmer	2(2.2)	0(0.0)
	Public servants	2(2.2)	0(0.0)
	Driver	1(1.1)	0(0.0)
	Teacher	2(2.2)	0(0.0)
	Retired	3(3.3)	0(0.0)
Total		91 (100.0)	1(1.1)

Discussion

When a HIV positive individual is infected with Epstein Barr virus, there is a high chance of health deterioration which may eventually lead to death. This is due to the immune exhaustion caused by the HIV (Balasubramaniam et al., 2019) and the pathogenic and malignant potential of the EBV (Kolawole et al., 2017). EBV has been reported to be a notable opportunistic infection affecting HIV cohorts causing malignancies and disorders (Anejo-Okopi et al., 2019).

The prevalence obtained in this study (1.1%) was low compared to earlier studies done in different parts of the country. Studies in Ogbomosho, Jos, Zaria and Abakaliki recorded an EBV IgM prevalence rate of 4%, 6.5%, 6.6% and 22% respectively (Bishop & Adegoke, 2016; Kolawole et al., 2017; Okonko et al., 2020). Several factors may have contributed to the varying prevalence rates such as regional differences, assay type (Cookey et al., 2023), male to female ratio, variation in sample size of the studies and the influence of antiretroviral therapy used in the treatment of HIV patients (O'Sullivan et al., 2002).

Consistent with the study of Hjalgrim et al. (2007), Schaftenaar et al. (2014) and Jarmai et al. (2020), a higherprevalence of anti EBV IgM was obtained in females. This is at variance with the reports of Abdollahi et al. (2014) and Chakraborty et al. (2010) where the prevalence was dominant in the male population. Though not completely clear, high prevalence in females has been postulated to arise from frequent contact with children (Anejo-Okopi et al., 2019), high sexual activeness and the notion that women mount more vigorous antibody and cell-mediated immune response following infection than in men ((Kolawole et al., 2017).

When a comparison was made between the age groups and the anti-EBV IgM positivity, 31-45years was observed to be the highest. This finding agrees with the studies carried out in Abakaliki (Okonko et al., 2020) and Jos and disagrees with the reports of Cookey et al., 2023 in which age group 21-30 years had the highest prevalence. Though some studies reported a gradual increase or decrease of EBV prevalence with respect to age, this study proved different.

It is well noted that occupation and educational background are part of the known risk factors affecting the epidemiological pattern of EBV infection. In this study, a seropositivity rate of 6.3% and 3.8% was obtained from students and those with secondary school educational background.

This corroborates with previous studies by Pereira et al. (2021) who reported how unsatisfactory education is a risk factor congruent with HIV infection due to the lack of aggregate knowledge about prevention mechanisms of STIs and unprotected sexual exposure. Also, Smatti et al. (2018) in his review, pointed to the fact that the age of primary infection of EBV varies with the socio-

economic factors which was reflective by crowdedness and poor hygiene standards.

Hence, higher education levels and higher family income were considered protective factors against both mono-infection and co-infections of EBV.

Conclusion

Immunoglobulin M antibodies against Epstein Barr Virus has been shown to be present among HIV patients in this study. Better understanding of the burden and epidemiology of the disease can be ascertained by adopting a comprehensive approach to surveillance and conducting appropriate studies to assist in defining the EBV susceptibility profile in HIV patients in Calabar, Nigeria.

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

Statement of ethical approval

All authors hereby declare that all experiments have been examined and approved by the University of Calabar Teaching Hospital (UCTH) Research Ethic committee and have, therefore, been performed following the ethical standards laid down in the 1964 Declaration of Helsinki.

Statement of informed consent

"All authors declare that informed consent was obtained from all individual participants included in the study."

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