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Research Article

Detection of Epstein-Barr Virus Antibodies in Some Nigerian Women with Breast Lumps

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Abstract

Breast cancer is the most frequently diagnosed cancer and leading cause of cancer death in females worldwide. Epstein-Barr virus (EBV) has been detected in subsets of breast cancers, implicating the virus as a probable cause of the cancer. The study was carried out to detect antibodies to EBV in women with or without breast lumps attending some hospitals in Zaria, Kaduna State, Nigeria. The study was a hospital-based and case-control study that combined the use of a structured questionnaire and analysis of blood samples obtained from 90 consenting women. The samples were screened for EBV-Viral Capsid Antigen (VCA) IgG and EBV-Early Antigen (EA) IgG using Enzyme-Linked Immunosorbent Assay. The EBV-VCA IgG was detected with similar rate of 82.2% (37/45) for women with and without breast lumps ($P=0.636$). The EBV-EA IgG was significantly detected in more than half (51.4%: 18/35) of the women with breast lump ($P=0.005$, $OR= 2.467$). There was no significant association between presence of the antibodies and all the socio-demographic and risk factors studied, except for breast feeding where EBV-EA IgG was detected significantly more ($P= 0.014$). Detection of EBV-VCA IgG indicate infection has occurred with EBV at some time recently or in the past among these women; while detection of the antibodies more in women with lumps could signify a relationship.

Key Words: Epstein - Barr virus, Antibodies, Breast Lumps, Women, Nigeria

INTRODUCTION

Epstein-Barr virus (EBV), also called human herpes virus 4, is an enveloped virus having linear double-stranded DNA as its genome (Bonnet *et al.*, 1999). The virus belongs to the family Herpesviridae which is divided into three subfamilies (α , β , and γ) (Knipe *et al.*, 2007).

Approximately 95% of the world's population sustains an asymptomatic life-long EBV infection which spreads via contact with infectious body fluids, such as saliva, blood and semen (CDC, 2021a). The virus spreads by saliva through kissing, sharing drinks and food, using the same cups, eating utensils, or toothbrush and having contact with toys that children have drooled on (CDC, 2021b). Epstein-Barr virus can cause infectious mononucleosis also known as mono. The virus persists in the memory B-cell pool of normal healthy individuals and any disruption of this interaction results in virus-associated B-cell tumors (Shah and Young, 2009). Epstein-Barr virus is associated with the development of different epithelial malignancies including nasopharyngeal carcinoma (Murray *et al.*, 1996).

The burden of cancer in Nigeria remains controversial. However, Nigeria records over 100,000 new cases of cancer

annually and breast cancer is the second leading cause of death in women after lung cancer (Akpanika, 2016).

As the aetiology and progression of breast cancer remain incompletely understood, novel routes of disease pathogenesis are important to consider. Viral pathogens have not been much explored, but recent interest has focused on EBV. Studies of an association of this virus with breast cancer have had inconsistent results, marked by varying EBV presence and the absence of certain viral characteristics found in other EBV-related malignancies. Therefore, this study intends to further investigate the association between EBV and breast cancer by exploring the relationship between breast lumps and the presence of antibodies to EBV. The presence of these antibodies could indicate an on-going infection; and an active infection in women with breast lumps could predispose to breast carcinogenesis (Tim, 2016). The study also aims at determining the possible risk factors that may be associated with breast lumps as this will provide useful information for prevention of breast cancer.

MATERIALS AND METHODS

Study Area, Design and Population: This was a hospital-based, case-control study conducted in some selected hospitals

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in Zaria, Kaduna State. The target population included 90 women (45 with old and new breast lumps and 45 without breast lumps) within the age of 18-75 years old attending the selected hospitals. Women without breast lumps served as the control population. Ethical Approval was obtained before the study commenced as well as informed consent from the respondents prior to sample and data collection.

Sample Collection and Processing: Using convenient sampling, a total of 90 women were selected and enrolled in this study. About 5ml of venous blood was collected from the women. The blood was centrifuged and the serum harvested and stored at -200C until analysed.

Sample Analysis: The sera were allowed to equilibrate to room temperature prior to analysis. All the serum samples were tested for EBV viral capsid antigen (VCA) IgG while only 70 samples were tested for EBV-EA IgG due to insufficient sample after running the first round of ELISA. The samples were tested using ELISA Kits with 100% specificity and sensitivity obtained from Diagnostic Automation/Cortez Diagnostics, Woodland Hills USA according to the manufacturer’s instructions.

Data Analysis: Data were analysed using the frequency procedure of Statistical Analysis System (SAS) version 9.2 at 0.05 level of significance and 95% confidence interval. Relationships between variables were determined using Pearson Chi Square.

RESULTS

Analysis of the study population by socio-demographic features revealed that more than half (52.2%: 47/90) of the respondents were between ages 15-29 years and were mostly married women (83.3%: 75/90). About a half (47.8%: 43/90) of them did not go beyond the secondary level of education.

The EBV-VCA IgG was detected with the same prevalence in both women with breast lumps (82.2%: 37/45) and those without breast lumps (82.2%: 37/45) (P = 0.636) (Table 1). Out of the 35 sera from women with breast lumps tested for EBV-EA IgG, 18 (51.4%) were positive, while only 6 (17.1%) of the 35 sera from women without breast lumps were positive. The EBV-EA IgG was significantly associated with the presence of breast lumps (P = 0.005, OR= 2.467).

Analysis of the result based on the type of lump showed that 81.8% (27/33) of the women that had benign lump were positive for EBV-VCA IgG while 83.3% (10/12) of those with malignant lump had EBV-VCA IgG, with no statistically significant association (P = 0.632) (Figure 1). Similarly,

53.8% (14/26) of the women with benign lump had EBV-EA IgG while only 44.4% (4/9) of those with malignant lump had EBV-EA IgG with no statistically significant difference (P = 0.345).

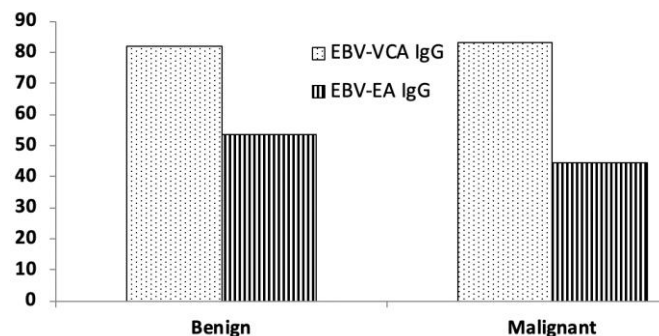


Figure 1: Seroprevalence of Antibodies to Epstein - Barr virus in Relation to Type of Lump among Women with and without Breast Lumps Attending some Hospitals in Zaria, Kaduna State
EBV- Epstein-Barr virus; VCA- Viral Capsid Antigen; EA- Early Antigen; IgG- Immunoglobulin Gamma

Analysis of the result based on socio-demographic factors revealed that women within age group 30-44 years had the highest prevalence of EBV-VCA IgG (87.5%: 14/16) while those within age group 45-59 years had the highest prevalence of EBV-EA IgG (53.8%: 7/13). There was no statistical association between the antibodies and age (Table 2). Looking at their educational status, 44.4% of the women that were positive for EBV-EA IgG did not go beyond the primary level of education (P = 0.117).

There was no statistically significant association between the antibodies and all the socio-demographic and risk factors studied, except for breast feeding that was significantly associated with EBV-EA IgG (P = 0.014).

DISCUSSION

Results obtained from this study shows that 82.2% of both women with breast lump and those without breast lump had IgG antibodies to EBV-VCA. Majority of the women in this study had circulating antibodies to EBV. This indicates that they had, at one point in their lives, been infected with EBV, hence had developed the antibodies. Also, the fact that the virus is easily transferable from one person to another by mere contact with body fluids, could explain the high prevalence of the circulating antibodies. This finding agree with the submission that, by adulthood, virtually everyone has been infected with EBV and has developed immunity to the virus (Shah and Young, 2009).

Table 1: Seroprevalence of Antibodies to Epstein - Barr virus among Women with and without Breast Lumps attending some Hospitals in Zaria, Kaduna State, Nigeria

Women	Total	VCA IgG			EA IgG			
		Positive	% Prevalence	P value	Total	Positive	% Prevalence	P value
Lumps	45	37	82.2	0.636	35	18	51.4	0.005
Without Lumps	45	37	82.2		35	6	17.1	

EBV- Epstein-Barr Virus, EA- Early Antigen, IgG- Immunoglobulin Gamma, VCA- Viral Capsid Antigen

Table 2:

Seroprevalence of Epstein - Barr virus in Relation to Socio-Demographic Factors among Women with and without Breast Lumps Attending some Hospitals in Zaria, Kaduna State

Demographic factor		EBV-VCA IgG				EBV-EA IgG			
		Total	No. Positive	Prevalence (%)	P value	Total	No. Positive	Prevalence (%)	P value
Educational status	Primary	12	10	83.3	0.145	9	4	44.4	0.117
	Secondary	43	33	76.7		59	20	33.9	
	Tertiary	35	31	88.6		2	0	0	
Marital status	Single	12	12	100	0.421	9	1	11.1	0.415
	Married	75	59	78.7		58	21	36.2	
	Separated	3	3	100		3	2	66.7	
Age group (Years)	15-29	47	40	85.1	0.406	34	10	29.4	0.414
	30-44	16	14	87.5		14	5	35.7	
	45-59	18	13	72.2		13	7	53.8	
	60-74	7	6	85.7		7	1	14.3	
	75-89	2	1	50.0		2	1	50.0	

EBV- Epstein-Barr virus; VCA- Viral Capsid Antigen ; EA- Early Antigen ; IgG- Immunoglobulin Gamma

Table 3:

Seroprevalence of Antibodies to Epstein - Barr virus in Relation to Risk Factors among Women with and without Breast Lumps Attending some Hospitals in Zaria, Kaduna State

Risk Factors		EBV VCA IgG				EBV EA IgG			
		Total	No. of Positive	% Prevalence	P value	Total	No. of Positive	% Prevalence	P value
Fever	Yes	86	70	81.4	0.212	67	23	34.3	0.251
	No	4	4	100		3	1	33.3	
Sore Throat	Yes	37	29	78.4	0.243	32	9	28.1	0.752
	No	53	45	84.9		38	15	39.5	
Malaria	Yes	90	74	82.2	0.604	70	24	34.3	0.308
	No	0	0	0		0	0	0	
Kissing	Yes	85	69	81.2	0.421	66	24	36.4	0.951
	No	5	5	100		4	0	0	
Alcohol	Yes	6	5	83.3	0.837	6	2	33.3	0.014
	No	84	69	82.1		64	22	34.4	
Breast Feeding	Yes	26	21	80.8	0.837	20	11	55	0.014
	No	64	53	82.8		50	13	26	

Key:

EBV- Epstein-Barr virus; VCA- Viral Capsid Antigen; EA- Early Antigen; IgG- Immunoglobulin Gamma

Epstein-Barr Virus-EA IgG was detected more in women that had breast lumps than in those without breast lumps (OR = 2.467). Anti-EA IgG appears in the acute phase of illness and generally falls to undetectable levels after three to six months, therefore in many individuals, detection of EBV-EA IgG is a sign of active infection (CDC, 2021b). The active state of this infection could have enhanced the development of the lumps seen in these women (Fields and Knipe, 2001). Although more than 80 percent of breast lumps end up being benign (Burstein, 2020), these lumps could be a manifestation of cancer and they therefore need to be properly managed (National Cancer Institute, 2021).

This study reported no statistically significant association between most of the risk factors studied (kissing, fever, sore throat, malaria and alcohol) and the presence of EBV IgG. However, a significant association was found between breast feeding and EBV-EA IgG. Ibrahim *et al.* (2015) reported high load of EBV in breast milk, suggesting the presence of the virus in breast cells and the high possibility of the virus being transferred to infants. There are many conditions that can cause lumps in the breast while breast-feeding. These include, engorgement, plugged duct, and mastitis (Galan, 2018). Therefore, the presence of lumps during breast feeding can

easily be overlooked, but where the lump persists, then further diagnostic approaches will be required as the lumps could be a sign of breast cancer (Galan, 2018).

Several hypotheses have been presented to explain the relationship between breastfeeding and breast cancer risk. One of these hypotheses suggested that estrogen and progesterone, by increasing the mitotic activity in breast cells, can have a role in the increased risk of breast cancer. Therefore, it is assumed that lactation amenorrhea (achieved through exclusive breast feeding in the first 6 months after birth) can impact the reduction of breast cancer by reducing hormone levels (Furberg *et al.*, 1999, WHO, 2014, National Cancer Institute, 2021). For this reason, the history and duration of breastfeeding have been considered as a possible protective factor (AAP and ACOG, 2006). The findings of this study did not agree with this submission. Although the pattern and duration of breast feeding were not captured in this study, the higher rate of EBV-EA IgG in women that were breast feeding could have been as a result of reactivation of previous infection that was acquired before or during pregnancy.

In conclusion, Epstein-Barr virus early antigen was found to be associated with breast feeding and breast lumps in this study. The presence of EBV early antigen indicates active state

of infection. Persistence of EBV in breast could promote breast changes that might enhanced development of lumps; and lumps could be cancerous if they persist.

Further studies is recommended to ascertain the possible relationship between Epstein-Barr virus, breast feeding and breast cancer which will provide useful data and insight on prevention of breast cancer.

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