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Research Article
Plasma Immunoglobulin-G (IgG) Against Epstein Barr Virus Nuclear Antigens Among University Students in Port Harcourt, Nigeria

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Abstract

Background and Objective: Although the high prevalence of EBV has been well documented in Africa, such data are sparse from Port Harcourt, Nigeria. This study was done to evaluate Epstein-Barr Nuclear Antigens (EBNA) among University students in Port Harcourt, Nigeria. **Materials and Methods:** Ninety (90) plasma samples were tested to examine the seroprevalence of EBV in University students. Plasma samples were tested for antibodies specific to EBV by IgG ELISA assays. Differences in seropositivity rates were evaluated using the Chi-square test. **Results:** Of the 90 University students tested, 78 (86.7%) were males and 12 (13.3%) were females, with ages ranging from 17-33 years. Among the University students tested, the overall seroprevalence of EBV was 95.6%. Age, marital status, occupations, drinking habits, stressful events, overseas travel, sexually activeness, oral sex, anal sex, sexual activities, condom use, ABO blood groups, history of allergies, blood transfusion, tissue and organ transplants and surgery were not independent determinants ($p>0.05$) while sex, educational level, smoking habits, penetrative intercourse and kissing were independent determinants ($p<0.05$) for EBV infections among University students in Port Harcourt, Nigeria. **Conclusion:** The results presented herein indicate that EBV infections are hyperendemic among university students in Port Harcourt, Nigeria and suggest primarily a horizontal route of transmission of the EBV infections in Nigeria. Students under 25 years should be the primary target population of public health measures in the future. This study demonstrated that the seropositivity of EBV significantly rose with age, sex and educational level.

Key words: Antibodies, Epstein-Barr virus, hyperendemic, nuclear antigens, prevalence, plasma immunoglobulin-g, seropositivity, DNA viruses, herpesviridae

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Epstein-Barr Virus (EBV) is one of the opportunistic pathogens that affect Human Immunodeficiency Virus (HIV) infected individuals and it is the leading cause of death and cancer in humans[1,2]. The EBV is an important global human pathogen in the herpes virus family[3,4]. The EBV is one among the group of double-stranded DNA viruses, infecting all across the globe[4,5]. It is also one of the eight human herpesviruses that establish life-long persistent infection in humans[5].

The EBV belongs to the family of herpesviridae and serves as the dominant cause of acute infectious mononucleosis in young children and adolescents[6-10]. Primary acute EBV infections are largely asymptomatic and the latent infection could persist for the lifetime[6,9]. In rare circumstances, chronic active EBV infections might lead to severe morbidities and death in previously healthy individuals[9,11,12]. Some EBV infections have been associated with the hemophagocytic syndrome, a severe inflammatory illness, characterized by prolonged fever, cytopenia and liver dysfunction[9,13]. Moreover, the virus is causally linked to several malignancies, including Burkitt's lymphoma, Hodgkin lymphoma, tumours in HIV-infected patients and nasopharyngeal carcinomas[9,14].

The majority (>90%) of the adult human population carries asymptomatic infection of EBV[10,15]. The EBV are oncogenic viruses with a long latency period in healthy hosts and will reactivate from dormancy when the hosts are immunosuppressed[15]. Primary infections with these viruses in the immunocompetent host are generally asymptomatic[15].

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[1,16,17]. The EBV investigation is done using blood to recognize an EBV disease. The investigation detects the existence of EBV immune response, which are antibodies that the body's immune system releases to attack invading of an antigen, screening current and precedent infection[18]. This study was designed to Evaluate Epstein-Barr virus (EBV) antibodies among university students in Port Harcourt, Nigeria.

MATERIALS AND METHODS

Study area: A cross-sectional research study was conducted among university students in Port Harcourt, Rivers State, Nigeria. Port Harcourt lies along the Bonny River in the Niger Delta region of Nigeria with its coordinates: 4°53'23"N 6°54'18"E and covers an area of 360 km^2. Port Harcourt metropolis consists of Obio/Akpor Local Government Area and Port Harcourt Local Government Area[19], which comprise of largely Ikwerre ethnic with several other ethnic groups from all around Nigeria.

Study design: The study was carried out at the Virus Research Unit, Department of Microbiology, University of Port Harcourt, Nigeria from January to November, 2019. The design of this study was to investigate the prevalence of EBVNA IgG antibodies among university students in Port Harcourt, Rivers State, Nigeria. A cross-sectional study was conducted on university students who attends the University of Port Harcourt, Port Harcourt, Nigeria. Ethical considerations and approval for the study was obtained from the University of Port Harcourt Research Ethics Committee following the ethics for research involving human subjects.

Study population: The study population was University students in Port Harcourt, Rivers State, Nigeria. At most, 90 students were selected and enrolled for the study. While the entire University students in Port Harcourt, Rivers State, Nigeria were the target population to which the findings of the study were extrapolated. The demographic details relevant to the study were obtained. Consecutive sampling of the University students in Port Harcourt, Rivers State, Nigeria to total 90; this was ensured that sampling was representative of the students in Port Harcourt. A sampling of university students in Port Harcourt, Rivers State, Nigeria was randomly done irrespective of age, gender and tribe etc, from various levels in the University. All University students in Port Harcourt, Rivers State, Nigeria was eligible for the study. Bonafide students of the University were included, whereas, students who had incomplete data like age, sex, level and duplicate records were excluded from the study.

Sample collection: About 5 mL venepuncture blood was collected in EDTA BA Vacutainer TM anti-coagulant tubes (BD, Franklin Lakes, USA). The blood samples were collected by vein puncture technique using aseptically procedure. The blood samples were then dispersed into the labelled sample bottles. Plasma specimens were separated by centrifugation at 3000 rpm (resolution per minute) for 5 min. The plasma was stored at -20°C and was used for the laboratory analyses.

Laboratory diagnosis and quality control: Plasma was tested at the Virus Research Unit, Department of Microbiology, University of Port Harcourt, for the presence of IgG antibodies to EBV Nuclear Antigens (EBNA, IgG antibodies to viral nuclear

antigen, Dia. Pro, Milano, Italy), following the manufacturer's instructions. Positive and negative standard sera, accompanying the kit were included in each assay. All samples with non-reactive results to EBNA kits were considered negative.

Serological analysis of EBVNA IgG antibodies: Detection of Epstein-Barr Virus Nuclear Antigen (EBVNA) IgG antibodies (qualitative assay of IgG antibodies) was done using Dia Pro according to the manufacturer's instructions. Washing was done automatically using an ELISA washer (ELx50, Biotek, USA). Plates were read using an ELISA plate reader (ELx808i, Biotek, USA) at an absorbance of 450 and 630 nm.

Interpretation of results: Samples with a concentration lower than 5 arbU mL^{-1} were considered negative for anti-EBNA IgG antibodies. Samples with a concentration ranging 5-10 arb U mL^{-1} were considered in the grey zone. Samples with a concentration higher than 10 arbU mL^{-1} were considered positive for anti-EBNA IgG antibodies.

Data collection and analysis: Data on socio-demographic variables, behavioural variables, clinical variables and laboratory test results were collected from the University Students. Data were then cross-checked for completeness and entered into SPSS version 20 software for analysis. Descriptive statistics were performed and the results were presented in tables. Chi-Square test was fit to identify factors associated with the prevalence of IgG antibodies EBV nuclear antigens. A p-value $\leq$0.05 was considered to be statistically significant.

RESULTS

Socio-demographical characteristics of the university students: A total of 90 University students participated in this study. The age ranges of the participants were from 17-33 years. The age groups 17-25 years constituted the largest populations making up 77.8% while age groups 26-33 years were the least (22.2%). The majority (78) were males (86.7%) and males were 12 (13.3%) as shown in Table 1. None were married (0.0%) while they were predominantly singles constituting 100.0%. Based on educational background, 18 (20.0) and 72 (80.0%) of the participants had secondary education and tertiary education, respectively. Based on occupational status, a lower percentage were employed/working-class students 15 (16.7%) while a larger percentage of them were mainly unemployed students only 75 (83.3%) as shown in Table 1.

Overall prevalence of IgG antibodies against Epstein Barr virus nuclear antigens (EBVNA) among university students studied: Of the 90 University students studied, 86 (95.7%) tested positive for EBVNA as shown in Table 1-3. Table 1 shows the prevalence of IgG antibodies against EBVNA to the various sociodemographic characteristics of the University students studied.

Age-related prevalence of IgG antibodies against EBVNA: The study showed no age-related statistical differences (χ^2 = 0.959089, df = 1, p = 0.7478) in the prevalence of IgG antibodies against EBVNA among the University students studied. Age group 17-25 years 67 (95.7%) had a slightly higher prevalence rate of IgG antibodies against EBVNA than age group 26-33 years 19 (95.0%) as shown in Table 1.

Sex-related prevalence of IgG antibodies against EBVNA: A higher prevalence rate prevalence of IgG antibodies against EBVNA was found among females 12 (100.0%) than in males 74 (94.9%) as shown in Table 1. However, this difference was not statistical associated (χ^2 = 0.974383, df = 1, p = 0.1467).

Marital status-related prevalence of IgG antibodies against EBVNA: Table 1 shows the prevalence of IgG antibodies against EBVNA to marital status. A higher prevalence of IgG antibodies against EBVNA was observed among the singles (95.7%) as none of the participants was married. Statistically, marital status was not significantly associated with the prevalence of IgG antibodies against EBVNA (χ^2 = 0.0172, df = 1, p = 0.0771).

Prevalence of IgG antibodies against EBVNA to educational background: The level of education of the participants had a significant relationship (χ^2 = 0.007870, df = 1, p = 0.00434) with the prevalence of IgG antibodies against EBVNA. A higher prevalence of IgG antibodies against EBVNA was observed among participants with tertiary education (97.2%, n = 70) compared to those with secondary education (88.9%, n = 16) as shown in Table 1.

Prevalence of IgG antibodies against EBVNA to occupational status: Table 1 shows the prevalence of IgG antibodies against EBVNA to occupation. A higher prevalence of IgG antibodies against EBVNA was found among unemployed 73 (97.3%) compared to those employed 13 (86.7%). Statistically, there was no significant relationship between occupation and prevalence of IgG antibodies against EBVNA (χ^2 = 0.945885, df = 1, p = 0.3010) (Table 1).

Table 1: Prevalence of IgG antibodies against Epstein Bar virus nuclear antigens to the socio-demographic characteristics of the students

Variables	Groups	No. of tested	No. of positive (%)	Chi-square test
Age groups	17-25	70	67 (95.7)	$\chi^2 = 0.959089$
	26-33	20	19 (95.0)	df = 1, p =0.7478
Sex	Male	78	74 (94.9)	$\chi^2 = 0.974383$,
	Female	12	12 (100.0)	df = 1, p = 0.1467
Marital Status	Married	0	0 (0.0)	$\chi^2 = 0.0172$, df = 1, p = 0.0771
	Singles	90	86 (95.6)	
Educational Status	Secondary	18	16 (88.9)	$\chi^2 = 0.007870$, df = 1, p = 0.00434
	Tertiary	72	70 (97.2)	
Occupational Status	Unemployed	75	73 (97.3)	$\chi^2 = 0.945885$, df = 1, p = 0.3010
	Employed	15	13 (86.7)	
Total		90	86 (95.6)	

Table 2: Prevalence of IgG antibodies against Epstein Bar virus nuclear antigens to the behavioural characteristics of the students

Variables	Groups	No. of tested	No. of positive (%)
Smoking Habits	Yes	14	14 (100.0)
	No	76	72 (94.7)
Drinking Habits	<12x in lifetime	39	37 (94.9)
	<12x per year	33	31 (93.9)
	>12x per year	17	17 (100.0)
Stressful events during secondary school	Yes	39	39 (100.0)
	No	51	47 (92.2)
Stressful events during university	Yes	47	46 (97.9)
	No	43	40 (93.0)
Travelled out of Nigeria	Yes	10	10 (100.0)
	No	80	76 (95.0)
Sexually active	Yes	65	62 (95.4)
	No	25	24 (96.0)
Oral sex	Yes	40	38 (95.0)
	No	50	48 (96.0)
Anal sex	Yes	7	6 (85.7)
	No	83	80 (96.4)
Sexual activity	Penetrative intercourse	69	66 (95.6)
	Non-penetrative intercourse	21	20 (95.2)
	Genital contact without penetrative intercourse	0	0 (0.0)
Condom Use	Never	20	19 (95.0)
	Seldom	47	45 (95.7)
	Always	23	22 (95.6)
Kissing Level	Wet kissing	72	70 (97.2)
	Dry kissing	18	16 (88.9)
Total		90	86 (95.6)

Table 3: Prevalence of IgG antibodies against Epstein Bar virus nuclear antigens to the clinical characteristics of the students

Variables	Groups	No. of tested	No. of positive (%)
Blood group	A	10	10 (100.0)
	B	9	9 (100.0)
	AB	0	0 (0.0)
	O	71	67 (95.7)
Experience allergies during secondary school	Yes	9	9 (100.0)
	No	81	77 (95.1)
Experience allergies during university	Yes	16	16 (100.0)
	No	74	70 (94.6)
History of blood transfusion	Yes	0	0 (0.0)
	No	90	86 (95.6)
History of tissue transplants	Yes	0	0 (0.0)
	No	90	86 (95.6)
History of organ transplants	Yes	0	0 (0.0)
	No	90	86 (95.6)
History of surgery	Yes	0	0 (0.0)
	No	90	86 (95.6)
Total		90	86 (95.6)

Behavioural characteristics of the university students: Other risk factors include smoking habits, drinking habits, sexual activity and kissing level. Fourteen (15.6%) of them had smoking habits while 76 (84.4%) of them were non-smokers. Regarding drinking habits, 39 (43.3%) of them drinks less than 12x in their lifetime, 33 (36.7%) of them drinks less than 12x per year and 17 (18.9%) drinks more than 12x per year (Table 2). In response to stressful events during educational days, 39 (43.3%) had stressful events during secondary school education while 51 (56.7%) had no such experience. In the same vein, 47 (52.2%) of them had stressful events during this University education while 43 (47.8%) had no such experience. Only 10 (11.1%) of them have travelled outside Nigeria before and 80 (88.9%) have not. Results also showed that 65 (72.2%) of the University students under study were sexually active while 25 (27.8%) of them responded no to questions on being sexually active. Also, 40 (44.4%) of them have had oral sex while 50 (55.6%) of them have not and only 7(7.8%) of them have had anal sex while 83 (92.2%) of them have not. In terms of sexual activity, a higher percentage (76.7%, n = 69) of them do have penetrative intercourse and 21 (23.3%) of them had non-penetrative intercourse while none of them has had genital contact without penetrative intercourse. Regarding condom use during sexual intercourse, 20 (22.2%) of them never used condoms, 47 (52.2%) of them had seldom condom use and 23 (25.6%) always use condom during sexual intercourse. Also, 72 (80.0%) students engaged in wet kissing while 18 (20.0%) engage in dry kissing (Table 2).

Prevalence of IgG antibodies against EBVNA to the various behavioural characteristics of the university students studied: Table 2 shows the prevalence of IgG antibodies against EBVNA to the various behavioural characteristics of the University students studied.

Prevalence of IgG antibodies against EBVNA to smoking habits: Table 2 shows the prevalence of IgG antibodies against EBVNA to smoking habits. A higher prevalence of IgG antibodies against EBVNA was found among those with the smoking habit (100.0%, n = 14/14) compared to non-smokers (94.7%, n = 72/76). Statistically, there was a significant relationship between smoking habits and the prevalence of IgG antibodies against EBVNA ($p<0.05$).

Prevalence of IgG antibodies against EBVNA to drinking habits: Table 2 shows the prevalence of IgG antibodies against EBVNA to drinking habits. Higher prevalence of IgG antibodies against EBVNA was found among those with drinking habits greater than 12x per year (100.0%, n = 17/17) compared to those of them with drinks habit of less than 12x in a lifetime (94.9%, n = 37/39) and less than 12x per year (93.9%, n = 31/33). However, this difference is not statistically associated ($p>0.05$).

Prevalence of IgG antibodies against EBVNA to stressful events during secondary school education: A higher prevalence of IgG antibodies against EBVNA was found among those who had stressful events during secondary school education (100.0%, n = 29/39) than those with no such experience (92.2%, n = 47/51). However, this difference is not statistically associated ($p>0.05$) (Table 2).

Prevalence of IgG antibodies against EBVNA to stressful events during university education: In the same vein, the prevalence of IgG antibodies against EBVNA was found among those of them who had stressful events during this University education (97.9%, n = 46/47) than those with no such experience (93.0%, n = 40/43). However, this difference is not statistically associated ($p>0.05$) (Table 2).

Prevalence of IgG antibodies against EBVNA to oversea travel: A higher prevalence of IgG antibodies against EBVNA was found among those who have travelled outside Nigeria before (100.0%, n = 10/10) than those who have not (95.0%, n = 76/80). However, this difference is not statistically associated ($p>0.05$) (Table 2).

Prevalence of IgG antibodies against EBVNA to sexually activeness: A higher prevalence of IgG antibodies against EBVNA was found among those who were not highly sexually active (96.0%, n = 24/25) than those who were (95.4%, n = 62/65). However, this difference is not statistically associated ($p>0.05$) (Table 2).

Prevalence of IgG antibodies against EBVNA to oral sexuality: A higher prevalence of IgG antibodies against EBVNA was found among those who were not engaged in oral sexual activity (96.0%, n = 48/50) than those who engaged in oral sexual activity (95.0%, n = 38/40). However, this difference is not statistically associated ($p>0.05$) (Table 2).

Prevalence of IgG antibodies against EBVNA to anal sexuality: A higher prevalence of IgG antibodies against EBVNA was found among those who were not engaged in anal sexual activity (96.4%, n = 80/83) than those who engaged in anal sexual activity (85.7%, n = 6/7). This difference was not statistically associated ($p>0.05$) (Table 2).

Prevalence of IgG antibodies against EBVNA to sexual activity: In terms of sexual activity, a higher prevalence of IgG antibodies against EBVNA was found among those who engaged in penetrative intercourse (95.6%, n = 66/69) compared to those who engaged in non-penetrative intercourse (95.2%, n = 20/21). However, this difference is statistically associated ($p<0.05$) (Table 2).

Prevalence of IgG antibodies against EBVNA to condom use: Regarding condom use during sexual intercourse, a higher prevalence of IgG antibodies against EBVNA was found among University students who seldomly used a condom during sexual intercourse (95.7%, n = 45/47) compared to those who always use a condom (95.6%, n = 22/23) and those never used condoms (95.0%, n = 19/20) during sexual intercourse (Table 2). However, this difference is not statistically associated ($p>0.05$).

Prevalence of IgG antibodies against EBVNA to kissing level: Regarding kissing level, a higher prevalence of IgG antibodies against EBVNA was found among University students who engaged in wet kissing (97.2%, n = 70/72) compared to those who do dry kissing (88.9%, n = 16/18) (Table 2). This difference was statistically associated ($p<0.05$).

Clinical history/characteristics of the university students: To clinical history/characteristics of the University students studied, a higher percentage of them (78.9%, n = 71) had O ABO blood group, 10 (11.1%) belonged to blood group A (15.6%) while 9 (10.0%) of them belong to blood group B while there was none (0.0%) belong to blood group AB. Regarding allergies, 9 (10.0%) experienced allergies during secondary school education while 81 (90.0%) had no such experience. In the same vein, 16 (17.8%) of them had experienced allergies during this University education while 74 (82.2%) had no such experience. None of the University students studied had a history of blood transfusion, tissue transplants, organ transplants and surgery (Table 3).

Clinical History/characteristics of the university students studied: Table 3 shows the prevalence of IgG antibodies against EBVNA to the various clinical history/characteristics of the University students studied.

Prevalence of IgG antibodies against EBVNA to ABO blood grouping: Regarding the ABO blood group, a higher prevalence of IgG antibodies against EBVNA was found among University students who had ABO blood group A (100.0%, n = 10/10) and B (100.0%, n = 9/9) compared to those who were ABO blood group O (95.7%, n = 67/71) and AB (0.0%) (Table 3). This difference was not statistically associated ($p>0.05$).

Prevalence of IgG antibodies against EBVNA to history of allergies during secondary school education: Regarding the history of allergies during secondary school education, a higher prevalence of IgG antibodies against EBVNA was found among those who experienced allergies during secondary school education (100.0%, n = 9/9) than those who had no such history of allergies (95.1%, n = 77/81). However, this difference is not statistically associated ($p>0.05$) (Table 3).

Prevalence of IgG antibodies against EBVNA to history of allergies during university education: Regarding the history of allergies during University education, a higher prevalence of IgG antibodies against EBVNA was found among those who experienced allergies during University education (100.0%, n = 16/16) than those who had no such history of allergies (94.6%, n = 70/74). However, this difference is not statistically associated ($p>0.05$) (Table 3).

Prevalence of IgG antibodies against EBVNA to history of blood transfusion: Regarding the history of blood transfusion, 95.6% (n = 86/90) prevalence of IgG antibodies against EBVNA was observed among University students with no history of blood transfusion (Table 3).

Prevalence of IgG antibodies against EBVNA to history of tissue transplants: Regarding the history of tissue transplants, 95.6% (n = 86/90) prevalence of IgG antibodies against EBVNA was observed among University students with no history of tissue transplants (Table 3).

Prevalence of IgG antibodies against EBVNA to history of organ transplants: Regarding the history of organ transplants, 95.6% (n = 86/90) prevalence of IgG antibodies against EBVNA was observed among University students with no history of organ transplants (Table 3).

Prevalence of IgG antibodies against EBVNA to history of surgery: Regarding the history of surgery, 95.6% (n = 86/90) prevalence of IgG antibodies against EBVNA was observed among University students with no history of surgery (Table 3).

DISCUSSION

The study showed an overall prevalence of IgG antibodies against Epstein Barr Virus Nuclear Antigen (EBNA) among university students in Port Harcourt, Nigeria to be 95.7%. This value is comparable to the value reported by Balfour *et al.*[7,8] in a prospective study of EBV-naïve University of Minnesota undergraduates, the incidence of primary EBV infection among freshmen. Balfour *et al.*[7,8] reported that 63.0% of students were antibody positive. This demonstrated the significant disease burden of primary EBV infection in EBV-naïve college freshmen. Such students were ideal subjects for a vaccine study whose primary endpoint is protection from infectious mononucleosis[7,8]. The presence of EBVNA IgG antibodies in a patient sample could be an indication of no history of EBV infection, history of previous infection and reactivation which confirms that they have been exposed[20].

The appearance of EBVNA IgG antibodies in these University students indicate that there was a history of the previous infection. The observed prevalence (95.6%) was high compared to earlier studies both in Jos (6.53%), South West which recorded (4%)[10] and also findings in Zaira, North Nigeria with a prevalence of (6.6%)[21]. The 95.7% is higher than the 90.4% reported by Abdollahi *et al.*[22] conducted at Tehran, Iran.

The herein reported significantly higher seroprevalence of EBV in University students in Port Harcourt, Nigeria suggests that sexual transmission might play an important role among high-risk sexual behaviour populations, such as sexually active individuals. The herein significantly higher seroprevalence of EBVNA IgG antibodies in university students is consistent with one previous study in Ghana[15,23]. However, the herein reported comparably high seroprevalence of EBV during both adolescence and adulthood suggests that their transmission might occur primarily through horizontal, non-sexual, contact[15]. Indeed, this is particularly evident in African populations where high prevalence rates have been observed in infants and children, with seroprevalence rates similar to that observed in adults[15,24,25].

This seroepidemiology study among university students supports the view that this viral infection is primarily transmitted non-sexually in West Africa[15]. Therefore, non-sexual transmission mainly through close interpersonal (especially between mother and child and among siblings) contact of non-intact skin or mucous membranes with blood containing secretions or saliva, maybe the primary mode of transmission of EBV in West Africa particularly in Nigeria and Ghana, similar to that suggested in previous reports from endemic areas[15,24,25].

Age is one significant risk factor for the EBV seropositive rate and this is also shown in the previous study[9,26]. After the analysis, results revealed that those from the age group 17-25 years had a higher prevalence rate than those of 26-33 years. The study showed no age-related statistical differences in the prevalence of IgG antibodies against EBNA among the University students studied. The age group 17-25 years (95.7%) had a slightly higher prevalence rate of IgG antibodies against EBVNA than the age group 26-33 years (95.0%). This is in agreement with Okonko *et al.*[2] in a similar study in Abakaliki, Nigeria[2]. Studies from various patients with regards to age has shown a 100.0% of $\leq$20 years old, 100.0% of $\geq$age 41, 90.9% between 21-30 years old and 97.1% of 31-40 were positive for EBNA IgG with no statistical significance between the ages. The findings are in terms with the reported by Abdollahi *et al.*[22] at Tehran and Iran. Similar findings were also found by Balfour *et al.*[8], who reported in their study that the prevalence in 2009-2010 by age group.

Also, cases of inactive EBV contamination could result in re-triggering via the lowering down of the host's internal defence network through contamination by HIV. Age-specific EBV antibody prevalence data from the National Health and Examinations Surveys indicate that white children could be vaccinated as late as 12 years of age, whereas, non-whites need to be vaccinated before they are 6 years old[8]. However, gender does not affect the EBV seropositive rate in the present and other studies[8,9,15,27]. This study showed sex-related association ($p = 0.001$) in the prevalence of IgG antibodies against EBNA. A higher prevalence rate of prevalence of IgG antibodies against EBNA was found among females (100.0%) compared to males (94.9%). This is in agreement with what was earlier reported by Okonko *et al.*[2] in a similar study in Abakaliki, Nigeria. The values reported for gender here is similar to the values reported based on gender in Abakaliki, Nigeria with females being high compared to male, 96.8 and 95.4%, respectively[2]. This is not in agreement with what was reported for females (29.8%) and males (70.2%) in an earlier study by Abdollahi *et al.*[22] in Tehran, Iran. This study shows that the seroprevalence of EBNA IgG antibodies among university students based on gender was statistically significant. This study also noted that females had a higher prevalence of EBNA IgG than males, although their infection status of EBV could not be confirmed in the study. A similar phenomenon was described by other EBV serological studies[9]. This is generally following the notion that females mount more vigorous antibody and cellular responses to infection or vaccination than males[28]. Although underlined mechanisms involving high EBV antibody levels in females remain unclear, the link between the high level of anti-nuclear antigen (NA)-2, rather than the anti-VCA IgG level[9] and the risk of multiple sclerosis in females was noted by Ascherio *et al.*[29].

A higher prevalence of IgG antibodies against EBNA was observed among the singles (95.7%) as none of the participants was married. This study also noted that higher education level was associated with the EBV seropositive rate in Port Harcourt, Nigeria. The level of education of the participants had a significant relationship ($p<0.05$) with the prevalence of IgG antibodies against EBNA. A higher prevalence of IgG antibodies against EBNA was observed among participants with tertiary education (97.2%) compared to those with secondary education (88.9%). The low maternal and household educational level being significant risk factors were previously reported[9], but the information about the effect of an individual's educational level on the EBV acquisition was limited[8,26,30].

The seroepidemiological pattern of infectious diseases could be affected by the public health policy, hygiene behaviour and socio-economical status. Similar results have been shown in other studies, although the definition of urbanization could vary in different countries and studies[9,30,31]. A higher prevalence of IgG antibodies against EBNA was found among unemployed (97.3%) compared to those employed (86.7%). Though statistically, there was no significant relationship between occupation and prevalence of IgG antibodies against EBNA. An estimate of the higher percentage of Nigerian population reside in urban areas and the high urbanization rate might result from the decline of rural industry and the continuous flow of people from rural to urban areas and this situation might as well partly explicate the high percentage of students who studied, worked and residents in the study area.

Other behavioural risk factors, including kissing, smoking habit and sexual activity, have been associated with the EBV seropositive rate[1,8,9,30]. In this study, a statistically significant relationship between smoking habits and the prevalence of IgG antibodies against EBNA ($p<0.05$) was observed. A higher prevalence of IgG antibodies against EBNA was found among those with the smoking habit (100.0%) compared to non-smokers (94.7%).

A higher prevalence of IgG antibodies against EBNA was found among those with drinking habits greater than 12x per year (100.0%) compared to those of them with drinks habit of less than 12x in a lifetime (94.9%) and less than 12x per year (93.9%). Also, a higher prevalence of IgG antibodies against EBNA was found among those who had stressful events during secondary school education (100.0%) than those with no such experience (92.2%). In the same vein, the prevalence of IgG antibodies against EBNA was found among those of them who had stressful events during this University education (97.9%) than those with no such experience (93.0%). A higher prevalence of IgG antibodies against EBNA was found among those who have travelled outside Nigeria before (100.0%) than those who have not (95.0%).

A higher prevalence of IgG antibodies against EBNA was found among those who were not highly sexually active (96.0%) than those who were (95.4%). A higher prevalence of IgG antibodies against EBNA was found among those who were not engaged in oral sexual activity (96.0%) than those who engaged in oral sexual activity (95.0%). A higher prevalence of IgG antibodies against EBNA was found among those who were not engaged in anal sexual activity (96.4%) than those who engaged in anal sexual activity (85.7%). In terms of sexual activity, a higher prevalence of IgG antibodies against EBNA was found among those who engaged in penetrative intercourse (95.6%) compared to those who engaged in non-penetrative intercourse (95.2%). Regarding condom use during sexual intercourse, a higher prevalence of IgG antibodies against EBNA was found among university students who seldomly used a condom during sexual intercourse (95.7%) compared to those who always use a condom (95.6%) and those never used condoms (95.0%) during sexual intercourse. This is in agreement with what was reported by Chen *et al.*[9] in Taiwan. This suggests the transmission of the virus through sexual activity[32].

While regarding kissing level, a statistical association ($p<0.05$) was observed. A higher prevalence of IgG antibodies against EBNA was found among university students who engaged in wet kissing (97.2%) compared to those who do dry kissing (88.9%). This agrees favourably with Chen *et al.*[9], who implicated deep kissing as a significant risk factor for EBV seropositivity. In another study, a similar percentage of university students had primary EBV infection in a 3-year follow-up period, in whom as high as 77% presented as infectious mononucleosis and deep kissing was a significant risk factor[8].

Regarding the ABO blood group, a higher prevalence of IgG antibodies against EBNA was found among university students who had ABO blood group A (100.0%) and B (100.0%) compared to those who were ABO blood group O (95.7%) and AB (0.0%). Regarding the history of allergies during secondary school education, a higher prevalence of IgG antibodies against EBNA was found among those who experienced allergies during secondary school education (100.0%) than those who had no such history of allergies (95.1%).

Regarding the history of allergies during secondary school education, a higher prevalence of IgG antibodies against EBNA was found among those who experienced allergies during secondary school education (100.0%) than those who had no such history of allergies (95.1%). Regarding the history of allergies during university education, a higher prevalence of IgG antibodies against EBNA was found among those who experienced allergies during university education (100.0%) than those who had no such history of allergies (94.6%). In a study by Chen *et al.*[9], a cohort study among university students showed that 46.0% (110 in 241) of seronegative subjects experienced EBV seroconversion during their time in college.

An important issue that has major public health implications is the possibility of transmission of EBV through blood transfusion[15,33], especially in hyperendemic countries. Regarding the history of blood transfusion in this study, a 95.6% prevalence of IgG antibodies against EBNA was observed among university students with no history of blood transfusion.

Regarding the history of tissue transplants, a 95.6% prevalence of IgG antibodies against EBNA was observed among university students with no history of tissue transplants. Regarding the history of organ transplants, a 95.6% prevalence of IgG antibodies against EBNA was observed among university students with no history of organ transplants. Regarding the history of surgery, a 95.6% prevalence of IgG antibodies against EBNA was observed among university students with no history of surgery.

CONCLUSION

The study showed a high proportion of 95.7% of university students were exposed to the EBV infection either during childbirth, early children or delayed acquisition in adulthood. The method is based on the use of an affinity-purified native EBNA antigen to provide the assay with the highest specificity to EBV. Results from this study also showed that there is a high rate of prevalence among the age of 17- 25 years. In summary, this study demonstrated that the seropositive rate of EBV significantly rose with the educational level, smoking habits, penetrative intercourse and kissing.

SIGNIFICANCE STATEMENT

This study discovers the presence of IgG antibodies to EBV in 95.7% of the university students that can be an indication of past infections or previous exposure to the virus, which may have gone undetected. This study will help the researcher to uncover the critical area of using ELISA to unmask the possible etiological role of this virus in causing several malignancies, including Burkitt's lymphoma and infectious mononucleosis in students that many researchers in Nigeria were not able to explore routinely. Thus, a new diagnostic assay on this viral infection may be arrived at to be employed routinely in resource-limited settings like ours.

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