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RESEACH ARTICLE

HUMAN PAPILLOMAVIRUS AS A BIOMARKER FOR HEAD AND NECK CARCINOGENESIS

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Abstract:
Background: Head and neck squamous cell carcinomas (HNSCCs) develop from the mucosal epithelium in the nose, oral cavity, pharynx and larynx and are the most common malignancies that arise in the head and neck. The study Aim to identify the role of Human papillomavirus in Head and Neck Carcinogenesis. **Methods:** The present study is a retrospective cross-sectional study conducted in Aminu Kano Teaching Hospital of Kano City, Nigeria. A total of ninety-six (96) Formalin fixed paraffin embedded (FFPE) tissue blocks of histologically diagnosed head and neck cancers processed between year 2020 to 2023 were retrieved. The nucleic acid was extracted using (Biopsin) kit for DNA/RNA extraction, and the extracted nucleic acid was then taken to real time multiplex PCR, using (Bioflux PCR kit), all performed according to manufacturer's instructions. Data obtained was analysed using SPSS (version 20.0) and presented in tabular form. **Results:** The samples were from patients across wide age groups and from both genders, across the commonest head and neck cancer sited, namely laryngeal, nasal, oropharyngeal, pharynx, larynx and salivary glands. We found that (57.3%) of all head and neck cancers (HNC) in the study were associated with Human Papillomavirus (HPV). It was established that (71.9%) of all cases with HNC are males. It was also established that nasal cancer was the most common cancer among all HNC in the study, with 69.8% cases. Participants in the age of group of (21-30) were the most frequently affected age group (34.6%). HPV59 was the most common oncogenic genotype (28.1%) among all the tested genotypes in the study, followed by HPV51. **Conclusion**, we found that HPV 59, was the most frequently encountered genotype of HPV positive head and neck cancers in the study population and the nasal region is the most vulnerable affected site of origin of the disease.

Key words: Head Neck Squamous Cell Carcinoma, Formalin Fixed Paraffin Embedded, Polymerase Chain Reaction, Human Papilloma Virus, Deoxyribonucleic Acid, Kano, Nigeria.

INTRODUCTION

Head and neck squamous cell carcinomas (HNSCCs) develop from the mucosal epithelium in the oral cavity, pharynx and larynx and are the most common malignancies that arise in the head and neck (Zhou et al., 2024). Increasingly, tumours that arise in the oropharynx are linked to prior infection with oncogenic strains of human papillomavirus (HPV), primarily HPV-16, and, to a lesser extent, HPV-18 and other strains (Tabatabaeian et al., 2024). As the most common oncogenic HPVs, HPV-16 and HPV-18, are covered by FDA-approved HPV vaccines, it is feasible that HPV-positive HNSCC could be prevented by successful vaccination campaigns worldwide (Madhukar and Subbarao, 2023). HNSCCs of the oral cavity and larynx are still primarily associated with smoking and are now collectively referred to as HPV-negative HNSCC (Wu et al., 2024). No screening strategy has proved to be effective, and careful physical examination remains the primary approach for early detection (Konin et al., 2024). Although a proportion of oral pre-malignant lesions (OPLs), which present as leukoplakia (white patches) or erythroplakia (red patches), progress to invasive cancer (Han et al., 2024), the majority of patients present with advanced stage HNSCC shows no clinical history of a pre-malignancy (Tabatabaeian et al., 2024).

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MATERIALS AND METHODS

The study is a retrospective cross-sectional study conducted in Aminu Kano Teaching Hospital of Kano City. An approval was obtained from ethical committee of Bayero University College Health Sciences. A total of ninety-six (96) Formalin fixed paraffin embedded blocks (FFPEB) of known head and neck cancers processed within the year 2020 to 2023 where retrieved. Any Head and Neck FFPEB processed within the year in questioned found negative for head and neck cancers are excluded from the study. A tissue section of (5µm) thickness were sectioned using rotary microtome, the tissue section was placed in Eppendorf tube containing 2mls of xylene, it was then incubated at
$56^{0c}$for thirty minutes and 3mls of absolute alcohol were added was added and then centrifuge at one 10000 rpm for five minutes, the supernatant where discarded. Then alcohols were added in descending to full hydration, then 500µl Phosphate Buffer Solution where added and it was shake thoroughly, it was centrifuged at 12000g for 5min the supernatant where then discarded and the deposit is now subjected to extraction. The nucleic acid was extracted using (Biopsin) kit for DNA/RNA extraction, the extracted nucleic acid is then taken to real time multiplex PCR, using (Bioflux PCR kit), all performed according to manufacturer's instructions.

Data Analysis

Data obtained was analysed using SPSS (version 20.0) statistical package and presented in tabular form.

Results

All the samples are for patient across different age groups of (5-90) years, and genders, across the commonest head and neck cancer origin, namely Laryngeal, Nasal, Oropharyngeal, Pharynx, Larynx and Salivary gland tumours.

We found that (57.3%) of all Head and Neck Cancers (HNC) in the study area are associated with Human Papillomavirus (HPV) (Table: 1). It was established that (71.9%) of all people with HNC are males (Table 2). It was established that Nasal Cancer is most occurring Cancer among all HNC in the study area with (69.8%) (Table3). Age group of (21-30) are the most vulnerable age group in the study area with (34.6%) (Table4). HPV59 is highest oncogenic genotype (28.1%) among all the tested genotype in study area (Tabl5).

Table:1. Human Papillomavirus Status in the Study Samples

Reaction	Frequency(n)	Percentage (%)
NEG	41	42.7
POS	55	57.3
Total	**96**	**100.0**

Keys: NEG – negative and POS – positive

Table:2. Distribution Head and Neck Squamous Cell Carcinoma Based on Gender

Gender	Frequency	Percentage
F	27	28.1
M	69	71.9
Total	**96**	**100.0**

Key: F- Female and M- Male

Table:3. Distribution of Human Papillomavirus Based on Anatomic site

Anatomic site	Frequency	Percentage
Larynx	18	18.8
Nasal	67	69.8
Orop	7	7.3
Pharynx	3	3.1
S/gland	1	1.0
Total	**96**	**100.0**

Key: Orop – Oropharyngeal and S/gland – Salivary gland

Table: 4. Distribution of HNC Based on Age.

Age range	Frequency	Percentage
5 – 10	6	6.2
11 – 20	4	4.2
21 – 30	33	34.4
31 – 40	8	8.3
41 – 50	13	13.6
51 – 60	17	17.8
61 – 70	9	9.3
71 – 80	5	5.1
81 – 90	1	1.0
Total	**96**	**100.0**

Table:5. Shows Distribution of Human Papillomavirus Genotype in the Study Samples.

Genotype	Frequency	Percentage
6	2	2.1
11	3	3.1
16	1	1.0
51	3	3.1
56	1	1.0
58	2	2.1
59	27	28.1
61	1	1.0
39,58	1	1.0
51,16	1	1.0
56,66	1	1.0
59,16	1	1.0
59,45	1	1.0
59,58	1	1.0
31,59,45	1	1.0
59,45,51,66	1	1.0
59,45,68,66	1	1.0
59,68,39,66	1	1.0
66,26,73,82	1	1.0
66,82,26,73	1	1.0
59,45,51,39,68	1	1.0
59,45,51,66,39	1	1.0
59,31,45,51,68,66	1	1.0
Total	**96**	**100.0**

Discussion

The recognition of high-risk HPV infection (primarily type 16) as a risk factor for development of Head and Neck Squamous Cell Carcinoma (HNSCC) is of prognostic importance (Du et al., 2024). There is increasing interest in approaching HNSCC as 2 distinct types, HPV-positive and HPV-negative disease (Muniz et al., 2024). This distinction is driving advances in our understanding of the biology, mutational landscape, predictors of response to treatment, and survival outcomes for these 2 distinct types of HNSCC (Mastronikolis et al., 2024). There is a distinct biology and molecular phenotype among HPV-positive Oropharyngeal squamous cell carcinoma (OPSCC) when compared with HPV-negative HNSCCs (Guo et al., 2024).

In this research we studied 96 archival tissue blocks of different types of head and neck squamous cell carcinoma. Using HPV Genotyping Real-time PCR Kit we investigate qualitatively the presence of human papillomavirus (HPV) type 6, 11, 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68; the kit can be used for detection (BIOFLUX). We found out that 55(57.3%) are positive for Human Papillomavirus while 41(42.7%) of the samples were negative for Human Papillomavirus this. This shows that Human Papilloma virus is the common risk factor of majority of head and neck squamous cell carcinoma. In addition, there is a higher prevalence of HPV-positive HNSCC in men (71.9%) compared with women (28.1%). This finding is in concomitant with work of Galati *et al.* (2022) in France on HPV head and neck cancers that found HR-HPV as the major risk factor of Head and neck squamous cell carcinoma. Vietía *et al.* (2014) in Venezuela reported that (61%) of Head and neck cancers are caused by Human Papillomavirus that is slightly below our finding; Garau *et al*. (2020) in France found that 40-60% of all head and neck cancers are caused by Human Papillomavirus which is also slightly below our finding. This might be due to association with other established risk factors such as smoking and alcohol consumption (Benítez et al., 2024). More over the result of Spence *et al.* (2016) in Toronto found that (25%) of Head and neck squamous cell carcinoma are associated with Human papillomavirus which disagree with our finding which may be due to awareness and health status among different study populations. However, in this our research we have find out that, the most common genotype circulating among head and neck cancer patient in the study area is HPV59 with (28.1%) followed by HPV11 and HPV51 with (3.1%) each, and HPV6 and HPV58 with (2.1%) each. All others with (1%) only, even-though there are multiple co-infections, this finding agrees with finding of Molina *et al.* (2020) in Maxico who reported that HPV59 is one of the major cause of cancer as such he advises for its inclusive in vaccination. Also Tumban (2019) reported that HPV59 as high risk HPV contributed majorly in the development of head and neck cancers among Maxican study population. Jessica *et al.* (2010) in America reported that majority of nasal cancers are HPV59 associated. More-over Gallison *et al.* (2008) in Texas reported that HPV16 as an independent and the only genotype responsible for head and neck cancers, which is contrary to our finding. The differences might be due to the use of HPV-16 insitu hybridization alone to stratify cases as HPV-positive or HPV-negative which might have misclassified tumour HPV status, the control population may not adequately represent the true prevalence of exposures of interest in the general population. Another factor might be due to differential recall bias among case subjects, residual confounding by sexual behaviour, or possible confounding by use of other

substances which could have influenced observed associations between marijuana use and HPV-16–positive HNSCC.

Conclusion

Head and neck cancers are markedly phenotypic and clinically heterogeneous neoplasms of different histogenesis. It is therefore, of high importance to consider Head and Neck Cancer as one of the major health challenges that requires special intervention due its heterogeneous nature and multiple risk factors. Head and neck squamous cell carcinoma (HNSCC) includes several malignancies which may arise in the oral cavity, oropharynx, larynx, and hypopharynx. HNSCCs have been associated with well-established risk factors like tobacco smoking, alcohol use and viral infections, such as human papillomaviruses. We found that HPV 59, is the cause of HPV positive head and neck cancers in the study population. We deduct that Nasal region is the most vulnerable affect site of region of the disease.

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Data Availability:

The data is available in Aminu Kano Teaching Hospital Kano, which can only be accessed through ethical approval.

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Complicit of Interest

There is no complicit of interest in the study

Authors Contribution

YG: Initiate the analysis, Conduct the Laboratory and analysis, interpret the result, and write the manuscript.
BEO: Go through the manuscript and make some correction.

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References

BENÍTEZ, L., CASTRO-BARQUERO, S., CRISPI, F., YOUSSEF, L., CROVETTO, F., FISCHER, U., KAMERI, E., BUENO, C., CAMOS, M. & MENÉNDEZ, P. 2024. MATERNAL LIFESTYLE AND PRENATAL RISK FACTORS FOR CHILDHOOD LEUKEMIA: A REVIEW OF THE EXISTING EVIDENCE. *Fetal Diagnosis and Therapy*.

DU, M., LIN, Q., YAN, S., GAO, X., YANG, C., LI, Z., LIAO, W., YANG, A. & CHEN, S. 2024. Clinicopathologic characteristics of HPV-associated head and neck squamous cell carcinoma in Southern China: long-term retrospective study of 400 cases. *Therapeutic Advances in Medical Oncology*, 16**,** 17588359241242962.

GUO, T., ZAMUNER, F., TING, S., CHEN, L., ROOPER, L., TAMAYO, P., FAKHRY, C., GAYKALOVA, D. & MEHRA, R. 2024. Clinical and genomic characterization of chemoradiation-resistant HPV-positive oropharyngeal squamous cell carcinoma. *Frontiers in Oncology*, 14**,** 1336577.

HAN, P., LI, F., ZHANG, Y., GAO, L., ZHANG, G., GUO, Q., ZHU, Y. & SU, Q. 2024. B-ultrasound or CT-guided 3D-printing individualized non-coplanar template brachytherapy for the treatment of locally uncontrolled recurrent head and neck squamous cell carcinoma. *Advances in Dermatology and Allergology/Postępy Dermatologii i Alergologii,* 41**,** 41-48.

KONIN, J. G., LEBSACK, D., VALIER, A. & ISEAR JR, J. A. 2024. *Special tests for orthopedic examination*, CRC Press.

MADHUKAR, G. & SUBBARAO, N. 2023. Current and Potential Pharmacological Targets for Therapeutic Intervention in Head and Neck Squamous Cell Carcinoma. *Socio-Scientific Interaction in Diabetes and Cancer and Its Management***,** 63.

MASTRONIKOLIS, N. S., DELIDES, A., KYRODIMOS, E., PIPERIGKOU, Z., SPYROPOULOU, D., GIOTAKIS, E., TSIAMBAS, E. & KARAMANOS, N. K. 2024. Insights into metastatic roadmap of head and neck cancer squamous cell carcinoma based on clinical, histopathological and molecular profiles. *Molecular Biology Reports*, 51**,** 597.

MUNIZ, I. D. A. F., ARAUJO, M., BOUASSALY, J., FARSHADI, F., ATIQUE, M., ESFAHANI, K., BONAN, P. R. F., HIER, M., MASCARELLA, M. & MLYNAREK, A. 2024. Therapeutic Advances and Challenges for the Management of HPV-Associated Oropharyngeal Cancer. *International Journal of Molecular Sciences*, 25**,** 4009.

TABATABAEIAN, H., BAI, Y., HUANG, R., CHAURASIA, A. & DARIDO, C. 2024. Navigating therapeutic strategies: HPV classification in head and neck cancer. *British Journal of Cancer***,** 1-11.

WU, D., LI, Y., XU, P., FANG, Q., CAO, F., LIN, H., LI, Y., SU, Y., LU, L. & CHEN, L. 2024. Neoadjuvant chemo-immunotherapy with camrelizumab plus nab-paclitaxel and cisplatin in resectable locally advanced squamous cell carcinoma of the head and neck: a pilot phase II trial. *Nature Communications*, 15**,** 2177.

ZHOU, J., LIU, C., AMORNPHIMOLTHAM, P., CHEONG, S., GUTKIND, J., CHEN, Q. & WANG, Z. 2024. Mouse Models for Head and Neck Squamous Cell Carcinoma. *Journal of Dental Research***,** 00220345241240997.