High Risk Human Papillomavirus in Head and Neck *Squamous Cell Carcinoma* Patients at Kenyatta National Hospital, Kenya.

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Summary

BACKGROUND

Human Papillomavirus (HPV) has been associated with a subset of Head and Neck *Squamous Cell Carcinoma* (HNSCC) in particular *Oropharyngeal Carcinoma*.

OBJECTIVE

To determine the prevalence and *Clinicopathological* predictors of high-risk HPV among patients with HNSCC at Kenyatta National Hospital (KNH) Nairobi, Kenya.

MATERIALS

One hundred and sixty(160) patients who presented themselves to the ENT clinic between 2015 and 2017 with HNSCC had their history taken. A complete physical examination was done along with the appropriate *haematological* and radiological work-up. Two tissue biopsies were taken from the primary tumour for histology and real time polymerase chain reaction.

METHODOLOGY

One hundred and sixty(160) patients with HNSCC aged 16 to 87 years were recruited and set in groups of six. These groups were based on the primary site of the tumour present such as; Oral cavity, *Oropharynx, Nasopharynx, Hypopharynx, Larynx* and *Sinonasal*. There were 117 (73.1%) males and 43 (26.9%) female participants. Twelve 12(7.5%) patients tested positive for high risk HPV. The HPV genotypes detected were 56, 52 and 33. There were no predictors for HPV positivity.

CONCLUSION

High risk HPV prevalence was low among HNSCC patients at Kenyatta National Hospital. No HPV 16 nor 18. The positive patients did not have profiles that matched those of HPV-positive HNSCC globally.

Key Words: **Human papillomavirus, carcinoma, head and neck**

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Introduction

Cancer is a growing public health concern worldwide and more so in Africa. In Kenya, cancer is the third most common cause of death after infectious and cardiovascular diseases contributing 7% of the total mortality annually.

According to the Nairobi Cancer Registry, HNSCC was the second most common malignancy reported after cancer of the breast during the period 2000 to 2002 [1].

A rapid rise of cancer is projected in many developing countries due to increased exposure to risk factors including tobacco, alcohol, environmental *Carcinogens,* HIV, and Human Papillomavirus [2].

The overall global incidence of HNSCC has increased by about 30% since 1988. This has been attributed to an increase in HPV - related cancers, in particular, *Oropharyngeal Squamous Carcinoma* (OPSCC) [3].

The incidence of cancers attributable to HPV in Kenya, showed that, cancer of the cervix *uteri*, other *anogenital* cancers, Head and Neck cancers stand at 40 in every 100,000 women, 1.9 in every 100,000 male and 0.2 in 100,000 people respectively [4].

This is despite the fact that all three groups of cancer are caused by the same high risk HPV subtypes.

Worldwide, HPV related cervical cancer occurs predominantly in less developed countries. While North America and Northern Europe have the bulk of HPV attributable to Head and Neck cancers. [5].

Many and more countries are setting up HPV vaccination programs for both girls and boys as a measure of prevention on HPV attributable cancers.

Such programs can only be effective if the target population is well defined based on reliable statistics.

Sudy Site

The study was done at Kenyatta National Hospital the largest and oldest hospital in kenya. KNH is a public tertiary, referral hospital in East and Central Africa, specifically, The Republic of Kenya. It also serves as a hands on facility for the University of Nairobi.

Kenyatta National Hospital is situated in the capital City of kenya (Nairobi), just a few kilometers from the Central business.

It has a bed capacity of 2000 beds and catters for approximately 3000 out patients per day. Some of whom had, Ear Nose and Throat – Head and Neck (ENT-HN) related ailments. We recruited our participants from the KNH wards, ENT-HN and Maxillofacial Clinics.

Kenyatta National Hospital receives hundreds of patients 24hrs including those from the neighbouring countries like Uganda, Tanzania, Sudan, Somalia Ethiopia Eritrea, Rwanda, Burundi, DRC Republic etc.

Materials

One hundred and sixty(160) patients who presented themselves to the ENT Clinic between 2015 and 2017 with suspected Head and Neck *Squamous Cell Carcinoma* (HNSCC) at Kenyatta National Hospital from the wards, ENT-HN and Maxillofacial clinics were recruited.

There were 117 (73.1%) male and 43 (26.9%) female. Twelve (7.5%) patients tested positive for high risk HPV. The HPV genotypes detected were 56, 52 and 33.

Their demographic data and detailed history with a focus on risk factors for HNSCC as well as purported risk factors for HPV - positive HNSCC were taken and recorded.

Information concerning their characteristics and symptoms was as below;

(a). Smoking, alcohol and other substance use
(b). Exposure to irritants or radiation
(c) History in keeping with *gastro-oesophageal* or *Laryngopharyngeal* reflux
(d) Sexual Orientation and Practices was also sought.

Methodology

A total of the one hundred and sixty patients with HNSCC, confirmed by histology, aged between 16 and 87 years were enrolled in the study and set in groups of six .

The mean and median age was 51.6 and 54 years respectively. There were 117 (73.1%) male and 43 (26.9%) female.

The patients' groups of six, were based on the

primary site of the tumour present as indicated below;

a. *Oral cavity*
b. *Oropharynx*
c. *Nasopharynx*
d. *Hypopharynx*
e. *Larynx*
f. *sinonasal*

A complete Ear Nose and Throat – Head and Neck (ENT-HN) examination was done. Standard *haematological* tests for all HNSCC patients, comprised of full;

a. Haemogram,
b. Renal
c. Liver function tests
d. ELISA for HIV were requested.

Appropriate radiological work-up for the tumour in question including but not limited to CT-Scan and chest x-ray were carried out.

TNM staging as per AJCC 2010 for specific sites was done and group staging made.

Tumour biopsy specimens were taken as per the unit protocol for *histopathological* diagnosis. From each subject, two tissue biopsies were taken and one was fixed in 10% buffered formal saline for *histopathologica*l analysis.

The other sample was placed in a specimen bottle and frozen at -80°C. At least two hundred specimens were collected and frozen from which one hundred and sixty histologically confirmed HNSCC specimens were selected for DNA extraction.

DNA Extraction

Approximately 25 mg of each frozen tissue was cut into small pieces and placed in a 1.5 ml micro-centrifuge tube. 100 µl of Buffer ATL was added to each sample followed by 20 µl of *proteinase K.*

The contents were mixed by vortexing and incubated at 56°C in a shaking water bath until the tissues were completely *lysed* (overnight). The samples were then briefly centrifuged.

200 µl of Buffer AL was added to each sample and mixed by pulse-vortexing for 15 seconds then incubated at 70°C for 10 minutes. The mixture was briefly centrifuged to remove drops from inside the lid.

200 µl of 100% *ethanol* was added to the sample and mixed by pulse-vortexing for 15 seconds then centrifuged to remove drops from inside the lid.

The mixture was pipetted into the *QIAamp* Mini spin column in a 2 ml collection tube, which was closed and centrifuged at 8000 revolutions per minute (rpm) for 1 minute.

The *QIAamp* Mini spin column was placed in a clean 2 ml collection tube and the tube containing the filtrate discarded.

The *QIAamp* Mini spin column was opened carefully and 500 µl of Buffer AW1 added then closed again. The mixture was centrifuged at 8000 rpm for 1 minute. The tube containing the filtrate was discarded.

500 µl of Buffer AW2 was added to the *QIAamp* Mini spin column and the mixture centrifuged at 14,000 rpm for 3 minutes.

The QIAamp Mini spin column was placed in a new 2 ml collection tube and centrifuged at full speed for 1 minute to eliminate the Buffer AW2 carryover.

The QIAamp Mini spin column was put in a clean 1.5 ml centrifuge tube and the collection tube discarded.

200 µl of Buffer AE was added and incubated at room temperature for 1 minute before centrifuging at 8000 *rpm* for 1 minute.

This last step was repeated for increased DNA yields. The resultant eluate containing HPV DNA was stored at -20°C for HPV DNA genotyping.

HPV DNA Genotyping

Real Time PCR was performed on 10 microliters of the extracted DNA using HPV Genotype Real-TM Quant kit from SACACE as per the manufacturer's instructions.

The kit detects 14 genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) of high risk Human Papillomavirus.

For the sample to be tested as per the manufacturer's instructions, Reaction mix was made using;

1. Hot Start DNA *Polymerase*,
2. PCR - buffer - FRT
3. PCR mix-1

Four test tubes for each clinical sample, four tubes for K2 standards and four tubes for negative control were set up.

Ten microlitres of extracted DNA sample was added to each test tube. For each panel, 10 µl of controls and standards were prepared for cycling.

The test tubes were closed and transferred into the Real Time Thermal cycler (Rotor Gene Q, QIAGEN). The machine was programmed as per the kit's PCR cycling conditions.

PCR was performed and the results interpreted using the Rotor Gene Q software. A signal was considered to be positive if the corresponding *fluorescence* accumulation curves crossed the threshold line.

Data Inquiry

Data analysis was performed using SPSS version 21.0 statistical software. Descriptive analysis was performed on all variables and summarized into frequency tables and charts.

Odds ratios was done to test for the strength of association between Independent and Dependent variables.

Pearson Chi square was applied to categorical data to test for association between Independent and Dependent variables. Fishers exact test was employed when cell numbers were small.

RESULTS

A total of one hundred and sixty patients with HNSCC, confirmed by histology, aged 16 to 87 years were enrolled in the study. The mean and median age was 51.6 and 54 years respectively. There were 117 (73.1%) males and 43 (26.9%) females.

The patients were grouped into six, based on the primary site of the tumour as;

a. Oral cavity,
b. *Oropharynx,*
c. *Nasopharynx,*
d. *Hypopharynx,*
e. *Larynx*
f. Sinonasal.

Majority of the patients had *Carcinoma* of the *Nasopharynx* (38.8%), followed by *Larynx* (29.4%), Sinonasal (11.3%), Oral Cavity (8.8%), *Hypopharynx* (6.9%) and *Oropharynx* (5.0%).

The male to female ratio for all the study subjects collectively was approximately 3:1.

All tumour groups had more male than female except for Oral Cavity *Carcinoma,* where the ratio was 1:1. *Carcinoma* of the *larynx* had the largest male to female ratio of about 23:1.

Patients presented with different combinations of symptoms dictated by the primary site of the tumour. At least 30% of the patients presented with nasal obstruction, hoarseness, neck swelling and difficulties in breathing.

The exposure of patients to risk factors for HNSCC either in the past or current is summarized in ***Table 1.***
Some patients were exposed to more than one risk factor. Cigarette smoking and drinking beers were the most frequent risk factors. Few patients had used khat and marijuana.

The four patients who had previous irradiation to the neck were presenting with either residual or recurrent disease treated with radiotherapy within five years of the first course.

GERD and/or LPR symptoms were present in 6.9% of patients. Three patients reported exposure to irritants with one having recurrent throat irritation.

Table I: *Exposure to Risk Factors*

Risk factor	Frequency	Percentage (%)
Cigarette smoking	79	49.4
Tobacco chewing	3	1.9
Khat chewing	10	6.3
Marijuana use	3	1.9
Alcohol		
• Beer	60	37.3
• Spirits	17	10.7
• Wine	7	4.4
• Chang'aa (unpurified spirit)	15	9.4
• Unprocessed brews	37	23.1
Previous head and neck radiation	4	2.5
Exposure to irritants	3	1.8
GERD/LPR	11	6.9
Others	1	0.6

Only nine of the study subjects were not sexually active. These were mainly young patients aged nineteen (19)years and less.

None of the patients was homosexual or bisexual. There was, however, one patient who admitted to having engaged in anogenital sex. Of the 151 sexually active patients, 19 could not recall their age at sexual debut.

Two (1.3%) of the patients had early sexual debut defined as sexual debut at less than 15 years age [6].

Patients who had at least four lifetime sexual partners were classified as having had multiple sexual partners [7].

Based on this definition, 45(29.8%) of the sexually active patients had multiple sexual partners. Of the remaining 106 (70.2%) sexually active patients, 15(9.9%)could not remember how many lifetime sexual partners they had had.

The disease stage was determined on the basis of clinical and radiological examination. There were no distant metastases detected.

The Primary tumour (T), regional nodal staging (N) and distant metastases (M) as per the AJCC 2010 staging was done and a group staging for HNC done thereafter.

Only 12.6% of the patients had early tumours (stage I and II). Four (2.5%) patients had recurrent tumours. *Cystic lymph* nodes were only found in five patients.

Three of these patients with cystic lymph nodes had *Nasopharyngeal Narcinoma*, one Sinonasal and one Oral Cavity *Carcinoma.*

All tumours were assigned the WHO histological grading I to IV.
The results are presented in figure 1 below.

Majority tumours of the oral cavity, *oropharynx, hypopharynx* and *larynx* were of better differentiation than the *nasopharyngeal* and *sinonasal* tumours, which were mainly poorly differentiated or undifferentiated. The NPC specimens were separately assigned WHO type I NPC (6.4%), type II (17.7%) and type III (75.8%).

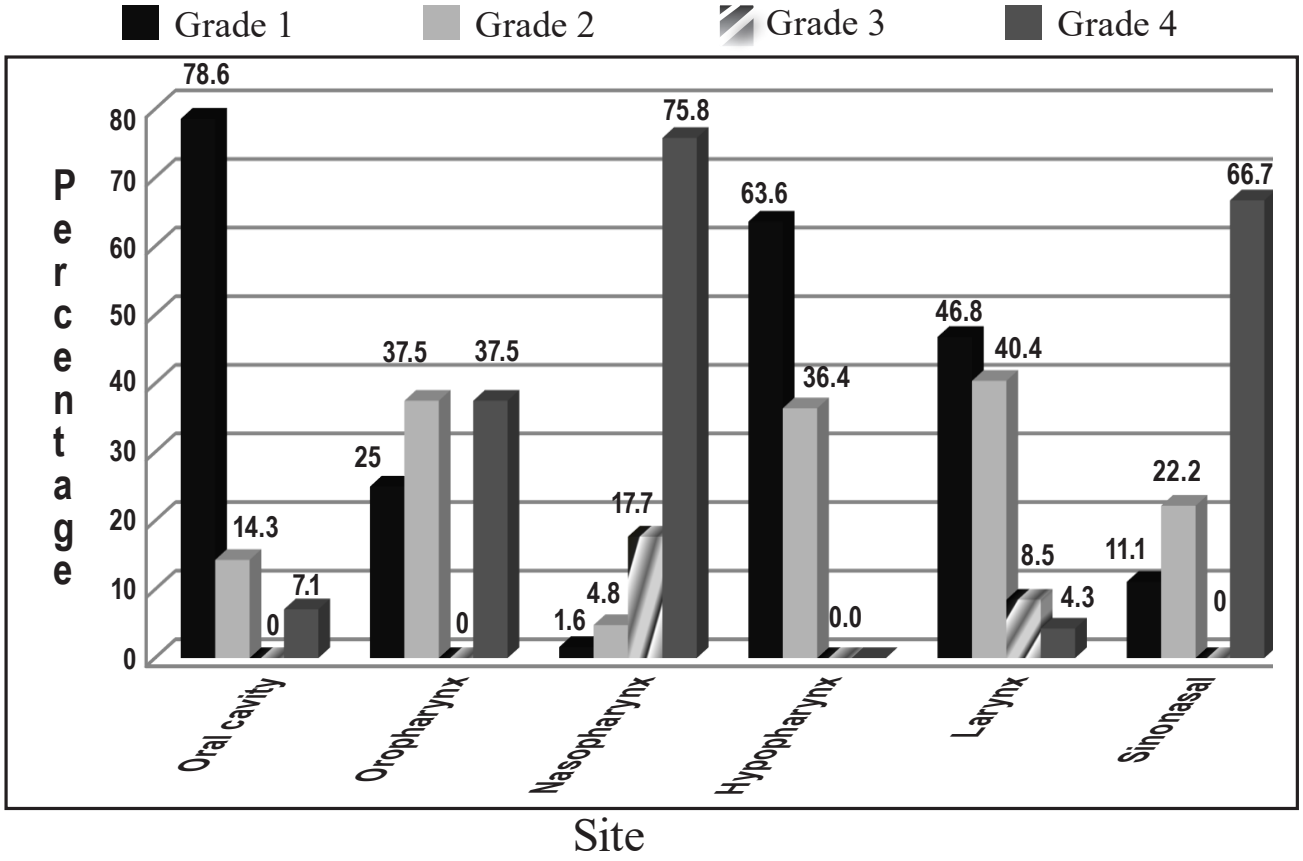

Figure I: *Histological Grading of Tumours*

Only 10 (6.3%) of the patients tested positive for HIV. These consisted of 4 female and 6 males aged 23 to 57.

All the six male had a history of smoking and used alcohol. The HIV positive patients who tested positive to the following ailments were as follows;

(a.) 6 had *Nasopharyngeal*
(b.) 2 had *Oropharyngeal*
(c.) 1 had *Hypopharyngeal*
(d.) 1 had *Laryngeal Carcinoma.*

Two 2 (20%) of the HIV positive patients tested positive for HPV. Both had *Nasopharyngeal Carcinoma* and HPV subtype 56.

Twelve 12(7.5%) of the study patients tested positive for HPV by real time Polymerase Chain Reaction.

The only high-risk HPV genotypes detected were 56 (10 patients) 33 and 52 (one patient each). No single patient tested positive for two or more genotypes.

The primary tumour types and profiles of patients that tested positive for HPV are shown in ***(Table 2)*** next page. There was no characteristic that was statistically significant with regard to HPV positivity.

CHARACTERISTIC	PCR		P VALUE	ODDS RATIO	CONFIDENCE INTERNALS	
	POSITIVE	NEGATIVE			LOWER	UPPER
Age: ≤ 60 years	6 (8.5%)	65 (91.5%)	0.7	1.27	0.393	4.144
> 60 years	6 (6.7%)	83 (93.3%)				
Gender: Male	6 (5.1%)	111(94.9%)	0.87	0.3	0.101	1.097
Female	6 (14.0%)	37 (86.0%)				
Tobacco Smoking: Yes	2 (2.4%)	80 (97.6%)	0.016	0.170	0.36	0.806
No.	10 (12.8%)	68 (86.9%)				
Tobacco chewing: Yes	1 (16.7%)	5 (83.3 %)	0.3	2.6	0.279	24.250
No.	11 (7.1%)	143 (92.9%)				
Miraa chewing: Yes	2 (20.0%)	8 (80.3%)	0.1	3.5	0.654	18.724
No.	10 (6.7%)	140 (93.3%)				
Marijuana use: Yes	0 (0%)	3 (100%)	1.0	1.083	1.035	1.133
No,	12 (7.6%)	145 (92.4%)				
Alcohol Drinker	4 (5%)	80 (95%)	0.231	0.425	0.123	1.473
Non-drinker	8 (10.5%)	68(89.5%)				
Previous head & neck Yes	1 (25.0%)	3 (75.0%)	0.2	4.39	0.421	45.830
irradiation : No	11 (7.1%)	145 (92.9%)				
Exposure to irritants: Yes	0 (0%)	3 (100%)	1.0	1.0	1.035	1.133
No.	12 (7.6%)	145 (92.4%)				
GERD/LPR: Yes	1 (9.1%)	10 (90.9%)	0.5	1.255	0.147	10.720
No	11 (7.4%)	138 (92.6%)				
Age of sexual debut: None/Early	2 (18.2%)	9 (81.8%)	0.206	2.98	0.560	15.953
Late	9 (6.9%)	121 (93.1%)				
No. of sexual partners: Multiple	3 (6.7%)	42(93.3%)	1.0	0.9	0.234	3.851
	7 (7.0%)	93 (93.0%)				
Site of tumour: Oral cavity	2 (14.3%)	12 (85.7%)	0.3	0.9	0.567	1.448
Oropharynx	0 (0%)	8 (100%)				
Nasopharynx	7 (11.3%)	55 (88.7%)				
ypopharynx	0 (0%)	11 (100%)				
Sinonasal	2 (11.1%	16 (88.9%)				
Histological grade: I	2 (5.7%)	43 (95.6%)	0.6	1.5	0.890	2.684
II	2 (16.7%)	33 (94.3%)				
III	1 (6.7%)	14 (93.3%)				
IV	7 (10.8%)	58 (89.2%)				
HIV Status: Positive	2 (20.0%)	8 (80.0%)	0.1	3.50	0.654	18.724
Negative	10 (6.7%)	140 (93.3%)				

Table II: *HPV Association with Patient and Tumour Factors*

DISCUSSION

HPV related Head and Neck cancers are associated with particular characteristics usually not present in HPV negative cancers.

These *Clinicopathological* profiles that are characteristic of HPV positive tumours include;

1. Male gender
2. Younger age
3. Higher socioeconomic status
4. *Marijuana* use
5. Minimal tobacco and/or alcohol consumption
6. Orogenital sex
7. Multiple sex partners
8. HIV co-infection
9. Early T stage
10. Advanced Nodal Stage with nodes being cystic.

The histology tends to be consistently poorly differentiated and non-keratinizing with basaloid features in contrast to the HPV- negative HNSCC that are moderately differentiated and keratinizing [8-9]

The profile of the HPV positive patients in this study is presented in ***Table II*** (Pg 7) behind. None of this patients had the typical characteristics of HPV patients as described in some literature.

Twelve 12(7.5%) of the study patients tested positive for HPV by Realtime polymerase chain reaction.

This is consistent with studies from the African continent, which have recorded a low prevalence of HPV in HNSCC [10-17].

A study done at KNH to determine P16 expression by IHC in 103 formalin - fixed paraffin embedded (FFPE) HNC blocks between 2008 and 2013 came up with an overall prevalence of 14.6%.

The majority were reported in specimens from the following as below;

The oral cavity	(46.67%),
The *larynx*	(26.67%)
The *pharynx*	(26.67%) [18].

The relatively high prevalence rates in these FFPE tissues can be attributed to the HPV detection method employed which has a high sensitivity (100%) but low specificity (79%) [19].

In Nigeria, HPV determination in 149 HNC FFPE specimens was attempted but PCR amplification and Linear Array genotyping was successful in only 49 and 17 specimens respectively. No HPV was detected in any of the specimens [14].

A multicentre cross-sectional study from Senegal assessing the prevalence of HPV in 117 Head and Neck Cancer specimens found only 4 cases (3.4%) with HPV DNA type 16, 35 and 45. None of the HPV positive patients showed P16INK4a over expression [15].

It is important to note that majority of the study subjects who had *laryngeal were* (64), oral cavity (41), with *oropharyngeal* and *pharyngeal* being only 5 and 7 respectively.

In Mozambique no HPV was found among the patients with HNC [16].

In Malawi a prevalence of HPV in HNC by p16 IHC of 17% was reported. Majority of these were either oral cavity or *oropharyngeal* cancer specimens [17].

The relatively high figure may, be attributed to the test modality.

Cameroon recorded low (5%) presence of HPV in oral swabs and rinses from HIV patients but higher (28.6%) HPV from OPSCC by p16 IHC and ISH [20].

Central African Republic yielded only 0.74% HPV by PCR on 25 HNC FFPE specimens [21].

Three different studies from Ghana have shown consistent findings for HPV in HNSCC (19.23% and 18%) with predominantly HPV 16 subtype but low positivity in OSCC (3.4%) [22, 23, 24].

Sudan has, in the latter years, reported unique results among African countries. Alternatively with some reports supporting HPV as a significant factor in oral cancer causation alongside *toombak* (tobacco species) use [25].

A study of 150 HNSCC patients showed an overall positivity of 4% predominantly HPV 16 and with more presence in oral cavity and *pharynx* [26].

In yet another Sudanese study, HPV was found by PCR sequencing in 39 (27%) of 145 FFPE oral cavity samples from *toombak* users. Non-users of *toombak* had 7% HPV positivity. Oral brushings from *toombak* users without oral cancer or *dysplasia* tested positive for HPV in 40%. of them. The authors concluded that HPV infections are common and may influence cancer development [27].

It is important to note that *toombak* has high levels of nicotine is an independent risk factor for HNC.

In another case, a control design study consisting of 40 oral SCC patients and 15 *benign* oral lesions in Sudan, HPV (four type 18 and two type 16) presence by PCR was 15% among cases, none within controls leading to the conclusion that, HPV 18 and 16 may have a causal role in oral SCC in Sudan [25-28].

In Morocco, HPV DNA was detected in 34% of 70 patients with *Nasopharyngeal Carcinoma* with 20.8% of them having HPV 31 and the rest HPV 59, 16, 18, 33, 35 and 45 [29].

Unlike the above two northern African countries who share an Arabian decent with Egypt, the latter has reported low (3.6%) HPV positivity in *laryngeal* cancer but high prevalence in both OPSCC (28%) and OSCC (37%) [30, 31].

Whether this variance is only influenced by the specific sites of study is not clear. What seems to emerge is that in North Africa and the greater Middle East there are varying HPV prevalence rates. The wide range is attributed to limited numbers and scope of studies [32].

Studies on HPV in cancer of the cervix have shown similarities between the North African countries and Europe attributing the similarities to geographical proximity [33].

A review of 60 published studies from 1995 to 2005 on the worldwide status of HPV in 5046 HNSCC specimens using PCR - based methods found an overall HPV prevalence of 25.9% with the individual sites having a prevalence of 35.6% for the *Oropharynx*, 24% for the *Larynx* and *Pharynx* and 23.5% for the Oral Cavity [34].

From the foregoing discussion, it is clear that HPV - related HNSCC has glaring disparities in its distribution.

The Low Prevalence Of HPV in African Countries

This might have been influenced by an interaction of several factors that have not been verified but can be extrapolated from other disease processes.

Among them are socio-cultural, genetic and environmental factors. Risky sexual behavior has been linked to oral HPV acquisition. This includes early sexual debut, multiple sexual partners and prior sexual practice of the partner.

Marital Status

Rather than the number of sexual partners. Marital status has been shown to be more predictive of oral HPV infection with higher rates occurring among divorced, separated and widowed individuals than the married or cohabiting persons [35].

Sexual Practices and Orientation

Generally divergent across the globe sexual practices and orientation raises concern in oral HPV infection. In America, 78% of men were reported to have engaged in oral sex compared to only 9% of Indian origin [36].

No statistics on these practices are available in Kenya and most of Africa but observations are that, they are less compared to the developed world. This may contribute to the low prevalence of HPV in HNSCC in Africa.

Racial disparity with regard to HPV positive HNSCC has been reported in America with lower prevalence among African Americans compared to white Americans.

Among the reasons fronted for the disparities are differences in sexual behavior, *marijuana* use, genetic differences between races, differences in host response to HPV infection and intratypic variation of HPV 16 within geographical areas [37].

A multi-institutional retrospective cohort study among black and white HNSCC patients showed HPV-inactive disease to be common among both black and white patients (31% and 38%) but HPV-active disease

to be less prevalent in black compared to white patients (0% versus 29%) [38].

In another study from America equal numbers of black and white adolescent males were sexually active but with whites 2.7 times more likely to have engaged in oral sex with a female and 1.4 times likely to have received oral sex from a female while the blacks were only 1.4 times likely to have engaged in genital-to-genital sex with a female. At the same time, adolescent white females were twice as likely as black females to have engaged in oro-genital sex [39].

These observations further confirm that other than environment, race is crucial in as far as sexual orientation and by extension HPV-related HNC is concerned. The trend is likely to be reproduced among whites and blacks on other continents including Africa.

It is noteworthy in this Kenyan study that all the patients were black and there was only one subject out of 160 who had admitted to have engaged in oro-genital sex.

Classification of Human Papillomavirus

Types, Subtypes and Variants are based on the L gene sequence similarities [40].

There are several *phylogenetic* variants of HPV 16 isolated in cervical cancer patients like the European, North-American-1, Asian, European-Asian, Asian-American, African-1, African-2, etc. [41].

Different biological properties of HPV 16 variants have been demonstrated in *vitro* and are thought to be responsible in part for variations in;

a. Persistence
b. *Pathogenicity*
c. *Carcinogenic* risk
d. *Immunogenicity*

of the virus among different populations [42].

The distribution of the variants is geographically and ethnically specific with the European type being global except for Sub-Saharan Africa where the African variants are more prevalent [43].

The European variant has been isolated among cervical cancer patients in Tunisia and Morocco. Perhaps reflecting the proximity of the two nations to the European continent [33].

Based on these observations, molecular sub-typing of High-risk HPV types may provide crucial information with regard to geographical and ethnical disparity in HPV-related HNSCC.

Several other logical explanations for low HPV presence in HNSCC in some areas have been proposed. These borrow heavily from other disease processes, but there is so far no evidence to support their role in HPV-related HNSCC.

One such explanation proposes that a genital HPV infection acquired before HPV exposure through oral sex may evoke an immune response that decreases the risk of oral HPV and therefore HPV-related HNSCC [44].

This may explain the high prevalence of HPV in cervical cancer but not in HNSCC in the same population. Geographical differences too, have been shown to influence distribution of certain diseases such as *Sickle* cell disease.

According to the malaria hypothesis, there exists malaria protection by *haemoglobin S* in certain geographical zones including Africa. This protection arises from both innate and acquired immunity to *Plasmodium. falciparum* [45].

It is, therefore thought that perhaps, an equivalent immunological protection against HPV infection by some yet to be determined factor may exist in some geographical regions to explain the low HPV-related HNC.

Gene - environment interaction may also influence disease distribution. This has been demonstrated in *oestrogen* receptor - negative breast tumours which are prevalent among the poor communities [46].

Based on these observations, it is postulated that different populations respond differently to given infections and this might be the case with HPV in head and neck cancer in some cases.

The most common HPV genotype associated with HNSCC is 16 followed by 18 [8, 34].

This study did not have either of these. Several other studies from Africa either had none or only a few of HPV 16 and/or 18. In Morocco, a study on HPV in *Nasopharyngeal Carcinoma* detected HPV in 34% of specimens with HPV 31 in 20.8% of the specimens and the rest being types 59, 16, 18, 33, 35 and 45 [29].

In Senegal, there were only 4 HPV positive cases out of 117 study subjects. The genotypes detected were 16, 35 and 45 but with no P16NK4a overexpression [15].

Analysis of FFPE tissues from *Nasopharyngeal Carcinoma* patients in Ghana was positive for HPV in 14(19.23%) cases 13 of which were type 18 and one type 31 [47].

This is in contrast to predominance of HPV type 16 in HNSCC specimens from the same country majority of which were from the *larynx* and oral cavity [22, 24].

Curiously, a study that evaluated HPV from OSCC in Ghana yielded HPV in only 3.4% of the specimens [23].

Two studies from South Africa, though with very few positive cases, had HPV types 18, 16 and 11 [10, 11].

A later study done in South Africa among male factory workers which sought to determine the oral and *Oropharyngeal* HPV strains and associated risk factors found a prevalence of 5.65%. Apparently the HPV types 16 and 18 were found in two men with a history of oral sex [48].

All characteristics considered, there was no patient or tumour factor from the current study predictive of HPV positivity.

Both HPV and HIV have been classified by the International Agency for Research on Cancer (IARC) as *Carcinogens*.

The association between HIV and HNSCC is unclear with higher prevalence of HNSCC in HIV positive patients being attributed to immunosuppression, opportunistic infections and infection with high-risk HPV types.

In this study HPV positivity was 20% in the HIV-positive HNSCC patients only and 6.7% among the HIV-negative patients.

The difference is wide although the numbers involved were too few and may not permit any statistical inference to be made. Perhaps this begs for further studies to be done to unravel the relationship.

In the meantime, this is one group of patients in whom HPV determination can be considered especially where the other features that characterize HPV positive HNSCC are present.

Conclusion

The prevalence of High-risk HPV among HNSCC patients at KNH is low (7.5%) with no HPV 16 or 18. The positive patients do not have profiles that match those of HPV-positive HNSCC globally and there were no predictors of HPV presence. The prevalence of HPV is relatively higher in the HIV- positive HNSCC patients.

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