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Epstein – Barr Virus Antibody Titres in Head and Neck Cancer

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Abstract: ***The study compares Epstein – Barr virus (EBV) antibody titres between head and neck cancer (HNC) cancer patients and healthy control persons. It also investigates the virus antibody titre among some HNC clinic-histopathologic factors. Sera samples were obtained from 100 HNC patients and 82 healthy controls. The samples were analysed and screened for IgG and IgA antibody against EBV VCA and EA using ELISA. The results showed that the EBV antibody levels were significantly higher in patients than controls except for IgA-VCA (P=0.380). The cancer type was significantly associated with EBV IgG-EA and IgA-EA, both had the highest titre in NPC. The oral cavity cancer (for IgG-EA) and salivary gland (for IgA-EA) cancer also had high antibody levels within the same titre range as NPC. Only IgA-EA was significantly associated with undifferentiated grade (p=0.006) and lymphoepithelioma histologic type of HNC (p=0.006). These factors were only attributed to 19 out of the 24 NPC in the study. The clinical stage of the HNC was not associated with the EBV antibody levels. Based on the results obtained, HNC patients harbour higher EBV antibody titres than control and there is possible involvement of EBV in the progression of these NPC, Oral cavity and salivary gland carcinogenesis. A further experiment on tissue samples is recommended to investigate the role of EBV in various HNC carcinogenesis.***

Keywords: EBV, HNC, ELISA, IgG, IgA

1. Introduction

Head and neck cancer (HNC) is the 6th-8th most common cancer in the world constituting 5-50% of all cancers globally. The estimated global incidence of all HNC is between 400-600,000 new cases per year and mortality rate of 223-300,000 death per year (Ferlay *et al*., 2010; Chaturvedi *et al.,* 2013). The cancer is proven to be endemic in southern China, southern Asia, to a lesser extent in Maghrebi Arabic region of North Africa and the Artic than in other region of the world (Thompson and kurzrock, 2004; Rickinson and Kieff, 2007; Saddiqui *et al*., 2012). In Nigeria, there is an increasing incidence of HNC per year with 20-24 new cases in northern part to 48 cases in Jos and 33-38 new cases in the south west (Lilly-Tariah *et al*., 2009). Also there is a prevalence increase from 0.9% to 1.3% in Port Harcourt (Onotai and Nwakolo, 2012).

Epstein-Barr virus (EBV) virus latent infection has been linked to several HNC malignant diseases that tend to occurs in the head and neck region as the oropharyngeal epithelium is the main site of the virus proliferation after infection (Ocheni *et al*., 2010). The virus has been strongly implicated in NPC carcinogenesis (Ocheni *et al*., 2010) and detected in other non nasopharyngeal HNC such as the palantine tonsil, tongue, pharyngolaryngeal, suppraglottid and the salivary gland caner (Greenspan *et al*., 1985; Young and Rickinson, 2004; Zheng *et al.,* 2010; Gupta and Metgud, 2013). The virus associated diseases are characterized by elevated IgG and IgA titres against viral capsid antigen (VCA) and early antigen (EA) expressed during the lytic cycle of EBV infection and replication (Wong *et al*., 2005; Majidi *et al.,* 2006; Abdulamir *et al.,* 2008).

The unequal prevalence of EBV in HNC throughout the world suggests complex etiology associated with genetic and environmental factors which vary in geographical distribution and genetic background (Eduardo *et al*., 2010; Gupta and Metgud, 2013). Most studies carried out are based in high endemic areas. The association of EBV infection and HNC development has not been studied thoroughly in this region (Nigeria) of the world given its low incidence. This study compares EBV antibody titres of HNC patients and healthy controls and also investigates the HNC clinical-histopathologic factors associated with EBV infection. This study will lead to better understanding of the significance of EBV in HNC and cast the way for further studies in the region.

2. Materials and Methods

This was a hospital based analytical cross sectional clinic-laboratory study. Serum samples were obtained from 100 histopathologically confirmed HNC patients attending the Ahmadu Bello University Teaching Hospital, Shika-Zaria Oncology clinic and 82 apparently healthy individuals (blood donors, patient visitors, health workers and students) who visited the hospital's Haematology laboratory for blood donation, blood grouping and other screen test. They had no history of cancer. The HNC enrolled were 24 nasopharyngeal, 19 oral cavity, 14 laryngeal, 12 sinonasal, 11 oropharyngeal, 11 salivary gland and 9 hypopharyngeal cancers. Participants were counselled and recruited prior to sampling, and only those that have signed the informed written consent as well as the questionnaire were sampled. The data sheet used for survey encompassed clinical-histopathologic data about patient disease that was obtained from consented patient's record with the official permission granted by ABUTH, Scientific and Health Research Committee. The data included cancer type, disease stage, cancer histology and grade. The serum samples were routinely stored at (-20°c) until used and then were evaluated for both IgA and IgG antibody levels to VCA and EA using ELISA kit test (IMMUNOLAB,GmbH,Otto-

Hahn-street, D-34123 Kassel). The cut-off value was used for demarcating sera into seronegative or seropositive following the manufacturer's instruction. The ELISA OD means titres were used for quantitative comparison.

3. Statistical Analysis

Data generated from this study was analysed using SPSS version 20.0. For qualitative categorized data (seroprevalence), the significance of association was measured by applying Chi square test. ELISA OD mean titres (quantitative categorized) were presented as mean ± 2 standard error. Only seropositive titres (i.e ELISA OD mean) were used for antibody level comparison in every parameter. Students T-test was used to compare the only seropositve IgG and IgA mean titres obtained from HNC patients and control. Other parameters assessed were cancer type, disease clinical stage, cancer histology and grade were compared using One-way analysis of variance (ANOVA). Duncan test was further used to indicate the significance of mean differences between a particular variable compared.

4. Results

(Table 1) list the levels of different classes of EBV antibody in patients and controls. All seroprevalence and antibody levels except for VCA (IgA) were significantly higher in HNC patients in comparison with healthy controls.

(Table 2) compare the antibody levels among various EBV seropositve head and neck cancer types. The seroprevalence of IgG-VCA was 100% for all cancer type. Only IgA-VCA was statistically significant with the highest seroprevalenece observed in nasopharyngeal and the lowest in salivary gland cancers. Nasopharyngeal cancer (NPC) had the highest antibody levels for all seropositive EBV antibodies compared to other cancers. Although it was only significant for IgG-EA and IgA-EA. The oral cavity (for IgG-EA) and salivary gland (for IgA-EA) cancers also had antibody level that were in the same range as NPC. Laryngeal cancer had the lowest titres.

(Table 3,4) compares the antibody levels among various seropositve HNC histologies and grades. The seroprevalence of IgG- EA, IgA-VCA and IgA-EA in HNC patients were not statistically significant with the histology and grade of cancer. Only IgA-EA antibody level was significantly associated with cancer histology and grade. lymphoepithelioma histologic type of cancer had the highest mean titres whereas undifferentiated grade type of cancer had the highest mean titre compared to other grades.

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able 1: Comparison of EBV seroprevalence and antibody titres between HNC patients and controls

Antibody	Seroprevalence n(%)			ELISA OD(means±s.e)		
	Patient n=100	Control n=82	p-value	Patient	Control	p-value
IgG-VCA	100(100.0)	54 (65.9)	0.000	1.989±0.05	1.482±0.07	0.000
IgG-EA	93 (93.0)	47 (57.3)	0.000	1.637±0.07	1.056±0.02	0.040
IgA-VCA	30 (30.0)	2 (2.4)	0.000	0.878±0.11	0.764±0.07	0.380
IgA-EA	75 (75%)	47 (57.0)	0.009	1.471±0.09	0.945±0.04	0.003

Seroprevalence; Chi square df=1; χ^2 value; IgG VCA=40.355, IgG EA=32.317, IgA VCA=23.617, IgA EA=6.375. ELISA OD; Student T-test value; IgG VCA=6.191, IgG EA=0.886, IgA VCA=2.150, IgA EA=5.530

Table 2: Comparison of EBV seroprevalence and antibody titres in various HNC types

Cancer type	Sample number	IgG-VCA	IgG-EA	IgA-VCA	IgA-EA
Seroprevalence n(%)					
Laryngeal	14	14 (100.0)	13(92.9)	2 (14.3)	9(64.3)
Oral cavity	19	19 (100.0)	17(89.5)	5 (26.3)	17(89.5)
Oropharygeal	11	11 (100.0)	11(100.0)	4 (36.4)	8(72.7)
Hypopharyngeal	9	9 (100.0)	8(88.9)	4 (44.4)	5(55.6)
Nasopharygeal	24	24 (100.0)	24(100.0)	13 (54.2)	19(79.2)
Salivary gland	11	11 (100.0)	11(100.0)	1(9.0)	8(72.7)
Sinonasal	12	12 (100.0)	9(75.0)	1(8.3)	9(75.0)
p-value*		-	0.123	0.024	0.534
ELISA-OD (means±s.e)					
Laryngeal		1.811±0.09	0.342 ± 0.05^{b}	1.466±0.14	0.860 ± 0.17^{b}
Oral cavity		2.118±0.08	1.214 ± 0.25^{a}	1.600±0.11	1.219 ± 0.15^{b}
Oropharygeal		2.065±0.11	0.730 ± 0.23^{ab}	1.346±0.11	0.973 ± 0.21^{b}
Hypopharyngeal		1.959±0.14	1.011 ± 0.51^{ab}	1.451±0.06	0.875 ± 0.17^{b}
Nasopharygeal		2.041±0.14	1.249 ± 0.28^{a}	1.823±0.13	1.734 ± 0.20^{a}
Salivary gland		1.865±0.10	0.790 ± 0.22^{ab}	1.799±0.00	1.494 ± 0.30^{a}
Sinonasal		1.938±0.12	0.202 ± 0.03^{b}	1.509±0.00	0.966 ± 0.19^{b}
p-value**		0.520	0.043	0.302	0.000

Seroprevalence**;** Chi square df=6; χ^2IgG VCA=(no statistical value), χ^2IgG EA=10.032, χ^2IgA VCA=14.523, χ^2IgA EA=5.078. Duncan test designated letters= (^{a,ab,b})

Table 3: Comparison of EBV seroprevalence and antibody titres in various HNC histology

Histology	Sample number	IgG-VCA	IgG-EA	IgA-VCA	IgA-EA
Seroprevalence n(%)					

SCC	62	62(100.0)	56(90.3)	14(22.6)	45 (72.6)
RS	3	3 (100.0)	3(100.0)	2(66.7)	3(100.0)
LEC	19	19(100.0)	19(100.0)	9(47.4)	15(78.9)
AC	3	3 (100.0)	3(100.0)	0(0.0)	2(66.7)
ACC	9	9(100.0)	8(88.9)	4(44.4)	8(88.9)
MEC	4	4 (100.0)	4(100.0)	1(25.0)	2(50.0)
p-value		-	0.685	0.131	0.590
ELISA-OD(means±s.e)					
SCC		1.986±0.05	0.751±0.13	1.579±0.08	1.274±0.09
RS		1.811±0.25	1.168±1.05	1.604±0.09	1.015±0.21
LEC		2.002±0.17	1.158±0.30	1.772±0.18	2.085±0.23
AC		2.215±0.07	1.329±0.09	-	1.848±1.21
ACC		2.132±0.08	1.050±0.31	1.576±0.17	1.376±0.19
MEC		1.618±0.19	0.434±0.27	1.560±0.00	1.980±0.76
p-value		0.483	0.558	0.824	0.006

Seroprevalence; Chi square df=5; χ^2 IgG-VCA=(no statiscal value), χ^2 IgG-EA=3.099, χ^2 IgA-VCA=8.503, χ^2 IgA-EA=3.722. ELISA-OD; one way ANOVA

KEY: SCC= Squamous cell carcinoma, RS= Rhabdomysarcoma, LEC=lymphoepthelioma carcinoma, AC=Adenocarcinoma, ACC= Adenocystic carcinoma, MEC=Mucoepidemoidcarcinoma

Table 4: Comparison of EBV seroprevalence and antibody titres in various HNC grade (differentiation)

Grade of cancer	*Sample number*	*IgG-VCA*	*IgG-EA*	*IgA-VCA*	*IgA-EA*
Seroprevalence n(%)					
Well differentiated	40	40(100.0)	38(95.0)	13(33.5)	29 (72.5)
Moderately differentiated	22	22 (100.0)	20(90.0)	5(22.7)	17(77.3)
Poorly differentiated	13	13(100.0)	11(84.6)	1(7.7)	8(61.5)
Undifferentiated	19	19(100.0)	19(100.0)	9(47.4)	15(78.9)
Grade x	6	6(100.0)	5(83.3)	2(33.3)	6(100)
p-value			0.372	0.372	0.531
ELISA-OD(means±s.e)					
Well differentiated		2.024±0.05	0.855±0.15	1.473±0.06	$1.335±0.10^{b}$
Moderately differentiated		2.030±0.89	0.811±0.02	1.756±0.16	$1.430±0.21^{b}$
Poorly differentiated		1.744±0.11	0.612±0.28	2.044±0.00	$1.256±0.26^{b}$
Undifferentiated		2.001±0.17	1.158±0.30	1.772±0.18	$2.085±0.23^{a}$
Grade x		2.098±0.18	0.845±0.60	1.604±0.09	$0.999±0.19^{b}$
p-value		0.369	0.702	0.263	0.006

Seroprevalence; Chi square; df=4; χ^2 IgG VCA=(no statistical value), χ^2 IgG EA=0.576, χ^2 IgA VCA=3.454, χ^2 IgA EA=0.053. ELISA-OD; one way ANOVA

Grade X= grade cannot be assessed.

(Table 5) compare antibody levels among head and neck cancer clinical stages. The seroprevalence and anti EBV antibody levels of were not significantly associated with the clinical stage of disease. Although the result showed an increase in EBV IgG-VCA, IgG-EA, IgA-VCA and IgA-EA mean titres as the disease advance in stage (i.e stage 2 to stage 4).

Table 5: Comparison of EBV seroprevalence and antibody titres in various HNC clinical stage

Clinical stage	Sample number	IgG-VCA	IgG-EA	IgA-VCA	IgA-EA
Seroprevalence n(%)					
Stage 2	7	7 (100.0)	7(100.0)	4(57.1)	5(71.4)
Stage 3	28	28(100.0)	26(92.9)	6(21.4)	21(75.0)
Stage 4	65	65(100.0)	60(92.3)	20(30.8)	49(75.4)
p-value		-	0.750	0.178	0.974
ELISA-OD(means±s.e)					
Stage 2		1.968±0.05	0.819±0.18	1.453±0.13	1.263±0.20
Stage 3		1.981±0.10	0.850±0.13	1.579±0.21	1.476±0.15
Stage 4		2.219±0.18	1.339±0.56	1.692±0.08	1.490±0.12
p-value		0.601	0.473	0.483	0.823

Seroprevalence; Chi square; df=2; χ^2 IgG-VCA= (no statiscal value), χ^2 IgG-EA= 0.576, χ^2 IgA-VCA=3.454, χ^2 IgA-EA=0.053. ELISA-OD; one way ANOVA

5. Discussion

In this study the seroprevalence and serum antibody levels of IgG and IgA against VCA and EA were significantly elevated in patients with head and neck cancer when compared with controls except for IgA-VCA. Similarly, Majidi *et al.* (2006) reported significantly elevated serum antibody levels against IgG-VCA, IgA-VCA and IgA-EA in HNC patients than controls. The results reflect the possibility that EBV seropositivity and elevated titres are associated with HCA development and the HNC patients habour higher titres than healthy individuals because of the cancer burden. In contrast to this result, Abdulamir *et al.*, (2008) and Morshed *et al.*, (2002) also reported no significant difference in anti-VCA titres between patients and controls.

The IgG anti VCA was the least specific serologic parameter in this study. It was elevated to high levels in the majority of HNC patients as observed by Majidi *et al.* (2006). The incidence was 100% for all cancer types as reported to range between 62–100% depending on the site of the lesion by Majidi *et al.* (2006). This titre elevation may represent an activation of a latent EBV infection occurring after a state of immunosuppression develops in the host from the effects of the tumor (Callaghan *et al*., 1983; Kondo *et al.*, 2004). In this study, the seroprevalence of IgA-VCA alone was associated with the NPC.

The IgG and IgA (VCA) antibody titres were not significantly associated with HNC cancer type ($p>0.05$) and this is in agreement with kamel *et al.*, (2003) who reported no association between EBV IgG-VCA level and HNC tumor site. These results disagrees with Abdulamir *et al.* (2008) who reported that the serum level of EBV IgG- and IgA-(VCA) antibodies were significantly higher in NPC group than other HNC. However NPC had the highest titres for IgG and IgA (VCA and EA) compared to other HNC. But it was only significant for IgG- and IgA- (EA). The seroprevalence and antibody titre of EBV supports several studies on EBV strong association with NPC carcinogenesis than non nasopharyngeal HNC cancers.

The oral cavity and salivary gland also had high antibody titres that were in the same range as NPC. The result agrees with Saemundsen *et al.* (1982) reported that the sera from patients with salivary gland carcinoma showed patterns of EBV IgG and IgA (VCA and EA) level within the same range seen in NPC patients. This suggests the EBV could be associated with non nasopharyngeal HNC particularly the oral cavity and salivary gland in this study. Other non nasopharygeal HNC having lower titres like the laryngeal, hypopharynx, within the same range in this study supports Majidi *et al* 2006 and Callaghan *et al*., 1983 who reported no difference in the antibody titers between various sites of head and epidermoid carcinoma

The serum level of EBV IgA-EA titres alone proved to be associated with tumor histology and grade. The results showed that EBV is more associated with lymphoepithelioma (LE) than other HNC histology type in the study. This results agrees with Abdulamir *et al.* (2008) who reported significant association of EBV serum IgG and IgA levels with tumor histology in NPC patients with lymphoepithelioma (LE) having higher antibody levels than NPC patients with squamous cell carcinoma (SCC) although no similar association was found between laryngeal cancer with LE and laryngeal cancer with SCC.

The EBV IgA-EA antibody titre was significantly higher in patients with undifferentiated cancer compared to others grades having antibody levels within the same range. This is supported by kamel *et al*. (2003) who reported that there was no significant difference of EBV antibody titres among well-, moderately- and poorly- differentiated HNC. More so all the HNC patients with LE histology and undifferentiated carcinoma are the 19 out of 24 nasopharygeal cases enrolled in the study. The evidence supports Adulamir *et al*. (2008) conclusion that EBV is clearly associated with undifferentiated NPC of LE histology than differentiated NPC of SCC histology.

In this study, the seroprevalence and antibody titres of EBV were not associated with clinical stage of the disease and this agrees with Luo *et al*. (2013) who reported that the positive rates of IgA (VCA and EA) were not related with HNC clinical stages. On the other hand, The EBV antibody titres increases as the disease progresses towards late stage as observed by Cai *et al*. (2010) and Sun *et al*. (2014) who concluded that IgA and IgG (VCA and EA) titre increases with advanced clinical stage of diseases which depicts increase burden of the disease that may affect the immune system of the patients.

6. Conclusion and Recommendation

Based on the present study there is an association between high EBV antibody titres and head and neck cancer particularly NPC, oral cavity and salivary gland cancer. The Undifferentiated–lymphoepithelioma of HNC histology was associated with EBV IgA-EA antibody titre. Further experiments on saliva and tissue samples could investigate the role, of EBV in tumorigenesis of HNC in Nigeria. The significance of one antibody and the insignificance of the other suggest the use of multiple anti EBV antibodies against more than one or more EBV antigen for diagnosis.

7. Authors' Contributions

Jibril, F.L*[1] carried out the sampling, Data sheet survey, obtained blood samples, ELISA assay, the statistical design, statistical analysis. Aminu-Mukthar, M.[2] was the major supervisor, whereas Jatau, E.D[3] and Usman, M.A.[4] were co-supervisors. They carried out the proofreading of the article language, integrity and approved the final manuscript.

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