Original Article

PREVALENCE OF HUMAN PAPILLOMA VIRUS IN CLINICO-HISTOLOGIC TYPES OF AMELOBLASTOMA SEEN IN LAGOS UNIVERSITY TEACHING HOSPITAL PATIENTS

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ABSTRACT

OBJECTIVE: Ameloblastoma in Nigerians presents as a locally aggressive lesion, requiring mutilating jaw surgery. The aetiology is unknown. The prevalence of Human Papilloma Virus (HPV) in ameloblastoma in scientific literature has been inconsistent. Strong association of HPV virus with oropharyngeal and cervical carcinoma has been reported. A high incidence of HPV reported in cervical smears of Nigerian females suggests risk of transmission through oral sex and kissing. In order to contribute to the possible implication of HPV in the aetiology of ameloblastoma, this study aimed to identify and determine the prevalence of HPV in clinico-histologic types of ameloblastoma in Nigerians.

METHODS: Sixty eight samples (35 formalin fixed tissues and 33 formalin fixed paraffin embedded tissues) were selected from a total of 193 cases of ameloblastoma seen during a 10-year period. haematoxylin and eosin sections confirmed histological diagnosis in each case. DNA was extracted from each of the 68 samples and polymerase chain reaction was used for genotyping HPV DNA, using type specific primers to identify HPV types 11, 16, 18, 31, 33, and 35.

RESULTS: HPV 35 DNA was detected in 3 out of the 68 samples (4.4%) of ameloblastoma, one from each of desmoplastic ameloblastoma (DA), solid multicystic ameloblastoma (SMA) and unicystic ameloblastoma (UA). The proportion of DA positive for HPV 35 (25%) was statistically higher than the proportion positive for SMA (1.67%). Chi-square, $X^2 = 9.11$, $df = 1$, $p = 0.0346$).

CONCLUSION: Detection of HPV 35 suggests the possible role the virus could play in the pathogenesis of ameloblastoma, which should be evaluated with more studies.

Key words: ameloblastoma, clinico-histologic-types, HPV 35.

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INTRODUCTION

Ameloblastoma is a benign locally invasive odontogenic epithelial tumour of unknown aetiology. While some studies reported no racial predilection globally,[1,2] differences in geographic and racial occurrence have also been reported in scientific literature.[3,4] It is a slow growing, locally invasive tumour that most often runs a benign course.[5] It affects both sexes with almost equal gender distribution [6] and all age groups with mean age of 37 years are affected. [7] Although, regarded as rare and the second most common odontogenic tumour in a predominantly Caucasian population,[8] Nigerian studies have documented ameloblastoma to be the most common odontogenic tumour.[9-11]

Nigerian patients are known for late presentation, [12] resulting in advanced tumours associated with pain and functional deficit. Attempts at research into the viral aetiology of ameloblastoma included production of jaw tumours that clinically mimicked the gross appearance of ameloblastoma and histologically resembled acanthomatous ameloblastoma by inoculation of mice with polyoma virus, [12] and development of ameloblastoma from the lining of cysts through transplantation of polyoma virus-infected tooth buds into mice.[13] Csiba *et al* [14] also described ultra-viral particles that resembled viruses in size and structure in a human ameloblastoma, while Gollard *et al* [15] induced an ameloblastoma tumour which strongly resembled ameloblastoma in a one day old Swiss Webster mouse with subcutaneously introduced polyoma virus. Further studies have been conducted in the United States of America, South Africa, Sweden, Iran, Italy, Venezuela, India and Egypt to investigate the role of HPV in the pathogenesis of ameloblastoma. These attempts have yielded controversial results, in which different HPV types, including types 6,11,16,18,31,33,42 types were observed, and prevalence of HPV in ameloblastoma reported to range from 32% to 100%. [16-26] HPV has also been associated with oral cancer with a prevalence ranging from 23.5% to 30%.[27,28] In addition, HPV has also been reported to be involved in potentially malignant oral disorders.[29] It is important to note that high prevalence (25.6%) of HPV has been reported in cervical cytologic smears of cervical cancer free Nigerian women, [30] thereby suggesting a possible risk of both the HPV positive Nigerian females and their male sexual partners to HPV infections.

Accurate diagnosis of HPV infection relies on the detection of viral nucleic acids using fresh and archived tissues.[31] The use of immunohistochemistry, [26] molecular techniques, such as polymerase chain reaction [18,20,32] and in-situ hybridization techniques [17,18] have been employed to detect HPV in ameloblastoma. The polymerase chain reaction is rapid and particularly sensitive method for examining DNA in paraffin embedded tissues.[32] Despite the degradation of DNA by fixatives, short DNA fragment yield are useful templates for PCR. [33] It is both highly sensitive and specific, [19] and allows for the identification of different types of HPV. [34] Immunohistochemical methods, on the other hand show strong sensitivity but lower specificity,[35] while in-situ hybridization methods are specific but less sensitive and the associated interpretation is often difficult.[36] In an attempt to contribute to knowledge and information on aetiologic agent implicated in development of ameloblastoma, this study aimed to identify and determine the prevalence of HPV types in ameloblastoma cases seen in Nigerian patients, using polymerase chain reaction method.

METHODS

Formalin fixed tissues (FFTs) and Formalin fixed paraffin embedded tissues (FFPETs) which had been stored for less than 10 years from the records of Department of Oral and Maxillofacial Pathology/Biology, LUTH were utilized. This ensures that enough genomic DNA is available from stored tissue. Available formalin fixed tissues (FFTs) and formalin fixed paraffin embedded tissues (FFPETs) of Ameloblastoma surgical specimens acquired from 2002 to 2012 were the target source of material for the study.

Collection of Formalin Fixed Tissue

2 g of representative samples of each of the 35 cases were cut. Each cut specimen was divided into 2 equal parts; 1g specimen of each was processed for HPV study, while the remaining 1g was processed to reconfirm the diagnosis of ameloblastoma.

Collection of Formalin Fixed Paraffin

Embedded Tissues (FFPET)

Ten 5 µm sections was prepared with a microtome from each of the 33 FFPET blocks
Sample-to-sample contamination was prevented by:
- Changing gloves between the cleaning of the microtome and the sectioning of each new block.
- Cleaning of both the microtome, microtome blade, between each paraffin block with (xylene) squirting freshly diluted 10% bleach onto gauze squares and carefully cleaning the microtome.
- Bleach wash was followed by an ethanol rinse to prevent corrosion of the microtome.
- The blade was removed and carefully wiped clean of all debris with clean, (xylene) bleach-soaked gauze.

DNA Extraction from Formalin Fixed Tissues (DNeasy Tissue extraction protocols Qiagen, Valencia, CA USA)

HPV DNA was extracted from ground tissue of the samples using QIAGEN kit.
Reagents were equilibrated at room temperature and prepared according to manufacturer's instructions and yielded pure DNA.

DNA Extraction from Formalin Fixed Paraffin Embedded Tissues

Prior to DNA extraction using Qiagen kit protocols according to manufacturer's instructions, Formalin Fixed Paraffin Embedded Tissues was deparaffinized with xylene as described below:
Section was placed into a 1.5 mL eppendorf tubes containing 300 µL xylene. The tubes were sealed and placed into a 65°C water bath for 1 minute. Two minutes of mixing followed by a 1minute equilibration was repeated 3 times. The tissue was pelleted by centrifugation at 12,800*g* and the xylene removed with a clean Pasteur pipette. Then, 300 µL xylene was again added and vortexed for 2 minutes followed by a 1minute equilibration. This procedure was repeated 3 times. The tissue was further pelleted, and 600 µL of 99.5% ethanol was added and mixed slowly for 10 minutes. The tissue was again pelleted by centrifugation at 12,800*g* and the supernatant removed with a clean Pasteur pipette. This step was repeated once, after which 50 µL of acetone was added and the open tube was put in a water bath at 60°C to increase evaporation of the acetone.

Polymerase Chain Reaction (amplification, genotyping)

5ml of extracted DNA of each sample was added to the PCR master mix, which comprises water, 1x buffer, 0.2mM dNTPs (Deoxyribonucleoside triphosphates), 0.3 µm forward and reverse primers (for HPV genotypes 11,16,18,31,33 and 35) and 0.04 units/mi Taq polymerase (enzyme). Each assay included controls for PCR contamination (water blanks taken through extraction and reagent water) and known positive HPV control. Amplicons were electrophoresed using 1.5% agarose at 120 V for 30 minutes. Gel image was captured and analysed using Bio Doc Gel Analyser.

RESULTS

A total of 68 cases of ameloblastoma were used for the study samples (35 samples of FFT and 33 samples of FFPET). These showed an equal gender predilection (34 cases [50.0%] in male and 34 cases [50.0%] in females) with M: F=1:1. Age range of patients: ranged from 3.5years to 55years. In addition, the tumour showed a strong mandibular site predilection of 95.6% (65 cases). The most common histologic ameloblastoma variant observed was the solid multicystic variant (88.0%) followed by the unicystic (6.0%) and desmoplastic variant (6.0%)

From 35 samples of FFTs of ameloblastoma that were screened for HPV types 11,16,18,31,33, and 35, only 2 samples (5.71%) were positive for HPV 35. From 33 samples of FFPETs of ameloblastoma screened for HPV types 11, 16, 18, 31, 33, and 35, only 1 sample (2.85%) was \

Table 1: Demographic and Histological Distributions of HPV Positive Ameloblastoma

Case no	Gender	Age (years)	Tissue Fixation	HPV Genotype	Clinico-Histologic Type	Growth Pattern Type
6	Female	43	FFT	35	Unicystic	Follicular
12	Male	38	FFT	35	Desmoplastic	Follicular
20	Female	25	FFPET	35	SMA	Plexiform

SMA - Solid Multicystic Ameloblastoma
FFT- Formalin Fixed Tissues
FFPET-Formalin Fixed Paraffin Embedded Tissues

Table 2: Distribution of HPV 35 Status in Clinico-Histologic Types of Ameloblastoma

Clinico-histologic Types of Ameloblastoma	HPV 35 status	
	Positive n (%)	Negative n (%)
Desmoplastic and unicystic	2 (25.0)	6 (75.0)
Solid multicystic ameloblastoma	1 (1.67)	59 (98.33)
Total	3 (4.4)	65 (95.6)

$\chi 2 = 9.11$,df =1 ,p = 0.0346 (Fisher's exact)

positive for HPV 35. Overall, 3 (4.4%) out of the 68 samples of ameloblastoma were positive for HPV 35, while other samples were negative for all the tested HPV genotypes. With 3 HPV 35 positive cases from 68 cases of ameloblastoma evaluated, the prevalence of HPV 35 positive cases in this study was 4.4%.

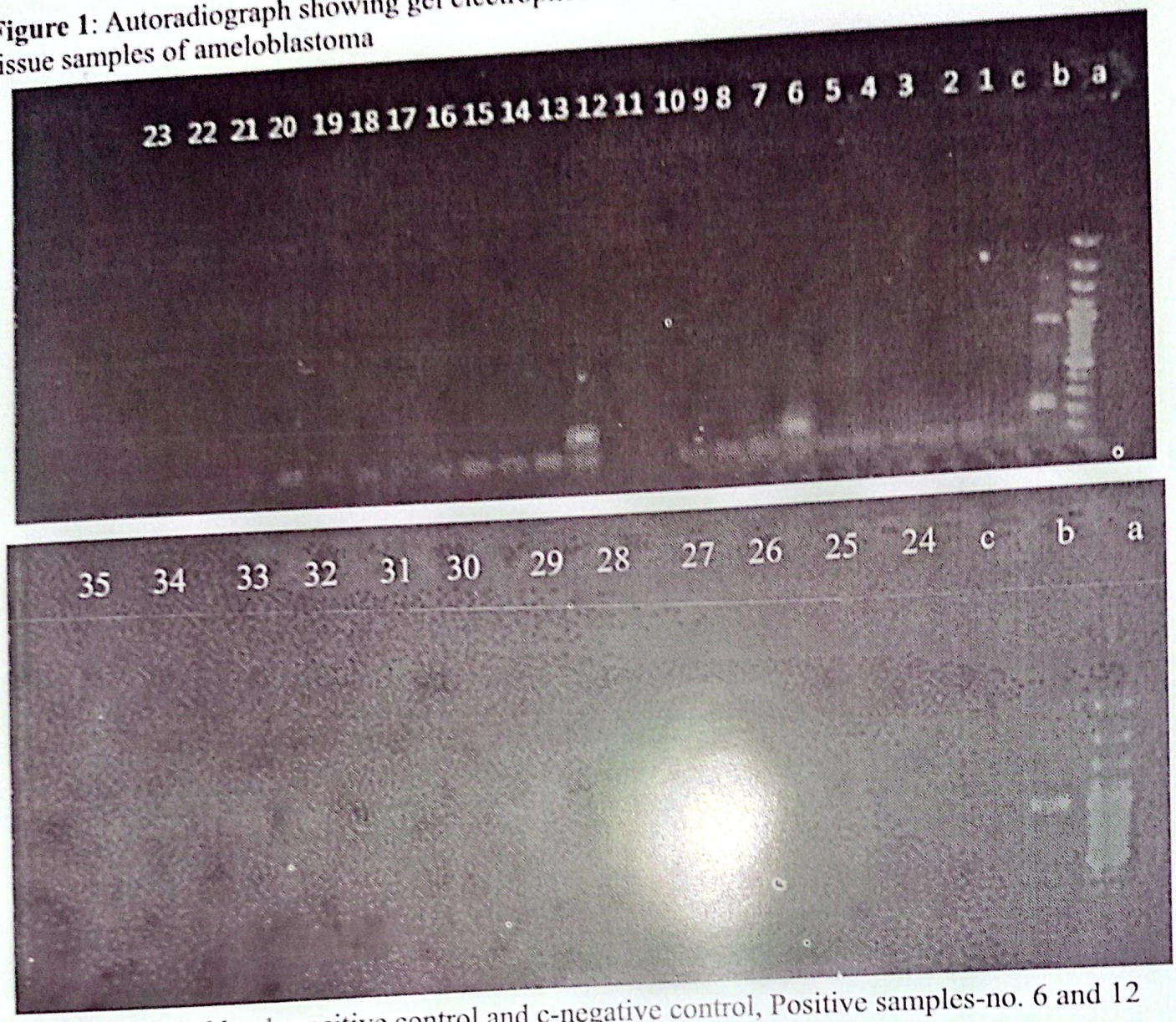
Positive results appeared as single high molecular weight band of 231 base pairs of DNA (samples numbers 6 and 12, Figure 1 and sample number 20, Figure 2). One sample each of desmoplastic ameloblastoma (DA), solid multicystic ameloblastoma (SMA) and unicystic ameloblastoma (UA) were positive for HPV 35 (Table 1). There is an association between the clinico-histologic types of ameloblastoma and HPV 35 prevalence.

The prevalence in the solid multicystic ameloblastoma (1.67%) is significantly lower than in Desmoplastic / Unicystic Ameloblastoma (25%) [p=0.0346] (Table 2).

DISCUSSION

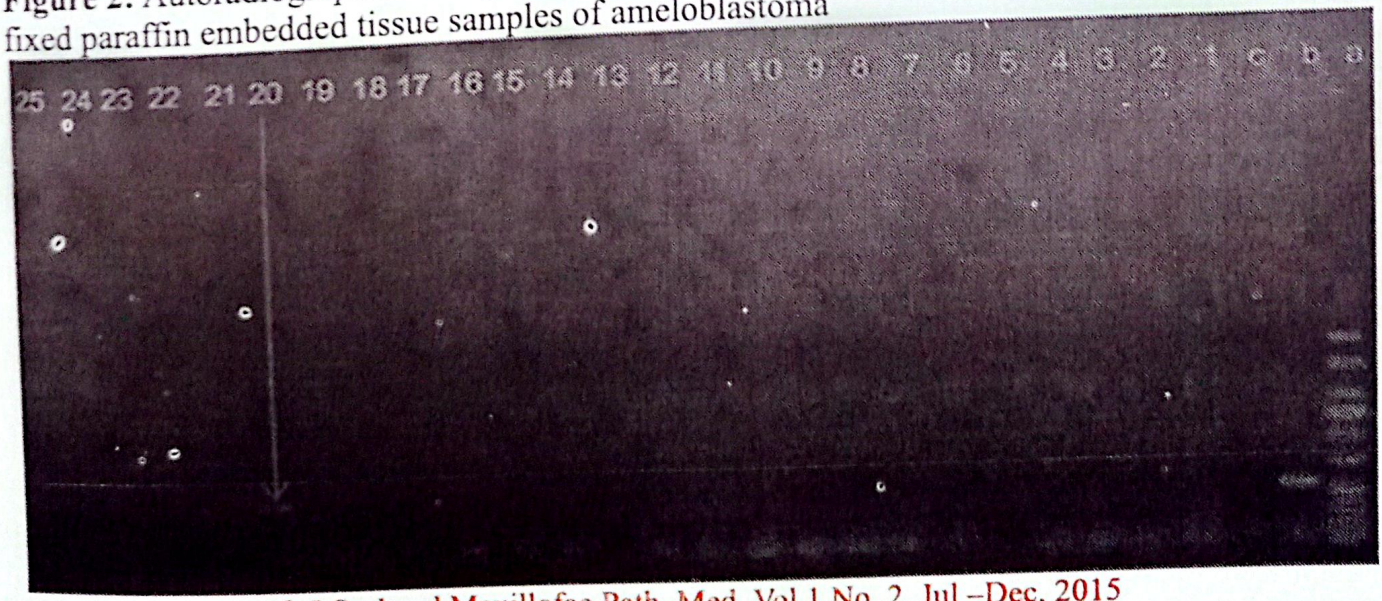
Most previous studies did not test for HPV 35 but other HPV genotypes and prevalence gotten from other genotypes, varied from 32% to 100%.[16-26] Prevalence of 4.4% for HPV 35 in ameloblastoma observed in this study does not have any parallel with studies in the scientific literature. However, previous study that investigated HPV 35 did not get any positive result.[21] Khan [17] reported HPV 16 and 18 in one sample of peripheral ameloblastoma, while Van Heerden et al, [18] found HPV (type 18) in one sample of ameloblastoma.

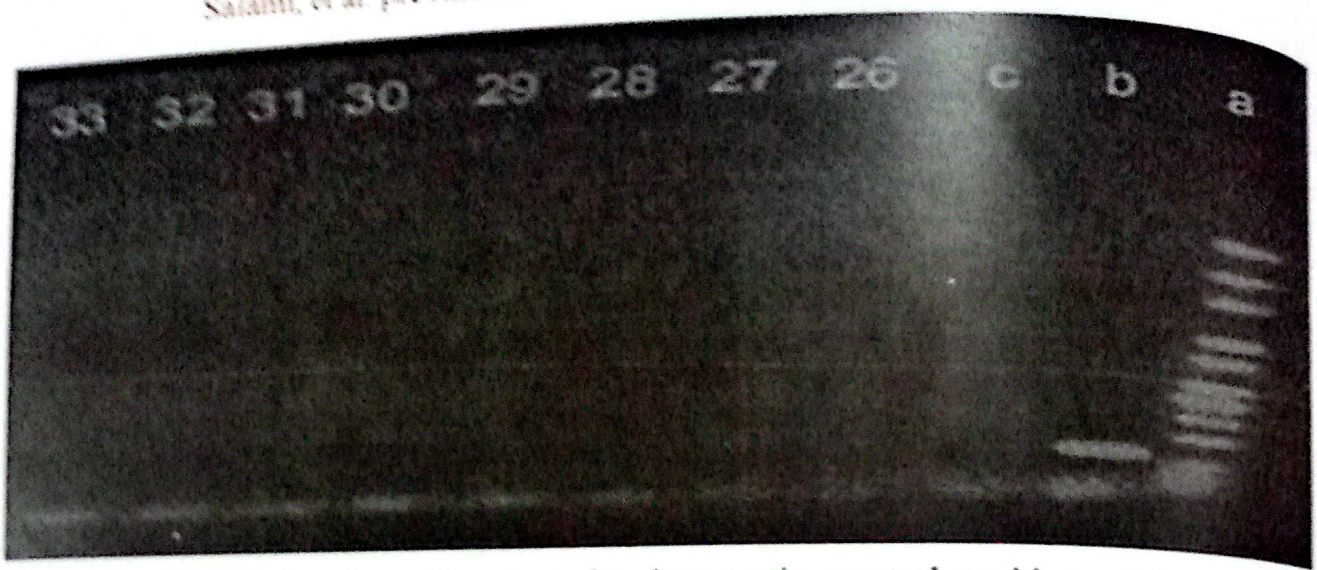
Figure 1: Autoradiograph showing gel electrophoresis expression of HPV 35 in 35 formalin fixed tissue samples of ameloblastoma



Key: a-DNA ladder, b-positive control and c-negative control, Positive samples-no. 6 and 12

Figure 2: Autoradiograph showing gel electrophoresis expression of HPV 35 in 33 formalin fixed paraffin embedded tissue samples of ameloblastoma





Key: a-DNA ladder, b-positive control and c-negative control, positive sample-no. 20

HPV types 6, 33, and 42 accounted for a prevalence of 33% of HPV in 18 samples of ameloblastoma studied by Correnti et al. [24] In the series, HPV type 6 accounted for 4 of the 6 samples positive for HPV, and these 4 were unicystic ameloblastoma. It is interesting to note that while only one case of unicystic ameloblastoma in our series was positive to HPV 35, 4 cases of unicystic ameloblastoma were positive for HPV 6 in a study by Correnti,et al.[24] This may suggest that HPV 6 is more important than HPV 35 in the development of unicystic ameloblastoma. Future studies in the Nigerian environment should therefore focus on comparative analysis of the prevalence of HPV 6 and HPV 35 in Nigerian series of ameloblastoma.

Sand et al [18] reported prevalence of 44.4% (8 from 18 samples of ameloblastoma). All the 8 samples were positive for HPV 18, while 5 samples from the 8 positive for HPV 18 were also positive for HPV 6 and HPV 11. HPV 6, 11, 16, and 31 accounted for prevalence of 32% of 100 samples of ameloblastoma reported by Azad et al. [23] HPV 6 alone accounted for 79.1% of the samples positive for HPV in the series of Azad et al.[23] These differences obtained from the various studies may be attributed to geographic variations in the distribution of HPV genotypes, detection techniques utilized to detect HPV DNA and number of samples used for the study. Perhaps the low prevalence observed in our series may also be due to degradation of DNA by the formalin fixative. Further studies regarding this hypothesis may be conducted.

HPV 16, 31, 35 and 58 were reported with a prevalence of 26.3% in cervical smears of 932 sexually active Nigerian females.[37] Nweke et al [38] reported HPV 35 as one of the high risk HPV types detected in HIV positive Nigerian women. Clifford et al [30] has reported that Nigeria recorded the highest HPV prevalence in a pool of 11 countries, where cervical smears of women without any obvious clinical abnormalities were examined. The proportions of HPV 35 (12·3%) was considered high in the series.

High risk HPV (a group to which HPV 35 also belongs) has been implicated in the development of oral and Oropharyngeal Squamous cell carcinoma, [39] although HPV 16 has been the most widely reported. The high prevalence of HPV 35 in cervical cancer in Nigeria [37] and Burkina Faso [40] puts the male partners, the carrier and their off springs at risk for oral HPV infections through oral sex, kissing and child birth. This suggests the possible role this HPV genotype could play in development of ameloblastoma, despite the low prevalence of 4.4% observed in this series.

The positive association observed between the HPV status and clinico-histologic types of

ameloblastoma in this series, further suggests the possible role that HPV 35 could play in the pathogenesis of ameloblastoma. In consideration of HPV 35 in different growth patterns of ameloblastoma, the prevalence rate of 2 out of 38 (0.05%) for follicular ameloblastoma was not significantly different from prevalence rate of 1 out of 16 (0.06%) with respect to plexiform pattern. This suggests that the growth pattern of ameloblastoma might not influence the prevalence of HPV 35 in ameloblastoma.

The implication of a significantly higher prevalence of HPV 35 in Desmoplastic / unicystic ameloblastoma than SMA in this series is not clear, but it will be interesting to test similar parameters for HPV type 6 and see whether similar trend occurs.

It can be concluded from this study that HPV 35 detected for the first time in this population may be implicated in the aetiology of ameloblastoma in Nigerians. It is recommended that future research should attempt to determine whether HPV 35 is involved in the initiation or progression of ameloblastoma. If it is involved at the initiation stage, patients at risk could be vaccinated against HPV 35 to prevent ameloblastoma developing. If it is involved at the progression stage of the tumour, then intra-lesional injection of vaccine may probably slow down the tumour progression and form part of the management. Further research that will utilize FFPET exclusively, on a larger sample size is recommended.

Conflict of Interest: None declared

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