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Prevalence of Epstein-Barr Virus in Some Women Suspected with Breast Cancer in Kaduna State, Nigeria

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Abstract

Epstein - Barr virus has been associated with several types of epithelial carcinomas; it promotes the oncogenic action of cultivated breast cancer cells through stimulation of HER2 signaling cascades. This work was aimed at determining the seroprevalence of EBV and some risk factors associated with the infection in some women suspected with breast cancer attending Ahmadu Bello University Teaching Hospital, Kaduna state, Nigeria. Serological screening for EBV antibodies was done using Enzyme Linked ImmunoSorbent Assay (ELISA) IgM. Of the one hundred and seventy eight patient samples, one hundred and seven patients were positive for EBV (60.1%). Highest prevalence of 71.7% was recorded in women in the age group 40-49 years old, 70.5% in women with secondary education. Married women in monogamous marriages had highest prevalence of 65.6% and 69.8% respectively. Highest prevalence of 67.2% was recorded in women who had children; women with 1-5 numbers of children also had highest prevalence of 74%. There was a significant association of EBV IgM seroprevalence ($p<0.05$) with marital status, having children and also number of children the women

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had. EBV is believed to be a leading contributor to Hodgkin lymphoma, and significant associations have been found between the frequency of Hodgkin lymphoma and breast cancer, which suggests that EBV may also promote some breast cancers.

Keywords: Epstein - Barr virus; ELISA IgM, Breast cancer

INTRODUCTION

Epstein Barr Virus (EBV) is a widespread gamma-herpesvirus with approximately 95% of healthy adults having been infected (Mentzer et al., 2022). Typically, primary infection with EBV occurs during childhood and is asymptomatic. However, when the infection occurs in adults, it can lead to infectious mononucleosis (IM) (Santpere et al., 2014). Symptoms of the infection may include fever, fatigue, sore throat, and lymphadenopathy. In some cases, severe complications such as airway obstruction, myocarditis, hemolytic anemia, thrombocytopenia and ophthalmologic complications can occur, which may be fatal for some patients (Johannsen & Kaye, 2010).

EBV is also a tumorigenic virus and has been linked to a wide range of malignancies, such as nasopharyngeal carcinoma (NPC), Burkitt lymphoma, post-transplant lymphoproliferative diseases (PTLDs), gastric carcinoma, malignant follicular dendritic cells and Hodgkin's lymphoma (Tzellos & Farrell, 2012; Santpere et al., 2014 and Young et al., 2016). Additionally, there have been reports linking the virus to multiple sclerosis (MS) (Ascherio et al., 2001). EBV is believed to be a leading contributor to Hodgkin lymphoma, and significant associations have been found between the frequency of Hodgkin lymphoma and breast cancer, which suggests that EBV may also promote some breast cancers (Yasiu et al., 2001). Nevertheless, the role of EBV is disputed; while some studies confirmed the presence of EBV in breast cancer, others did not (Al-Moustafa et al., 2016 and Sinclair et al., 2021).

The primary route of transmission for EBV is through the oral route. However, it has been reported that organ transplantation and blood transfusion can also lead to the spread of EBV (Trottier et al., 2012). Infected epithelial cells can also be found in the uterine cervix or semen, suggesting the possibility of EBV spread through sexual contact (Papesch & Watkins, 2001). EBV can also be spread through kissing, sharing personal

items like toothbrushes and eating utensils, and sharing food and drinks with an infected individuals (CDC, 2016).

Breast cancer is currently the most common type of cancer and the leading cause of cancer death in women worldwide, with 2.26 million cases recorded in 2020 and 685,000 deaths (WHO, 2021). In Nigeria, breast cancer cases were historically low, but they are now on the rise due to urbanization and lifestyle changes. Currently, it is the leading cause of cancer related deaths, accounting for approximately 23% of all cancer cases and around 18% of deaths in the country (GLOBACOM, 2020). The etiology of breast cancer is not yet fully understood, but it is partially attributed to environmental factors, including viruses like Epstein-Barr virus (EBV). EBV is closely linked to endemic Burkitt lymphoma in sub-Saharan Africa and has recently been identified as a cause of breast cancer in Sudan (Tang et al., 2010 and Yahia et al., 2014). Epstein-Barr virus (EBV) is associated with 38% of all virus-associated neoplasia and has also been incriminated in the development of breast cancer (Huo *et al.,* 2012).

The potential relationship between breast cancer and EBV is important because it can not only broaden our understanding of breast cancer etiology but also be applied to early detection of breast cancer, as well as breast cancer prevention and treatment. There is a dearth of information on risk factors associated with cancer due to factors militating against data collection, documentation and statistics (Oladimeji et al., 2015). The aim was to determining the seroprevalence of EBV and the risk factors associated with the infection in some women suspected with breast cancer attending Ahmadu Bello University Teaching Hospital, Kaduna state, Nigeria.

METHODS

Study area

This study was conducted in Kaduna State, which is located in North Central Nigeria. The state is situated between latitude 10°C 38'58" N and 10°C 25'36" north of the equator, and longitude 7°C 22'14" east and 7°C 32'00" east of the Greenwich Meridian (Yusuf, 2015). Kaduna State is divided into three Senatorial Zones: Northern, Central and Southern zones. It consists of twenty-three Local Government Areas. For this study, we used Ahmadu Bello University Teaching Hospital (ABUTH), located in Shika-Zaria, Kaduna State, Nigeria. ABUTH is one of the referral centers in the Northern part of

Nigeria. The samples for this study were obtained from patients who visited the oncology clinic at ABUTH. We obtained ethical clearance from the Medical Ethical and Scientific Committee of the Ahmadu Bello University Teaching Hospital as well as the Ministry of Health, Kaduna State before commencing this study. We also sought consent from the participants, and only those who gave their consent were included in the study.

Questionnaire Administration

To collect data, we administered structured questionnaires to the patients who agreed to participate. The questionnaires aimed to gather personal information, demographic data, clinical data and risk factors associated with EBV.

Sample collection and analysis

A sterile needle and syringe were used to make a vein puncture and collect 2ml of blood. The blood was then dispensed into labeled containers (plain bottles) and allowed to clot for 30minutes. After clotting, the blood was centrifuged at 1000rpm for 10minutes to separate the serum. The serum was carefully transferred into labeled sterile serum screw capped containers using a pipette. The serum was then stored at -20°C for further analysis.

All samples and kit reagents were brought to room temperature (20-25°C) and mixed thoroughly by gently swirling before use. The procedure followed the manufacturer's instructions. The samples were numbered based on the microtitre well, and then 1 in 81 dilutions were made (i.e. 10µl in 800µl) of test sera, cutoff calibrator, and control sera in Serum Diluent Plus. Diluted sera, cutoff calibrator, and controls (100µl) were dispensed into the appropriate wells. For the reagent blank, 100µl of Serum Diluent Plus was dispensed in the1A well position. The holder was tapped to remove air bubbles and ensure proper mixing. All plates were incubated for 30minutes at room temperature (21° to 25°C). The liquid from the wells was removed by emptying the contents into a waste container, and the wells were washed three times with wash buffer. After washing, the wells were drained and dried by blotting with tissue. Enzyme conjugate (100µl) was then dispensed into each well, and the plates were incubated at room temperature for 30minutes. Excess enzyme conjugate was removed, and the wash process was repeated three times. After washing, the wells were drained and dried by blotting with tissue. Chromogen/substrate (100µl) was dispensed into each well and incubated at room temperature for an additional 15minutes. Finally, stop solution (100µl) was dispensed into each well to stop the reaction. The reaction was read using a microplate reader at an optical density of 450nm within

10minutes, following the manufacturer's instructions. The mean Calibrator Optical Density (O.D.) (mCOD) was calculated from the three calibrator determinations. The correction factor (CF) provided for each kit was then used to calculate the cutoff calibrator value (CCV) by multiplying the given correction factor by the mean calibrator O.D. The Immune Status Ratio (ISR) value was calculated by dividing the O.D. for the patient sera (POD) by the cutoff calibrator value.

That is: CCV = CF × mCOD

ISR = POD/CCV

Results were interpreted as follows:

Negative: ≤ 0.09 indicates no detectable EBNA-1 IgM antibody by the ELISA test.

Equivocal: 0.91-1.09 indicates that samples should be retested.

Positive: ≥1.10 indicates a significant level of detectable EBNA-1 IgM antibody, indicative of current or recent infection.

RESULTS

Out of the 178 samples analyzed, 107 tested positive for EBV IgM antibodies resulting in an overall prevalence of 60.10% (Figure 1).

The results were analyzed based on various socio-demographic factors (Table 1). Among women aged 40-49, the highest prevalence of EBV was observed, with 33 cases (71.7%), while the lowest prevalence was recorded among women over 50 years, with 14 cases (48.3%). In terms of educational status, women with secondary education had the highest prevalence, with 31 cases (70.5%), while women with tertiary education had the lowest prevalence, with 44 cases (54.3%). Women living in rural areas had a higher prevalence of EBV (54 cases, 61.4%) compared to those living in urban areas (53 cases, 58.9%). Among married women, the highest prevalence was observed, with 84 cases (65.6%), while single women had the lowest prevalence, with 15 cases (45.5%). when considering the type of marriage, monogamous marriages had the highest prevalence (60 cases, 69.8%), while women in polygamous marriages had the lowest prevalence (32 cases, 54.2%). The highest prevalence of EBV was found in women with children, at 84(67.2%), while lowest prevalence was recorded in women without children at 23(43.4%). Among women with between 1-5 children, the highest prevalence was observed, at 57(74.0%) while

the lowest prevalence was recorded among women with more than 11 children, at 0(0%). The data showed significant differences (P<0.05) between EBV and some socio-demographic factors which include marital status, having children and the number of children.

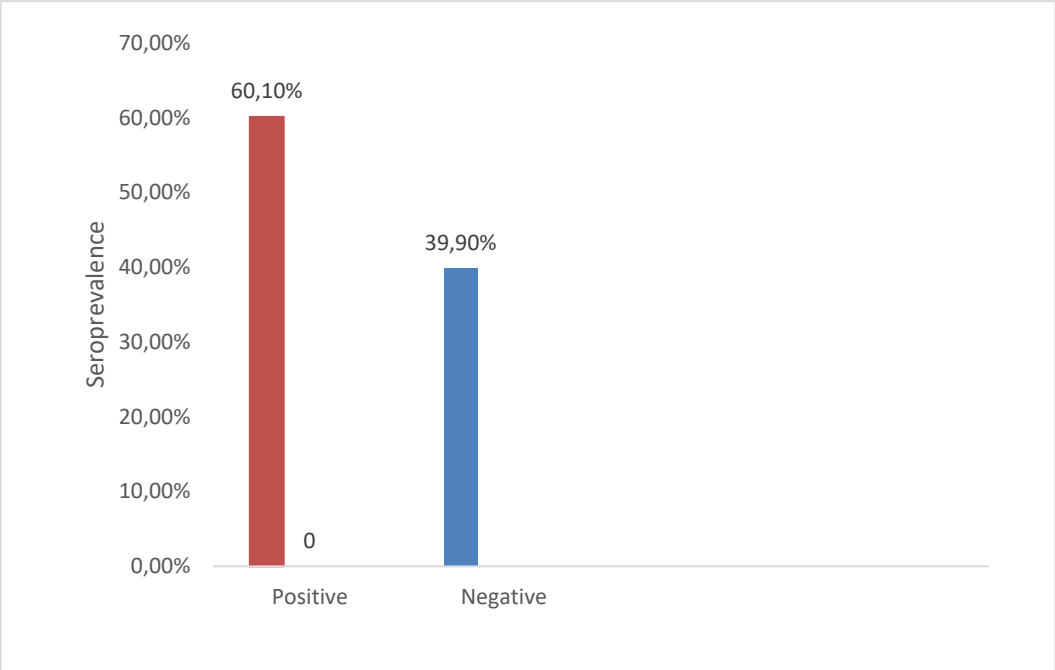


Figure 1: Seroprevalence of EBV (IgM) antibodies in some women suspected with breast cancer in Kaduna State, Nigeria.

Table 1: Seroprevalence of EBV in relation to socio-demographic factors in some women suspected with breast cancer in Kaduna State, Nigeria.

Variable	Number Examined	No. Positive (%)	P value
Age (years)			
<20	12	7(58.3)	0.179
20-29	38	19(50.0)	
30-39	53	34(64.2)	
40-49	46	33(71.7)	
>50	29	14(48.3)	
Total	**178**	**107(60.1)**	
Education			
Primary	17	11(64.7)	0.349
Secondary	44	31(70.5)	
Tertiary	81	44(54.3)	

Informal	36	21(58.3)	
Total	**178**	**107(60.1)**	
Place of Residence			
Urban	90	53(58.9)	0.512
Rural	88	54(61.4)	
Total	**178**	**107(60.1)**	
Marital Status			
Single	33	15(45.5)	0.049
Married	128	84(65.6)	
Divorced	17	8(47.1)	
Total	**178**	**107(60.1)**	
Type of Marriage			
Monogamous	86	60(69.8)	0.056
Polygamous	59	32(54.2)	
Total	**145**	**92(63.4)**	
Children			
Yes	125	84(67.2)	0.003
No	53	23(43.4)	
Total	**178**	**107(60.1)**	
No of children			
1-5	77	57(74.0)	0.002
6-10	43	27(62.8)	
>11	5	0(0.0)	
Total	**125**	**84(67.2)**	

Some risk factors were considered and analyzed (Table 2). Seroprevalence was highest in those who have been transfused with blood, at 30(61.2%), and the lowest values were found in those who have never been transfused with blood, at 77(59.7%). In terms of organ transplant, a seroprevalence of 107(60.5%) was obtained from those who have never undergone organ transplant. We observed a significant difference ($P<0.05$) between EBV and some risk factors.

Table 2: Prevalence of EBV in relation to some risk factors in some women suspected with breast cancer in Kaduna State, Nigeria.

Variable	Number Examined	Number Positive (%)	P value
Blood transfusion			
Yes	49	30(61.2)	0.852
No	129	77(59.7)	
Total	178	107(60.1)	
Organ transplant			
Yes	1	0(0.0)	0.218
No	177	107(60.5)	
Total	178	107(60.1)	

DISCUSSION

The Epstein-Barr Virus (EBV) is a widely spread herpesvirus that infects over 90% of adults worldwide (Hoover and Higginbotham, 2023). In our study, 60.10% of the population tested positive for EBV IgM indicating an acute infection. These women can be considered to have an active and ongoing infection, which may be a marker of biological aggressiveness in breast cancer (Mazouni et al., 2011). It is also believed that EBV infection may play a role in the early stages of breast carcinogenesis and increase the risk of breast cancer (Huo *et al.,* 2012). EBV infection might be a latent factor in the development of certain types of breast carcinoma (Huo *et al.,* 2012). Our results had a lower prevalence compared to a previous study by Aminu et al., (2021), which reported a prevalence of 82.20% for EBV.

In this study, the highest prevalence of EBV was found in women in the age group of 40-49years. This could be attributed to cumulative exposure over time, as individuals are more likely to encounter the virus through close contact with others during their lifetime. Additionally, waning immunity or reactivation of the virus could contribute to its persistence in this age group. Factors such as stress, compromised immune functions, sexual activity and unhygienic living conditions, which are possible routes of virus transmission may play a role in the higher prevalence in this age group (Crawford et al.,

2002 and Higgins et al., 2007). This result is in agreement with a previous study by Zhang et al., (2017).

In this study, the highest prevalence of EBV was recorded in women with secondary education, which could be due to lifestyle factors such as drinking alcohol, smoking, sleeping poorly and being sedentary (Bever, 2023). This result is in agreement with a previous study by Zhang et al. (2017). The lowest prevalence was recorded in women with tertiary education, which could be attributed to a higher level of education and increased awareness. The highest prevalence of EBV was found in women living in rural areas, which could be due to low personal hygiene, overcrowding, limited access to healthcare, close knit communities and low levels of education which can contribute to low awareness.

In this study, married women had the highest EBV prevalence. Hormonal changes (childbirth and pregnancy) and stress which influences the immune system could be possible reasons. Some studies have reported EBV in male and female genital secretions, which may be the cause of infection transmission during sexual contact. This result agrees with a previous study by Zhang et al. (2017). The highest prevalence of EBV was found in women in monogamous marriages which contradicts report of Crawford et al. (2006), who reported the highest prevalence in people with multiple sexual partners.

In this study, women who have children had the highest EBV prevalence. This could be attributed to the fact that in pregnant women reactivation of EBV frequently reoccurs because cellular immune responses are suppressed (Haeri et al., 2010).

Women with a history of blood transfusion had a higher EBV prevalence, suggesting that the virus could have been transmitted through the blood. This finding is consistent with a previous study by Nishiwaki et al. (2006). Women with no history of organ transplant had a higher EBV prevalence, indicating that the virus could have been acquired through other means, such as contact with infected saliva or blood transfusion.

CONCLUSION

In conclusion, the prevalence of EBV IgM (60.10%) in this study indicates an acute and ongoing infection, which could increase the risk of breast cancer. Marital status, having

children and the number of children were found to be statistically significant (P <0.05) in relation to EBV prevalence**.**

REFERENCES

Al-Moustafa, A.E., Al-Antary, N., Aboulkassim, T., Akil, N., Batist, G., & Yasmeen, A. (2016). Co-prevalence of Epstein-Barr virus and high-risk human papillomaviruses in Syrian women with breast cancer. *Human Vaccines and Immunotherapeutics* 12*(***7***):* 1936-1939. PMID: 27082145. DOI: 10.1080/21645515.2016.1139255.

Aminu, M., Muhammed, A., Magaji, S.J., Olaniyan, F.A., Usman, M.A., & Ella, E.E. (2021). Detection of Epstein-Barr Virus Antibodies in Some Nigerian Women with Breast Lumps. Archives of Basics and Applied Medicine, 9:19– 22.

Ascherio, A., Munger, K.L., Lennette, E.T., Spiegelman, D., Hernan, M.A., Olek, M.J., Hankinson, S.E., & Hunter, D.J. (2001). Epstein-Barr virus antibodies and risk of multiple sclerosis: a prospective study. ***JAMA.*** 286(24):3083–8. PMID: 11754673 doi: 10.1001/jama.286.24.3083.

Center for Disease Control (CDC), (2016). Epstein-Barr Virus and Infectious Mononucleosis. Available from: https://www.cdc.gov/epstein-barr/about-mono.html.

Crawford, D.H., Macsween, K.F., Higgins, C.D., Thomas, R., McAulay, K., Williams, H., Swerdlow, A.J., & Britton, K. (2006). A cohort study among university students: identification of risk factors for Epstein-Barr virus seroconversion and infectious mononucleosis. *Clinical Infectious Disease Journal.* 43(**3**):276–82. https://doi.org/10.1086/505400

Crawford, D.H., Swerdlow, A.J., Higgins, C., McAulay, K., Harrison, N., Williams, H., Britton, K., & MacSween, K.F. (2002). Sexual History and Epstein-Barr virus Infection. *Journal of Infectious Diseases*, 186(**6**):731-736. ISSN 0022-1899 DOI: https://doi.org/10.1086/342596

GLOBOCAN 2020: UICC: New global cancer data. December 17, 2020. Available at www.uicc.org/news/globocan-2020-new-global-cancer-data. Accessed June 16, 2021.

Haeri, S., Baker, A.M., & Boggess, K.A. (2010). Prevalence of Epstein-Barr Virus Reactivation in Pregnancy. *American Journal of Perinatology*, **27**(9):715-720. doi: 10.1055/s-0030-1253098.

Higgins, C.D., Swerdlow, A.J., Macsween, K.F., Harrison, N., Williams, H., McAulay, K., Thomas, R., Reid, S., Conacher, M., Britton, K., & Crawford, D.H. (2007). A Study of Risk Factors for Acquisition of Epstein-Barr virus and Its Subtypes. *Journal of Infectious Diseases*, 195(**4**):474-482. doi: 10.1086/510854.

Hoover, K., & Higginbotham, K. (2023). Epstein - Barr virus. [Updated 2023 Aug 8]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK559285/

Huo, Q., Zhang, N., & Yang, Q. (2012). Epstein-Barr Virus Infection and Sporadic Breast Cancer Risk: A Meta-Analysis. *PLoS One,* **7**(2):e31656. https://doi.org/10.1371/journal.pone.0031656

Johannsen, E., & Kaye, K. (2010). Epstein-Barr virus (Infectious Mononucleosis, Epstein-Barr Virus-Associated Malignant Disease and other Diseases) In: Mandell GL, editor. *Mandell, Douglas, Benett's Principles and practice of Infectious Diseases*. 7th Ed. Philadelphia: Churchil Livingstone; 1989–2005. 138, 1872-1890.e7\

Mazouni, C., Fina, F., Romain, S., Ouafik, L., Bonnier, P., Brandone, J.M., & Martin, P.M. (2011). Epstein-Barr virus as a marker of biological aggressiveness in breast cancer. *British Journal of Cancer*. 104(**2**):332–337. doi: 10.1038/sj.bjc.6606048

Mentzer, A.J., Brenner, N., Allen, N., Littlejohns, T.J., Chong, A.Y., Cortes, A., Almond, R., Hill, M., Sheard, S., McVean, G., Collins, R., Hill, A.V.S., Waterboer, T., & UKB Infection Advisory Board (2022). Identification of host-pathogen-disease relationships using a scalable Multiplex Serology platform in UK Biobank. Nature Communication. 13(1):1818. Doi: 10.1038/s41467-022-29307-3.

Nishiwaki, M., Fujimuro, M., Teishikata, Y., Inoue, H., Sasajima, H., & Nakaso, K. (2006). Epidemiology of Epstein-Barr virus, cytomegalovirus, and Kaposi's sarcoma-associated herpesvirus infections in peripheral blood leukocytes revealed by a multiplex PCR assay. *Journal of Medical Virology*. 78(**12**):1635–42. doi: 10.1002/jmv.20748.

Oladimeji, K.E., Tsoka-Gwegweni, J.M., Igbodekwe, F.C., Twomey, M., Akolo, C., Balarabe, H.S., Atilola, O., Jegede, O., & Oladimeji, O. (2015). Knowledge and beliefs of breast self-examination and breast cancer among market women in Ibadan, South West, Nigeria. *PloS one*, **10**(11):e0140904. https://doi.org/10.1371/journal.pone.0140904.

Papesch, M., & Watkins, R. (2001). Epstein-Barr virus infectious mononucleosis. *Clinical Otolaryngology and Allied Science*. 26(1):3–8. doi: 10.1046/j.1365-2273.2001.00431.x.

Santpere, G., Darre, F., Blanco, S., Alcami, A., Villoslada, P., Mar Albà, M., & Navarro, A. (2014). Genome-wide analysis of wild-type Epstein-Barr virus genomes derived from healthy individuals of the 1,000 genomes project. *Genome Biology and Evolution*. 6(**4**):846–60. doi: 10.1093/gbe/evu054.

Sinclair, A.J., Moalwi, M.H., & Amoaten, T. (2021). Is EBV associated with breast cancer in specific geographic locations? *Cancers (Basel),* **13***(4):* 819. doi: 10.3390/cancers13040819.

Tang, W., Harmon, P., Gulley, M.L., Mwansambo, C., Kazembe, P.N., & Martinson, F. (2010). Viral Response to Chemotherapy in Endemic Burkitt Lymphoma. *Clinical Cancer Resource*, **16**(7):2055–2064. doi: 10.1158/1078-0432.CCR-09-2424.

Trottier, H., Buteau, C., Robitaille, N., Duval, M., Tucci, M. and Lacroix, J., & Alfieri, C. (2012). Transfusion-related Epstein-Barr virus infection among stem cell transplant recipients: a retrospective cohort study in children. *Transfusion.* 52(12):2653–63. doi: 10.1111/j.1537-2995.2012.03611.x.

Tzellos, S., & Farrell, P.J. (2012). Epstein-Barr virus sequence variation-biology and disease. *Pathogens.* 1(2):156–74. doi: 10.3390/pathogens1020156.

World Health Organization (WHO), (2021). Breast cancer and the Nigerian Woman. https://healththink.org/breast-cancer-and-the-nigerian-woman/.

Yahia, Z.A., Adam, A.A., Elgizouli, M., Hussein, A., Masri, M.A., Kamal, M., Mohamed, H.S., Alzaki, K., Elhassan, A., Hamad, K., & Ibrahim, M.E. (2014). Epstein-Barr virus: a prime candidate of breast cancer etiology in Sudanese patients. *Infectious Agent and Cancer,* **9**(1):9. doi: 10.1186/1750-9378-9-9

Yasui, Y., Potter, J.D., Stanford, J.L., Rossing, M.A., Winget, M.D., Bronner, M., & Daling, J. (2001). Breast cancer risk and "delayed" primary Epstein-Barr virus infection. *Cancer Epidemiology Biomarkers Preview* 10(**1**):9-16. PMID: 11205495

Young, L.S., Yap, L.F., & Murray, P.G. (2016). Epstein-Barr virus: more than 50 years old and still providing surprises. *Nat Rev Cancer* 16(12):789–802. doi: 10.1038/nrc.2016.92.

Yusuf, S. (2015). Kaduna: Physical and Human environment. Department of Geography, Kaduna State University, Kaduna. Published by Shanono Printers and Publishers Kaduna.

Zhang, W., Wang, M.Y., Wei, X.L., Lin, Y., Su, F.X., & Xie, X.M. (2017). Associations of Epstein-Barr virus DNA in PBMCs and the subtypes with breast cancer risk. *Journal of Cancer.* 8(**15**):2944–9. doi: 10.7150/jca.20330