HIGH RISK HUMAN PAPILLOMAVIRUS, HUMAN IMMUNODEFICIENCY VIRUS AND EPSTEIN BARR-VIRUS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA PATIENTS AT KENYATTA NATIONAL HOSPITAL

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A thesis submitted in fulfilment of the requirement for the degree of Doctor of Philosophy in Otorhinolaryngology Head and Neck Surgery of the University of Nairobi

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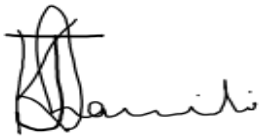
DECLARATION

This thesis is my original work and has not been submitted to any other institution for a degree award.

Signed:  Date: 15-03-2022

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To the Almighty God whose favour and grace has been my portion throughout the study.

To my husband John and children Daisy, Victor and Edgar for their unending support.

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ABBREVIATIONS

AJCC	American Joint Committee on Cancer
CD	Cluster of differentiation
CDC	Centres for Disease Control
CDK	Cyclin Dependent Kinase
CDKN2A	Cyclin Dependent Kinase Inhibitor 2A
CISH	Chromogenic in situ hybridization
DB	Dot blot
DNA	Deoxyribonucleic acid
E6AP	E6-associated protein
E6/E7	Early viral oncogene 6/7
EBV	Epstein-Barr virus
ECOG	Eastern Cooperative Oncology Group
ELISA	Enzyme-linked immunosorbent assay
ENT-HN	Ear Nose and Throat – Head and Neck
ERC	Ethics and Research Committee
FDA	Food and Drug Administration
FFPE	Formalin-Fixed Paraffin Embedded
FISH	Fluorescent in situ hybridization
GERD	Gastro-oesophageal reflux
gp	Glycoprotein
GP5+/6+	General primer P5+/6+
HAART	Highly Active Antiretroviral Therapy

HIV	Human Immunodeficiency Virus
HIV +	Human Immunodeficiency Virus positive
HNC	Head and Neck Cancer
HNSCC	Head and Neck Squamous Cell Carcinoma
HPV	Human papillomavirus
HR-HPV	High risk human papillomavirus
IARC	International Agency for Research on Cancer
IgA	Immunoglobulin A
IHC	Immunohistochemistry
ISH	In situ hybridization
KAVI-ICR	KAVI-Institute of Clinical Research
KNH	Kenyatta National Hospital
LCR	Long Control Region
LMP	Latent Membrane Protein
LPR	Laryngopharyngeal reflux
mRNA	messenger ribonucleic acid
NIAAA	National Institute on Alcohol Abuse and Alcoholism
NPC	Nasopharyngeal Carcinoma
OPSCC	Oropharyngeal Squamous Cell Carcinoma
PCR	Polymerase Chain Reaction
qPCR	Real time (quantitative) PCR
RB	Retinoblastoma tumour suppressor gene product
RNA	Ribonucleic acid

rpm	Revolutions per minute
SCC	Squamous cell carcinoma
SPSS	Statistical package for social sciences
STH	Southern transfer hybridization
TNM	Tumour, Node, Metastases
UADT	Upper Aerodigestive Tract
UoN	University of Nairobi
VCA	Viral Capsid Antigen

ABSTRACT

Background: Smoking and alcohol are the traditional well-known risk factors for head and neck cancer (HNC). In the recent past, human papillomavirus (HPV) has been recognized as risk factor for some head and neck squamous cell carcinomas (HNSCC) specifically Oropharyngeal Squamous Cell Carcinoma. The HPV is classified into 5 groups namely alpha, beta, gamma, mu and nu on the basis of the DNA sequence similarities. The alpha HPVs show tropism for cutaneous and mucosal epithelium and are further categorized into low and high-risk types depending on their ability to cause malignancy. The fifteen high risk human papillomavirus (HR-HPV) types associated with malignancy in the cervix, anogenital and head and neck areas are HPV 16, 18, 31, 33, 35, 39, 45, 51,52, 56, 58, 68, 69, 73 and 82. High risk HPV associated HNSCC is characterized by specific features not seen in HPV-negative HNC like male gender, younger age, higher socio-economic status and minimal or no alcohol and cigarette use among others. HPV status, when known, plays a role in the choice of treatment for HNSCC. This has not been adopted at Kenyatta National Hospital (KNH) for lack of supportive evidence of HPV presence in HNSCC at the institution.

Objectives: The primary objectives of the study were to determine the prevalence and genotypes of HR-HPV among patients with HNSCC at KNH as well as their clinical and pathological characteristics and predictors. The secondary objectives of the study were to determine the prevalence of HR-HPV among Human Immunodeficiency Virus (HIV) positive HNSCC patients and the presence of HR-HPV and Epstein Barr Virus (EBV) co-infection in the nasopharyngeal carcinoma (NPC) patients among the HNSSC patients at KNH; as well as the characteristics of the respective patients.

Study design: This was a descriptive cross-sectional study.

Study setting: The study was carried out at the Ear Nose and Throat – Head and Neck (ENT-HN) and Maxillofacial clinics and wards at KNH. The histology and HIV work up was carried out at the hospital's pathology and immunology laboratories respectively while polymerase chain reaction (PCR) test was done at the KAVI-Institute of Clinical Research (KAVI-ICR) laboratory.

Study Duration: The study was conducted between January 2015 and December 2018.

Study population: This comprised of patients with HNSCC who presented to the ENT-HN and Maxillofacial Departments of KNH during the study period and consented to participate in the study.

Materials and Methods: One hundred and sixty patients with HNSCC were enrolled. Their demographic details and medical history were taken. A complete ENT-HN examination was done. The appropriate haematological work-up, including HIV immunoassay, and radiological work-up for the tumours was done. Two tissue biopsies were taken from the primary tumour for histomorphological diagnosis and DNA extraction. The extracted DNA was subjected to HPV real time PCR for all patients and to EBV real time PCR for the sixty-two nasopharyngeal carcinoma patients.

Data analysis: This was performed using SPSS version 21.0 statistical software. Summary statistics such as means, median and standard deviations were used to describe the distribution of the continuous variables. Descriptive analysis was performed on all variables and summarized into

frequency tables and charts. Pearson Chi square was applied to categorical data to test for association between independent and dependent variables. Fisher's exact test was employed when cell numbers were small. A p-value less than 0.05 was considered significant.

Results: One hundred and sixty patients with HNSCC aged 16 to 87 years with median age of 54.0 years were recruited into the study. There were 117(73.1%) males and 43(26.9%) females. Sixty-two (38.8%) of the patients had nasopharyngeal carcinoma. Most 136(85%) of the patients were drawn from Nairobi and its environs with majority140(87.5%) presenting with advanced disease (stage ≥3). Twelve (7.5%) of the 160 HNSCC patients tested positive for HR-HPV. Of these, ten were HPV 56, one was HPV 52 and one HPV 33. There was no HPV type 16 or 18. Out of the 160 patients with HNSCC, 10(6.3%) tested positive for HIV and only two (20%) of the ten HIV positive patients both of whom had NPC, tested positive for HPV 56. There were no differences in clinical or pathological characteristics between HR-HPV and non- HR-HPV associated HNSCC. All 62 nasopharyngeal carcinoma patients tested positive for EBV while seven (11.3%) tested positive for both EBV and HR-HPV.

Conclusion: The prevalence of HR-HPV at KNH is low (7.5%) among HNSCC patients. Only three HR-HPV types 33, 52 and 56 were detected. There were no clinical or pathological predictors for HR-HPV associated HNSCC. In contrast there was high (20%) prevalence of HR-HPV among HIV positive HNSCC patients, and all the cases had HPV 56. There is 100% EBV and 11.3% HR-HPV presence in NPC patients at KNH with features consistent with high NPC incidence status.

Recommendations: Considering the low prevalence and the absence of HPV types 16 and 18 in HNSCC patients at KNH, the role of HR-HPV in HNSCC in this population is insignificant and

does not warrant routine testing for HR-HPV in all HNSCC patients. The relatively higher prevalence of HR-HPV among HIV positive HNSCC patients may be useful in directing the focus of future studies to these specific populations. Finally, due to the high prevalence of EBV in NPC, it may be prudent to adopt EBV serology in screening and follow up of NPC patients.

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

1.1.1 The global burden of High-Risk Human Papillomavirus

Cancer is a growing public health concern worldwide and more so in Africa. In Kenya, it is the third commonest cause of death after infectious and cardiovascular diseases, contributing 7% of the total annual mortality. According to the Nairobi cancer registry, HNC was the second commonest malignancy reported in males after cancer of the prostate and fifth in females after cancer of the breast, cervix uteri, eye and oesophagus in the period 2004 to 2008[1]. A rapid rise in cancers is projected in many low income countries due to increased exposure to risk factors including tobacco, alcohol, environmental carcinogens, HIV, and human papillomavirus (HPV)[2]. The overall incidence of HNSCC has increased over the last three decades. This has been attributed to an increase in HPV-related cancers, in particular, oropharyngeal squamous carcinoma (OPSCC)[3]. In the USA, the prevalence of HPV-positive OPSCC increased by 225% between 1988 and 2004 while in Europe the increase was from 40% before 2000 to 72% between 2005 and 2009[4, 5]. Sweden reported an increase in prevalence of HPV-associated oropharyngeal cancer from 23% in the 1970s to 57% in the 1990s and 93% in 2007 [6]. During the same period, the prevalence of HPV-negative OPSCC declined by 50% , this being related to a decline in smoking following successful implimentation of legislation to curb smoking and alcohol consumption[5]. Studies indicate that the dominance of HPV 16 in HNSCC is greater than that seen in cervical carcinoma (85-95% versus 50-60%) worldwide [7]. Currently, HPV, and particularly HPV16, is recognized as a risk factor for a subset of HNSCC with prevalence varying from 0 to 65% [4-15].

1.1.2 The Human Papillomavirus

The Human papillomavirus is a double stranded deoxyribonucleic acid (DNA) virus that, according to Centres for Disease Control (CDC), is the commonest sexually transmitted disease. It is a small virus with about 200 identified genotypes. The viruses are classified into five groups namely alpha (α), beta (β), gamma (γ), mu (μ) and nu(ν) on the basis of the DNA sequence similarities. The alpha HPVs show tropism for cutaneous and mucosal epithelium and are further categorized into low- and high-risk types depending on their ability to cause cancer. The fifteen high risk types associated with malignancy in the cervix, anogenital and head and neck areas are HPV 16,18, 31, 33, 35, 39, 45, 51,52,56,58, 68, 69, 73 and 82[16]. Classification into HPV genotypes is based on the type of epithelial cells infected and the ability to effect cellular transformation e.g. HPV1 infects cutaneous epithelial cells whereas HPV 6, 11, 16 and 18 infect mucosal epithelial cells of the upper aerodigestive and anogenital tracts. The effect of infection can either be productive or transformative. Productive infections caused by low-risk subtypes are associated with benign cellular proliferation and differentiation typically seen in condylomata and papillomas. Transformative infections are associated with high-risk HPV and are characterised by dysplastic lesions that may progress to neoplasia.

The HPV viral genome consists of three segments: the early (E), late (L) and the long control region (LCR) also called upstream regulatory region. The early genes (E1, E2, E4, E5, E6, E7 and E8) regulate, promote and support viral DNA transcription and replication. The late region (L1 and L2) form the viral capsid and the LCR contains the regulatory sequence that controls viral replication and transcription [17]. Whereas E6 and E7 are found in all HPV types, most studies tend to focus on

E6 and E7 from HPV 16 and 18 because they are the most frequent types isolated in cervical and OPSCC worldwide.

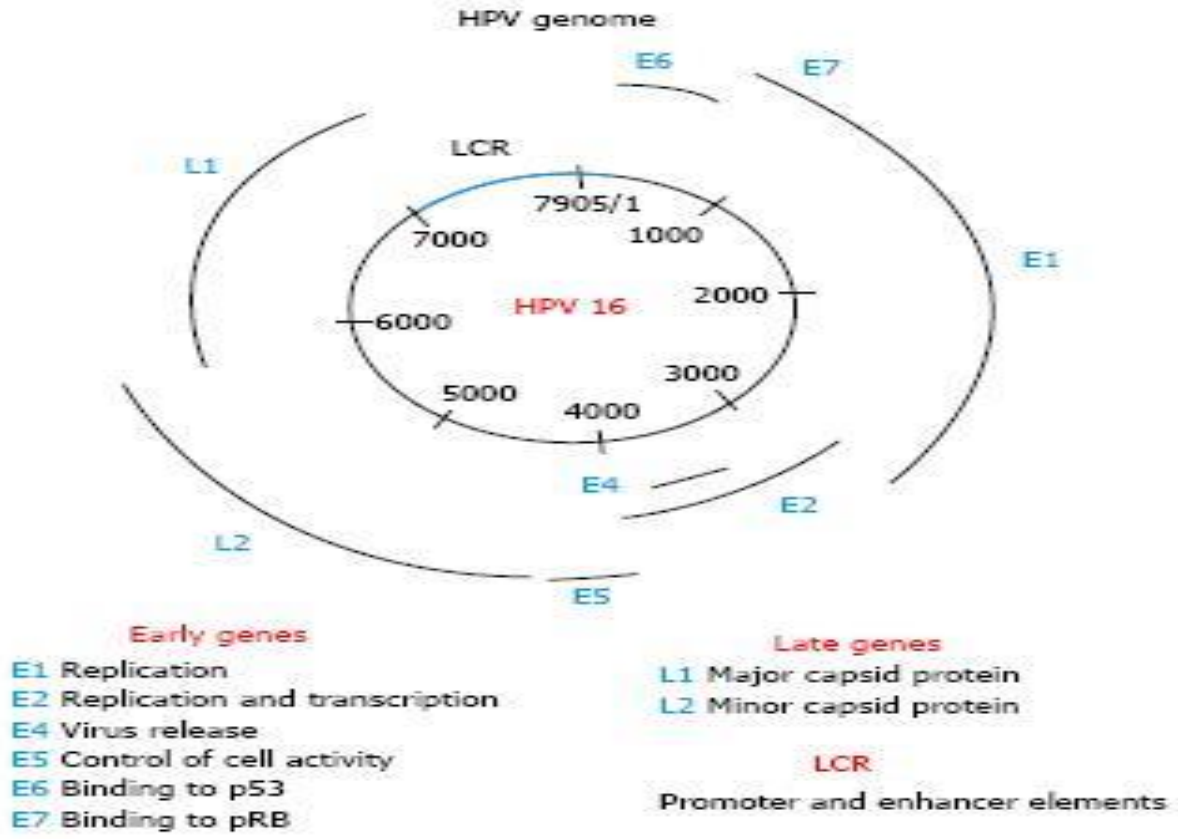


Figure 1: HPV Genome [18]

The oncogenic potential of each HPV genotype is determined by the strength of binding affinity for p53. HPV 16, for example, encodes for an E6 allele that binds p53 with twice the affinity of HPV 18 allele [19]. Only the HR-HPV E6 alleles bind to p53. Based on this observation, the global contribution of the various HR-HPV types has been reported as summarised in the table below:

Table 1. "The contribution of each high-risk human papillomavirus (HPV) type to head and neck cancer (HNC) cases worldwide" [20]

HPV Type	Worldwide Contribution to HNCs *
HPV16	70.7%
HPV18	14%–17%
HPV33	4.5%
HPV35	4.5%
HPV52	2.7%
HPV45	1.5%
HPV39	1.04%
HPV58	0.6%
HPV31	0.56%
HPV53	0.3%
HPV56	0.25%

1.1.3 Transmission of HR-HPV to the Head and Neck Sites

Infection of the upper aerodigestive tract (UADT) with HPV is thought to be mainly via oro-genital contact or perinatal transmission although, theoretically, any skin-to-skin contact may result in viral transmission. Literature has shown that oral sexual activities in association with increased number of oral sexual partners is responsible for high oral transmission rates. In addition, HPV infection arising from open-mouth or deep kissing has been reported in persons with no history of oral sex [21,22]. Other modes of oral transmission are self-inoculation through contaminated fingernails, vertical transmission from mother to child during birth or via breast milk, and horizontal transmission from the breast to spouse or vice versa [23-25].

1.1.4 Human papillomavirus Integration

Human papillomavirus is the commonest sexually transmitted infection based on reports from CDC. Despite this, most infections get cleared within a period of 18 months [26]. Persistent infection is thought to be necessary for progression to malignancy.

The initial infection requires access of viral particles to the basal layer cells via a break in the stratified epithelium. In the oropharynx, this infection may occur in the absence of any epithelial abrasion. This is because in the lymphoid tissue of the pharynx, the reticulated epithelium has a fenestrated discontinuous basement membrane that allows the immune cells access to antigens [27]. The virus initially infects an epithelial stem cell which in this case is the keratinocyte. Following attachment to the basal cell, the L1 capsid protein interacts with the basement membrane heparin sulphate proteoglycans leading to conformational changes in L1 and L2 with transfer of virion particles to the host cellular uptake receptors necessary for viral internalisation through endocytosis [28]. Uncoating, facilitated by the disruption of intracapsomeric disulphide bonds in the cell allows viral DNA to be transported into the nucleus. Within the nucleus the virions persist either in episomal form or get integrated into the host genome [29]. Initially, the virus will undergo a transient round of replication that results in 50 to 100 copies of viral genomes per cell. Subsequently, viral episomes get maintained within the undifferentiated basal cells by replicating along with the host cell chromosomes. E6 and E7 oncoproteins are expressed before the productive viral replication thereby driving the cell cycle entry and cell proliferation. During epithelial differentiation, the productive phase of the virus is activated resulting in the amplification of viral genomes to thousands of copies per cell in the suprabasal layers in addition to late gene expression and virion assembly and release [30, 31]. The restriction of viral gene expression and virion production to the

uppermost layers of the epithelium where there is no immune surveillance protects the virus from detection by the host immune mechanisms [17].

1.1.5 Carcinogenesis in HPV-Related Head and Neck Cancer

Majority of human cancers are attributed to alteration in the p53 and retinoblastoma gene (Rb) pathways of the cell cycle. These two are tumour-suppressor proteins that control apoptosis, DNA damage repair and cell cycle arrest. P53 is a protein product of TP53 gene. Its function is in regulation of the DNA damage response and transition of the G2/M cell cycle stage. The retinoblastoma protein confers its inhibitory effect on the cell cycle by regulating the nuclear accumulation of a mitogenic transcription factor E2F thereby controlling the G1 to S-phase cell transition. Figure 2 below shows how these proteins work. In (A) the physiologic regulation of the cell cycle is depicted. The CDKN2A gene encodes the $p14^{ARF}$ and $p16^{INK4A}$ proteins. The former inhibits MNM2 hence allowing p53 to activate p21 and stop the progression of G2/M checkpoint into mitosis. $P16^{INK4A,}$ on the other hand, inhibits the cyclin D1/CDK4 and cyclin D1/CDK6 complexes. Both complexes catalyse phosphorylation of the retinoblastoma protein thereby inducing it to release E2F family transcription factors to enter the nucleus and activate transcription of S-phase promoting transcripts. The Rb phosphorylation causes feedback inhibition of $p16^{INK4A}$ expression. (B) shows how the HPV E6 and E7 oncoproteins degrade P53 and Rb to dysregulate the cell cycle. In addition, Rb degradation causes loss of feedback inhibition of $p16^{INK4A}$ expression, hence the $p16^{INK4A}$ overexpression seen in HPV infection.

In HPV-driven HNSCC, two HPV viral oncogenes, E6 and E7, are involved in carcinogenesis and maintenance of malignant phenotypes. High-risk HPV types produce E6 and E7 that inactivate and degrade the p53 and Rb genes respectively thereby causing defects in apoptosis, DNA repair, cell cycle control and eventually leading to cellular proliferation and immortalization. The uncontrolled

cellular proliferation resulting from the two processes cause chromosomal instability and accumulation of genetic mutations, hence the resultant cellular transformation.

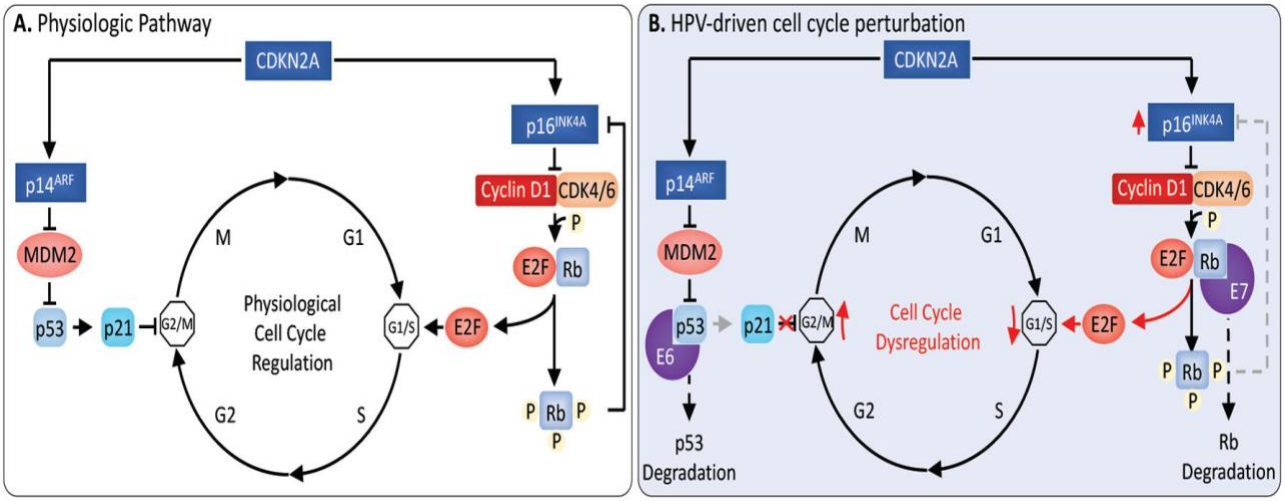


Figure 2: "Cell cycle perturbation by high risk human papillomavirus" [33]

1.1.6 Human Papillomavirus Detection Methods

Detection of HPV-DNA in cancer tissue does not imply viral involvement in the on-going carcinogenic process since the test cannot delineate between transient and potentially transforming infection. It has therefore been suggested that the only way to confirm a conclusive viral involvement is by measuring the levels of E6/E7 RNA. Although few commercial HPV E6 and E7 mRNA assays are available, there are challenges of stability of E6 and E7 mRNA in routinely collected clinical samples [34]. The effects of E7 on Rb are known to induce P16 up-regulation to levels that can be detected by immunohistochemistry (IHC). Detection of P16 is, therefore, a useful surrogate biomarker for HPV presence in cancer tissues [35]. P16 immunohistochemical methods are sensitive but do not differentiate HPV serotypes and so are associated with false positives that may arise from HPV-unrelated disturbances of retinoblastoma protein [36]. Methods of HPV detection in cells have evolved over time from electron microscopy to polymerase chain reaction (PCR). The

evolution and the fact that there is no standardized method of elucidating HPV status has, in part, contributed to inconsistent HPV prevalence estimates in tissues. There are three main categories of molecular assays, employed in the detection of HPV-DNA in tissues:

1. Non-amplified hybridization assays such as southern transfer hybridization (STH), dot blot (DB) hybridization and in situ hybridization (ISH). Large amounts of DNA are required for STH and the procedure is laborious and not reproducible. ISH is available in two forms: Fluorescent in situ hybridization (FISH); and chromogenic in situ hybridization (CISH). Although ISH has the advantage that it can be carried out on formalin fixed paraffin embedded tissue (PFFE) is limited by the inability to detect targets with low DNA copies.
2. Signal amplified hybridization assays such as hybrid capture assays is a non-radioactive signal-amplification method based on hybridization of HPV-DNA to labelled RNA probes in solution. The technique has the weakness of not providing evidence of viral integration as with PCR. It is FDA approved but was not designed for genotyping single HPV. It has low false-positive rates.
3. Target amplification assays such as PCR. PCR-based detection of HPV E6 oncogene expression in frozen tissue samples is regarded as the gold standard for HPV presence. PCR methods are very sensitive but require a high level of technical skill. They work best with fresh and frozen samples but are prone to false-positives arising from carry-over of product and contamination. In PCR, viral DNA is amplified in vitro by DNA polymerase to generate adequate amounts of target that is then either directly visualised on gels or detected by specific probe using traditional hybridization methods and using real time luciferase-based techniques. The FDA has approved a multiplex real time PCR based assay for diagnosis of high-risk HPV types, including 16 and 18.

A feasible algorithm that combines the strengths of complimentary assays has been proposed in clinical settings to offset the limitations of any one detection assay [15]. This algorithm incorporates P16 immunohistochemistry, which serves to eliminate the HPV-negative cases from any additional analysis. The P16-positive specimens are then tested for active HPV DNA using GP5+/6+ PCR.

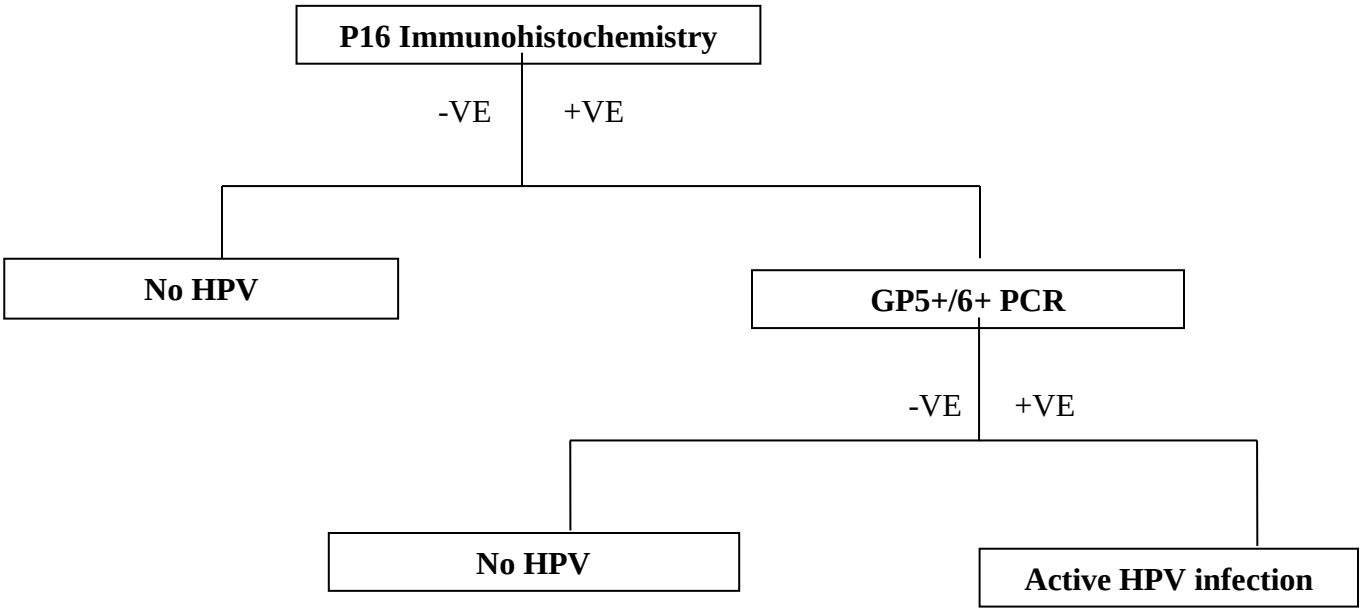


Figure 3: "Proposed flowchart for high throughput identification of HNSSC with a clinically relevant HPV infection on paraffin-embedded tissue sections with 100%sensitivity and specificity" [37]

1.1.7 Human Papillomavirus and other Viruses in Head and Neck Squamous Cell Carcinoma

Head and neck squamous cell carcinoma is the third commonest HNC among HIV patients after Kaposi's sarcoma and non-Hodgkin's lymphoma [38, 39]. The association between HIV and HNSCC is unclear with higher prevalence of HNSCC in HIV positive patients being attributed to immunosuppression, opportunistic infections, increased tobacco use and infection with high-risk HPV subtypes [40-42]. At the molecular level, HIV promotes HPV infection by disrupting of epithelial tight junctions and potentiating penetration of HPV into the basal epithelial cells [43]. The disruption is induced by HIV proteins (tat and gp120) as well as cytokines produced by HIV-infected cells. The same HIV tat proteins have been shown to upregulate the tumorigenic HPV-E6 and to downregulate the anti-tumorigenic p53 protein [44]. To escape immune surveillance and cause viral persistence, both viruses modify the host immune system through tumour-driven macrophage differentiation, cellular immune response compromise, imbalance between type 1 and type 2 T helper cells, and downregulation of dendritic cell activation and maturation [45]. At the clinical level, HIV and HR-HPV infection and persistence is favoured by the shared transmission route, HIV-induced immunosuppression, reduced HR-HPV clearance and reactivation of latent HR-HPV infection.

The presence of both EBV and HR-HPV in nasopharyngeal cancer cells has been reported but their interactive role is still a subject of study. Epstein-Barr virus infections tend to occur early in life and are followed by lifelong persistence of the virus. Except for the clinical form of the infection referred to as infectious mononucleosis, most EBV infections are asymptomatic. It is estimated that at least 90% of adults worldwide test positive for EBV by serology [46]. In the nasopharynx, EBV is thought to infect the epithelial cells of the fossa of Rosenmuller either via a surface protein, which is antigenically related to the B-cell C21 receptor or through IgA-mediated endocytosis [47, 48]. To

cause malignancy, the virus activates intracellular signaling involved in control of proliferation. This may require interaction with other risk factors such as failure of immune recognition, genetic aberrations, environmental and nutritional factors [49]. An early EBV protein named Z Epstein-Barr Virus Replication Activator expressed during the lytic replication of EBV has been shown to bind the p53 protein in a manner similar to HPV resulting in cell immortalization as well as viral replication [50]. Another point of interaction of the two viruses is thought to be through suppression of retinoblastoma protein. Studies have demonstrated that B Cells transfected with EBV latent membrane proteins lose the regulatory effects of the retinoblastoma protein [51]. More often, the EBV negative NPC tumours are the ones that tend to test positive for HR-HPV. Like EBV, HR-HPV positivity is associated with a better prognosis [52, 53].

1.2 STUDY RATIONALE

Based on the 2004-2008 Nairobi cancer registry, Head and Neck Cancer was the second commonest malignancy reported in males after cancer of the prostate and fifth in females after cancer of the breast, cervix uteri, eye and oesophagus; yet it has received very low public health attention in Kenya. Studies done in North America and Europe have indicated that HNSCC is significantly associated with HR-HPV as a risk factor.This association exceeds that of HR-HPV and cancer of the cervix[7]. The two cancers also share HPV 16 and 18 as the main genotypes involved.Vaccines against the two HPV types as well as HPV 6 and 11 have been approved and are in use for prevention of HPV-related cancer of the cervix worldwide as well as in Kenya. Many high income countries have rolled out programs for prophylactic HPV vaccination for boys and girls aged 9 to 26 years. In America, three vaccines are available for HPV prophylaxis against cervical, anogenital and oropharyngeal carcinomas.: bivalent (HPV 16/18), quadrivalent (HPV 16/18/6/11) and nonavalent

(HPV 16/18/6/11/31/33/45/52/58). Randomized clinical trials have shown at least 90% vaccine efficacy in the prevention of anogenital HPV infections and precancerous lesions [54]. An evaluation of the effects of HPV vaccination on the population level burden of oral HPV infections in the United States showed an 88% reduction in the prevalence of vaccine-type oral HPV 16/18/6/11 infections among vaccinated young adults. A reduction of 93% oral and 72% cervical HPV infection has been reported in Costa Rica among women who got the HPV bivalent vaccine with highest efficacy among the HPV "naïve"[55, 56].The biggest challenge with HPV vaccine trials with regard to HNC has been determination of clinically identifiable surrogate end points like precancers as these are difficult to detect in the oropharynx. In America, the acceptable end-point for efficacy trials is limited to oral cavity HPV infection prevention [57]. It is feasible that once the issue of vaccine efficacy trials is overcome, prevention of HPV-related HNC may become an indication for HPV vaccination. In order for similar interventions to be extrapolated to Kenya as is the case with cervical cancer, there is need to determine the magnitude of HR-HPV-related HNSCC and the genotypes involved.

Patients with HNC are treated with surgery, chemotherapy, radiotherapy or a combination of any of the modes. In KNH, chemotherapy, when offered is usually adjuvant. Radiotherapy is given either as primary therapy in selected early tumours or palliation in advanced disease. Surgery is the mainstay mode of treatment in resectable tumours. It is, however, disfiguring and associated with loss of organ function necessitating reconstructive surgery. In the developed countries, escalated organ-preserving treatment regimes involving chemoradiation have gained popularity. This approach has been noted to have good prognosis for HPV-related HNSCC. HPV status is thus increasingly being used to guide and direct HNSCC patient care in the high income countries. As

Kenya lays strategies to improve management of HNSCC, it will be necessary to determine the HPV status of patients as a guide to the management for improved functional outcomes. HPV detection methods are, however, associated with significant cost implications. Determination of predictors of HR-HPV-associated HNSCC will be useful in triaging patients for HPV testing thereby alleviating the cost implications attendant to HPV testing in all HNSCC. Use and popularization of chemoradiation for HPV-related HNSCC may confer the benefit of organ-preservation and possibly function-preservation to the patients.

1.3 RESEARCH QUESTIONS

Primary:

1. What is the prevalence, clinical and pathological characteristics of HR-HPV associated HNSCC patients at KNH?

Secondary:

2. What is the prevalence and characteristics of HR-HPV among HIV positive HNSCC patients at KNH?
3. What is the prevalence and characteristics of HR-HPV and EBV among NPC patients at KNH?

1.4 STUDY OBJECTIVES

1.4.1 Broad objective

To determine the prevalence and predictors of HR-HPV among HNSCC patients; the prevalence of HR-HPV among HIV positive HNSCC patients; and the prevalence of HR-HPV and EBV among NPC patients at KNH.

1.4.2 Primary Objectives

1. To determine the prevalence of high-risk HPV among patients with HNSCC at KNH
2. To describe the HR-HPV genotypes among patients with HNSCC at KNH
3. To determine the characteristics of HR-HPV positive HNSCC patients at KNH

1.4.3 Secondary Objectives

1. To determine the prevalence of HR-HPV among HIV positive HNSCC patients at KNH
2. To describe the characteristics of HIV positive HNSCC patients with HR-HPV at KNH
3. To determine the prevalence of HR-HPV and EBV co-infection among patients with NPC at KNH
4. To describe the characteristics of EBV positive PNS patients at KNH.

CHAPTER 2: LITERATURE REVIEW

4.1 Overview of Head and Neck Squamous Cell Carcinoma

Head and neck cancers form the seventh commonest malignancies globally [58]. Site and subsite distribution vary from country to country. According to the Nairobi cancer registry, which uses statistics from hospitals within Nairobi (Capital of Kenya), the commonest head and neck cancers in Nairobi in decreasing order are oral cavity, larynx, and nasopharynx [1]. Kenyatta National Hospital draws most of its patients from the environs of Nairobi [59]. Previous data from Kenyatta National Hospital gives the order from the most common head and neck malignancies by site as laryngeal cancer followed by the tongue, mouth and nasopharynx [60]. Majority of HNCs occur in the 6th and 7th decades except for nasopharyngeal carcinoma which has a bimodal peak in adolescence and 7th decade in low-risk populations [61, 62]. Males tend to be affected more compared to females [63]. The male: female ratio is even higher for laryngeal cancer, perhaps reflecting the differences in lifestyle with regard to alcohol and tobacco use between the genders. Two studies done in Kenya on laryngeal carcinoma showed a male: female ratio of 24:1 [64, 65]. This is in keeping with the reported alcohol and tobacco use among Kenyan patients of 80.8% and 56.4% respectively for males compared to 30.6% and 5.6% respectively for females [66]. Studies from the Kenyatta National Hospital have confirmed that smoking confers a positive risk for carcinoma of the larynx and oropharynx [65,67]. The HNSCC patients in Kenya tend to present with advanced disease for various reasons among them multiple referrals, missed diagnoses and inaccessible health facilities [68]. Kenyatta National Hospital is the only public hospital in the country with cancer treatment facilities. Most patients with cancer would have to cover long distances to access the facility. Studies have shown that most of the cancer patients managed at the hospital come from Nairobi and the neighbouring provinces [59, 68].

Head and neck squamous cell carcinoma is thought to arise from an interaction of various factors among them genetics, environmental and lifestyle factors. Cigarette smoking is the strongest independent risk factor for HNSCC and works in synergy with alcohol in cancer causation [69]. The recent addition of HPV to the risk factors for HNSCC has not been uniform or global. Overall, the prevalence of HPV in the head and neck region ranges from 0 -65% depending on the study population, combination of topographies studied, type of specimen analysed, method of specimen preservation and the DNA detection method employed. The association is strongest for oropharyngeal carcinoma, specifically the palatine and lingual tonsil [8, 9, 10]. Several other risk factors for HNSCC have been reported including betel nut chewing among the Asian community and *khat* (Catha edulis) on the African continent particularly the northern horn of Africa. *Khat* is a plant whose leaves are chewed for their simulating amphetamine-like effects. *Khat* contains a number of chemicals with the main one being cathinone. Its chemical structure is similar to amphetamine and it causes similar psychomotor stimulant effects as does amphetamine but on a lesser scale. In the oral cavity, *khat* chewing has been associated with the possibility of developing potentially malignant as well as malignant oral lesions [70].

4.2 High Risk HPV in Head and Neck Cancer

There is significant evidence that human papilloma virus may have a role in tumours of the head and neck with 20 to 25% of HNSCC having oncogenic HPV [10, 11]. The commonest genotype of HR-HPV associated with these tumours is HPV 16 followed by HPV 18 [10, 71]. There is evidence that the dominance of HPV 16 in HNSCC is greater than that seen in cervical carcinoma (85-95% versus 50-60%) worldwide [7]. The incidence of cancers attributable to HR-HPV in Kenya shows that cancer of the cervix uteri, other anogenital cancers and Head and Neck cancers stand at 33.8 in

100,000 women, 1.9 in 100,000 people and 0.2 in 10,0000 people respectively [72]. This is despite the fact that all three groups of cancer are caused by the same HR-HPV types. The increase in HPV presence in cervical cancer is attributed to multiple sexual partners while that in HNC is linked to oral sex, number of oral sex partners and open-mouth kissing. Anogenital cancers, like cervical cancer, are related to sexual transmission of the virus [73]. The difference in the incidence of the cancer in the three sites in Kenya may be due to tissue or viral preference factors, sexual orientation practices, as well as the accessibility to HPV testing.

Worldwide HR-HPV related cervical cancer occurs predominantly in less developed countries while North America and Northern Europe have the bulk of HR-HPV attributable Head and Neck cancers. [74]. This has been attributed to higher rates of oral sexual practice in the high economy countries compared to the sub-Saharan African countries (84% versus 47%) [75, 76]. There is also a variation in the distribution of HPV-associated cancers in the different anatomical sites of the UADT. The highest prevalence worldwide is in oropharynx (45.8%) followed by the oral cavity (24.2%) and larynx and hypopharynx (22.1%) [77]. This has been attributed to various factors namely:

1. Specimen: - HPV has been tested from a variety of specimens including swabs, oral washes, scrapings and tissue biopsies. In addition, the method of preservation of the specimen may influence the test result. Some specimens are fresh-frozen while others are formalin fixed paraffin-embedded (FFPE).
2. Detection assay: - Sensitivities and specificities of HPV detection assays vary a great deal. For instance, PCR tends to be more sensitive compared to ISH; while a nested PCR is even more sensitive than the regular PCR.

3. Analysed biomarker: -Some biomarkers are more sensitive for certain anatomical sites than others. It has been demonstrated that both E6/E7 mRNA and $p16^{INK4A}$ are more sensitive for the oropharyngeal site than they are for other UADT sites [(78)].

A meta-analysis covering reports from 1982 to 1997 to examine the risk of HPV detection in normal oral mucosa, pre-cancerous oral tissue and oral carcinoma showed that the pooled probability of detecting HPV in normal mucosa was 10%, benign leucoplakia 22%, intra-epithelial neoplasia 26.2%, verrucous carcinoma 29.5% and oral squamous cell carcinoma (SCC) 46.5%. The progressive increase in presence of HPV from normal mucosa through dysplastic tissue to carcinoma clearly supports the role of HR-HPV in oral SCC. A total of 4680 samples from 94 studies were included in the above analysis. HPV 16 and 18 were detected in 30% of oral SCC with other high-risk types being detected in less than 1% of the tumours. Several limitations are, however, noted in the study e.g. variation in prevalence between geographic regions, variation between head and neck sub-sites, different HPV detection methods, use of different primers, variable sample sources, and collection methods [(71)].

Termine et al did a search of 62 studies done between 1988 and 2007 to detect HPV DNA in oral and not site-specified HNSCC in 4852 paraffin-embedded samples using PCR and/or ISH. The objective of the study was to determine the effect of specific HNSCC site and HPV DNA detection method on the prevalence of HR-HPV in HNC. The pooled prevalence was 34.5% with a higher prevalence in oral SCC (38.1%) compared to not site-specific HNSCC (24.1%). HPV 16 was the most frequently reported genotype, often in association with HPV 18. PCR-based studies yielded higher prevalence rates compared to ISH (34.8% versus 32.9%). Among the studies considered, the

highest prevalence was among Asian countries, in particular Japan, with Africa posting very low prevalence of 0-11.9% [79].

A review of 60 published studies from 1995 to 2005 on the status of HPV worldwide involving 5,046 HNSCC that employed PCR-based methods to detect and genotype HR-HPV found an overall HR-HPV prevalence of 25.9%. The prevalence of HR-HPV by cancer site and the HPV16 and HPV18 subtype detection in HNSCC are summarised in Table 2. Apart from HPV16 and 18, other oncogenic HPV subtypes (31, 33, 35, 45, 51, 52, 56, 58, 59 and 68) were rarely detected in HNSCC. The same study revealed a higher prevalence of HR-HPV in oral SCC from Asia compared with other geographical locations [80]. This is in contrast to the findings of the multi-continental International Agency for Research on Cancer (IARC) study in which HR-HPV prevalence in oral and oropharyngeal cancers did not differ significantly among Europe, North and South America and Asia [81].

Table 2: "Prevalence of HR-HPV by cancer site & HPV16 and 18 subtype detection rates" [80]

Site	**Oral cavity**	**Oropharynx**	**Larynx & hypopharynx**	**Overall**
No. Studies	35	27	35	60*
Cases	2642	969	1435	5046
Overall HPV Prevalence (95%CI)	23.5 (21.9 - 25.1)	35.6 (32.6 - 38.7)	24.0 (21.8 - 26.3)	25.9 (24.7- 27.2)
HPV16 Detection (%)	16.0	30.9	16.6	
HPV18 Detection (%)	8.0	1.0	3.9	

*Does not add to total number of studies because some studies included multiple site

Many studies on high risk HR-HPV in HNC in Africa report low prevalence and often a variety of genotypes other than 16 and 18 (Table 3 below).

Table 3: HPV in Head and Neck Squamous Cell Carcinoma in Africa

Country (Year of publication)	**Tumor Site (Sample size)**	**Specimen type**	**Detection method**	**HPV Prevalence**	**HPV genotypes in bold (n=number)**
S. Africa (1995) [82]	HNSCC (66)	FFPE	ISH	1.50%	**18** (n=1)
S. Africa (1996) [83]	HNSCC (146)	FFPE	PCR	1.40%	**16**(n=1), **11**(n=0)
S. Africa (2006) [84]	HNSCC (59)	FFPE	PCR ISH	11.90% 0%	**18**(n=7)
S. Africa (2018) [85]	HNSCC (112)	Fresh	P16 IHC PCR	19.60% 6.30%	**11**(n=2), **16**(n=2), **18**(n=1), **31**(n=1), **45**(n=1)
Ghana (2014) [86]	HNSCC (78)	FFPE	PCR	19.23%	**16** (n=13), **18** (n=3)
Ghana (2019) [87]	HNSCC (100)	FFPE	PCR	18%	**16**(n=18), **18**(n=1)
Sudan (2012) [88]	HNSCC (150)	FFPE	PCR	4%	**16**(n=3), **18**(n=2), **33**(n=1)
Malawi (2017) [89]	HNSCC (55)	FFPE	P16 IHC	17%	
Nigeria (2016) [90]	HNSCC (17)	FFPE	PCR	0%	
Senegal (2013) [91]	HNSCC	FFPE	PCR	3.40%	**45**(n=2), **16**(n=1), **35**(n=1)
CAR (2019) [92]	HNSCC (25)	FFPE	PCR	0.74%	**16** (n=1)
Cameroon (2019) [93]	OPSCC (7)	FFPE	P16 IHC, ISH	28.60%	
Mozambique (2015) [94]	OSCC (29) OPSCC (22)	FFPE	P16 IHC, PCR	IHC=6.9%, PCR=0% IHC=0%, PCR=0%	
Ghana (2018) [95]	OSCC (88)	FFPE	PCR	3.40%	**16**(n=1), **18**(n=1), **52**(n=1)
Egypt (2019) [96]	OPSCC (32) OSCC (67)	FFPE	P16 IHC, ISH	28% 37%	
Sudan (1998) [97]	OSCC (28)	FFPE	ISH, PCR	0%	
Sudan (2010) [98]	OSCC (40)	FFPE	PCR	15%	
Sudan (2010) [99]	OSCC (145)	FFPE	PCR	27%	
Morocco (2018) [100]	NPC (70)		PCR	34%	34%; **31**(n=5),**59**(n=4), **16**(n=2), **18**(n=2), **33**(n=1),**35**(n=1), **45**(n=1)
Egypt (2017) [101]	LSCC (56)	FFPE	PCR	3.60%	

A multicentre cross-sectional study from Senegal assessing the prevalence of HPV in 117 Head and Neck Cancer found only 4 cases (3.4%) with HPV DNA type 16, 35 and 45. None of the HPV positive patients showed $P16^{INK4a}$ over expression [91]. It is important to note that majority of the study subjects were laryngeal (64), and oral cavity (41) with oropharyngeal and pharyngeal being only 5 and 7 respectively. Both Nigeria and Mozambique found no HPV among their patients with HNC [90, 94]. In Malawi a prevalence of HPV in HNC by p16 IHC of 17% has been reported. Majority of these were either oral cavity or oropharyngeal [89]. A similar study from Kenya where 103 FFPE blocks of HNC seen between 2003 and 2013 were subjected to p16 IHC revealed HPV prevalence of 14.6%. Majority of the specimens were from the oral cavity (46.67%) followed by pharynx and larynx (26.67% each) [102]. It is notable that p16 IHC is associated with high levels of false-positives attributed to disturbance of Rb by HPV-unrelated factors.

Sudan has, in the latter years, posted unique results among African countries with some reports supporting HPV alongside *toombak* use as an important factor in oral cancer causation [103]. *Toombak* is a type of highly addictive snuff made from ground leaves of a tobacco species called Nicotiana rustica used by native Sudanese. The paste is made by mixing the ground fermented leaves with sodium bicarbonate and water in the ratio of 4:1 for increased alkalinity which is thought to enhance nicotine absorption. The resultant snuff is rolled into balls that are put in the oral vestibule and left for two to three hours before being discarded. It is estimated that up to thirty replacements can be achieved per day. *Toombak* has nearly 100-fold higher concentration of tobacco-specific N-nitrosamines than the American commercial snuff brands. Its use has been associated with oral cavity inflammations, pre-malignant and malignant lesions. The observation that *toombak* use alongside HPV infection may contribute to HNC causation has been further

strengthened by another of the Sudanese studies where HPV was found by PCR sequencing in 39 (27%) of 145 FFPE oral cavity samples from *toombak* users with non-users of *toombak* having 7% HPV positivity. At the same time oral brushings from *toombak* users without oral cancer or dysplasia tested positive for HPV in 40%. The authors, therefore, concluded that HPV infections are common and may influence cancer development in association with *toombak* use [99]. It would appear that the use of *toombak* is associated with high HPV presence. This may be explained by the fact that chronic exposure of the oral mucosa to *toombak* results in alkaline burns [104]. HPV requires a break in the stratified epithelium to access the basal layers. The burns caused by the alkalinity in *toombak* may therefore be the point of facilitation for HPV acquisition. It is also important to take note of the high levels of nitrosamines in *toombak* which are carcinogenic with regard to oral cancer. The role of HPV in OSCC in Sudan has received further backing from a case control design study consisting of 40 OSCC patients and 15 benign oral lesions in which HPV (four type 18 and two type 16) presence by PCR was 15% among cases and 0% among controls [98]. 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 [100]. Unlike the above two northern African countries which share an Arabian decent with Egypt, the latter has reported low (3.6%) HPV positivity in laryngeal cancer [101]. This low figure may be related to the specific site of study; the larynx. What seems consistent is that in North Africa and the greater Middle East there are varying HPV prevalence rates. The wide range has been attributed to limited numbers and scope of work covered [96]. Studies on HPV in cancer of the cervix have shown similarities between the North African countries and Europe attributing the similarities to geographical proximity [105].

The predominantly low prevalence of HPV in HNSCC in most of Africa has been attributed to an interaction of socio-cultural, genetic and environmental factors extrapolated from other disease processes [106-114].Classification of human papillomavirus types, subtypes and variants are based on the L gene sequence similarities [108]. 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. [109]. Different biological properties of HPV 16 variants have been demonstrated in vitro and are thought to be responsible, in part, for variations in persistence, pathogenicity, carcinogenic risk and immunogenicity of the virus among different populations [115]. 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 [110]. 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 [105]. Based on these observations, molecular sub-typing of high-risk HPV types may provide useful information with regard to geographical and ethnic disparity in HPV-related HNSCC.

Significant disparities have been reported in HPV-related HNC with regard to race among African Americans and white Americans with the latter having higher prevalence. In a multi-institutional study involving the two races, HPV-inactive disease was high in both black and white patients (31% versus 38%) but HPV-active disease was less prevalent in black compared to white patients (0% versus 29%) [106]. Among the reasons fronted for the disparities were 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 [107].

Risky sexual behavior has been linked to oral HPV acquisition. These include early sexual debut, marital status, multiple sexual partners and prior sexual practice of the partner. Sexual practices and orientation are generally divergent across the globe and ethnic groups. A good example of this is a report of 78% of American men found to have engaged in oral sex compared to only 9% of Indian men [(107)]. Another study from America demonstrated that oral sexual behavior is the primary predictor of oral HPV infection and that differences in gender, age-cohort and race are responsible for the varied prevalence of oral HPV infection [(75)].

There exist other theories that explain variance in HPV-related disease distribution among populations. Some of these borrow heavily from other disease processes and have led to the postulation that different populations respond differently to given disease entities. One such explanation proposes that an immune response may be evoked by a genital HPV infection acquired before HPV exposure through oral sex and that this may decrease the risk of oral HPV and therefore HPV-related HNSCC [(112)]. 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* [(113)]. It is, therefore thought that perhaps, an equivalent immunological protection against HPV-related HNSCC by some yet to be determined factor may exist in certain geographical regions to explain the low prevalence of HPV in HNSCC in some populations. 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 [(114)].

4.3 Unique Features of HPV-Associated HNSCC

High risk HPV-positive tumours are thought to constitute a distinct epidemiological, biological and clinical subset of HNSCC. Patients with HR-HPV-associated tumours tend to have a distinct profile. Most authors seem to agree that patient characteristics associated with this group include younger age group (median of 57 years versus 64 years for HPV-negative tumours), higher socio-economic status, male gender, marijuana use, HIV infection, minimal or no tobacco and/or alcohol consumption. This group of patients have been shown to have a specific lifestyle characterised by a high lifetime number of both genital and oral sex partners and an early sexual debut [80,116-121]. In addition, HPV-positive tumours have been found to present at an early T stage and advanced nodal stage with cystic nodes and to be consistently poorly differentiated and non-keratinising with basaloid histology in contrast to HPV-negative HNSCC, which are more differentiated and keratinising [101-104]. Other observed features of HPV-positive HNSCC are lack of P53 mutations, better response to chemoradiation and a better prognosis [9, 119,122-128]. A few studies have found an association between HPV-positive HNSCC and increased alcohol and tobacco use, poorer survival in HPV-positive patients who are also smokers, increased distant metastases and female gender [8,119, 122]. The discrepancy has in some cases been attributed to small sample size and inability to consistently define and standardize smoking and alcohol intake status. Table 4 below shows the different characteristics between HPV-driven and HPV-negative head and neck cancers.

Table 4: "Comparison of HPV-Positive and HPV-Negative head and neck cancer" [108]

Characteristics	HPV-Positive	HPV-Negative
Age	Younger (<60 years)	Older (>60 years)
Sex	Male: female = 3:1	Male: female = 3:1
Risk factors	Sexual behaviour	Tobacco & Alcohol
Sites	Tonsil & Base of tongue	All UADT sites
Co-factors	Marijuana use	Poor oral hygiene
Status	Educated/ Higher SES	Less education / Poor SES
Disease stage	Early T & advanced N stage	Variable
Histology	Basaloid /poorly differentiated	Keratinized
P53	Wild type	Mutated
P16	Over-expressed	Low expression
Prognosis	Good	Poor

4.4 High Risk HPV and HIV in HNSCC

Nearly five percent of adult Kenyans have HIV [129]. The association between HIV and HNSCC is unclear with higher prevalence of HNSCC in HIV positive patients being reported. AIDS patients tend to suffer more from AIDS-defining tumours most of which are not SCC. With more HIV-positive patients using HAART, there is reported increase in non-AIDS defining cancers including HNSCC among HIV patients. This is attributed to mild to moderate immunosuppression among these patients on HAART, opportunistic infections, infection with high-risk HPV subtypes as well as higher cigarette use and other risky behavior among HIV patients [40-42]. Studies from renal

transplant patients have also shown that prolonged immunosuppression, even when only modest, is a risk factor for HPV-related carcinomas [130].

HNSCC is the third commonest HNC among HIV patients after Kaposi's sarcoma and non-Hodgkin's lymphoma [38, 39]. A study done in Kenya reported a prevalence of head and neck neoplastic lesions of 27% among HIV-infected patients distributed as 68% Kaposi's sarcoma, 17% SCC, 13% non-Hodgkin's lymphoma and 2% Burkitt's lymphoma [131]. In Uganda, HIV prevalence among head and neck patients excluding Kaposi's sarcoma, lymphomas and thyroid neoplasms was 15% against the national prevalence of 7.3% [132]. In the USA, HIV infection has been reported to increase the risk of HNSCC by approximately two to three times [133]. The presence of cervical intra-epithelial lesions in women has been shown to be proportional to the level of HIV-induced immunosuppression [134]. HIV-positive HNSCC patients tend to be young (mean age of 33 for females and 37 years for males) with advanced aggressive disease with resultant poor prognosis [132]. The head and neck regions at greatest risk for malignancy in HIV-positive patients are the larynx, oral cavity, oropharynx, lips, salivary glands and conjunctiva [135]. An increased risk for NPC in HIV positive patients has been reported with the highest risk being associated with the non-keratinizing histological type [136]. In Kenya, a prevalence of conjunctival SCC of 7.8% has been reported among HIV positive patients [137].

Few studies have been done on association of HPV and HIV+ HNSCC. A threefold risk of HPV infection in HIV infected patients, commonly with HPV 52, 51, 58, 35, 56, 53, 31 and 59 has been demonstrated. More often the infections with HPV tend to be multiple [138]. This, therefore, increases the risk of HR-HPV- related head and neck malignancies in HIV patients. In support of this observation, the incidence of HPV-positive oropharyngeal SCC in HIV patients in the United

States of America is reported to have risen from 0.0 cases per 100,000 person-years in the period from 1980-1989 to 3.9 and 6.5 cases per 100,000 person-years in the period 1990-1995 and 1996-2004 respectively [139].

McLemore found twenty four percent (6 out of 25) of HIV+ HNSCC patients to be positive for HPV with five being HPV16 and one HPV 26/29. Of note is the observation that despite 65% of the subjects having tumours of the larynx, 5 (83%) of the HPV positive ones were from the oropharynx [140]. This is in keeping with other authors who found no or minimal HPV in laryngeal tumours of HIV positive patients [141, 142]. A larger study involving six tertiary referral centres in USA found a prevalence of 30% HPV presence in HIV +VE HNSCCC with majority of these having oropharyngeal cancer [143]. Detection of HPV and EBV in the oral cavity of HIV-positive patients has also been noted to be higher than in HIV-negative patients [144].A study done on HPV types in Kenyan women with invasive cervical carcinoma by HIV status found more multiple-type infections in HIV positive women than in HIV negative women (37.2% versus 13.7%) but similar HPV type distribution. The only difference of borderline statistical significance was an excess of HPV 52 among HIV positive women (19.6% versus 5.2%) and low HPV 45(7.8% versus17.0%) [145].

4.5 High Risk HPV and EBV in Nasopharyngeal Carcinoma

Nasopharyngeal carcinoma (NPC) is a malignancy with unique geographical and ethnic distribution. It is the third commonest head and neck malignancy in Kenya [1]. There are three incidence levels of endemicity for NPC: low, intermediate and high endemic areas. Globally, NPC affects more males than females and is common in 6^{th} and 7^{th} decades. A bimodal age distribution is

seen in late childhood and 50 to 60 years in low risk areas. The first peak is thought to arise from a germ-line alteration while the second one might be related to lifestyle and environmental factors [146]. In the 1960s, Clifford published a lot on NPC in Kenya, noting that it accounted for 9% of all hospital admissions for malignancies. There was a male preponderance of 2.65: 1 with age peaks at 50-54 in males and 55-59 in females [147]. More recent studies done at KNH on NPC have, however, shown peaks at 31-40 and 41-50 years with a male: female ratio of 2.2:1 and 1:1 respectively [148, 149].

WHO has classified NPC into three categories based on histology: Type I is well to moderately differentiated carcinoma, type II is non-keratinizing carcinoma and type III is undifferentiated carcinoma. Some scholars have proposed grouping types II and III together on the basis of shared clinical features, strong association with EBV and lack of keratinization [150]. Low incidence areas tend to have more of the differentiated types in association with smoking [151]. Cigarette smoking is thought to act through its carcinogenic products including nitrosamines which cause DNA damage and via EBV reactivation [152]. It is estimated that nearly all NPC cases in endemic areas have EBV while the WHO type I associated with non-endemic areas is usually negative for EBV but positive for HPV. Association of EBV with the differentiated NPC types has, however, been reported in geographical regions with high incidence of undifferentiated NPC [151, 153].

The aetiology of NPC has for a long time been linked to an interaction between EBV and other viruses, genetic factors, diet and environmental factors. Some of these factors are thought to act as initiators while others are promoters of the carcinogenic process. According to Clifford, some of these factors in the Kenyan community were thought to include EBV, high altitude, polynuclear

aromatic hydrocarbons found in household smoke, and genetic factors like the "ABO" blood groups [147]. These presuppositions were based on the observation that most NPC patients in Kenya were from the cold highland areas where people lived in poorly ventilated huts where cooking and heating was done using wood fire. This reasoning has, however, been disputed on account that NPC has a male preponderance despite the fact that females do most of the cooking and have more exposure to household smoke. A study from Southern China among the boat people has also failed to support an inhaled carcinogen as a risk factor for NPC [154]. Available evidence supports EBV, genetics and salted fish as the main risk factors for NPC [155]. Lately, more viruses including HPV and cytomegalovirus have been associated with NPC, particularly HPV [156]. In the only high-volume study in which 956 patients with nasopharyngeal carcinoma were analyzed, 36% were HPV positive. The HPV-positive patients, like HPV-positive oropharyngeal patients, were younger, less likely to be uninsured and lived in more up-market places. They, however, presented with a more advanced T stage but not overall TNM stage. Their HPV status did not correlate with or predict the overall survival [156].

The association of HPV and oropharyngeal squamous cell carcinoma is an established fact [4]. The proximity and similarity of the oropharyngeal and nasopharyngeal sites have led to several hypotheses to explain the association of HPV with NPC. These include tropism to the pharyngeal tissue by HPV and local extension of oropharyngeal infection by HPV to the nasopharynx [18, 136]. The presence of both EBV and HPV in nasopharyngeal cancer cells has been reported but their interactive role is still unclear. More often, the EBV negative NPC tumours are the ones that tend to test positive for HPV. A number of EBV-positive NPC specimens have tested positive for HPV while others have demonstrated HPV positivity only in EBV-negative tumors of the nasopharynx

[52,100, 152, 153]. A study from Greece during the mid 1990s employed PCR techniques to determine the presence of EBV and HPV among 63 FFPE nasopharyngeal tissues. Thirty-two and nineteen percent of the specimens tested positive for EBV and HPV respectively with no co-infection. This led to the conclusion that HPV might be involved in the pathogenesis of EBV-negative SCC tumours of the nasopharynx [157].

Studies comparing endemic and non-endemic area findings seem to associate EBV positivity with endemic status and HPV positivity with non-endemic status. In America, a cohort study comparing EBV and HPV presence in endemic (Southern China) and non-endemic (USA) cohorts with NPC showed no HPV among the Southern China cases or Chinese American patients. All except 3 cases from the endemic cohort were EBV positive. All EBV negative cases were white Americans who also had high risk HPV and smoking association. There was no co-infection with both viruses [153]. This is similar to another study where HPV was detected in non-endemic (Danish) cohort but not in the endemic (Inuit) cohort [159]. A low incidence area in America also posted similar results where HPV positivity was reported in four of five subjects all of whom were EBV-negative and white Americans. The only EBV positive patient in the said study was HPV-negative, EBV positive and Korean [158].

Towards the end of the last century, co-existence of EBV and HPV was reported in 42% of 88 fresh NPC specimens from Taiwan by PCR. There were 51% HPV positive specimens of which 67% were high risk [160]. Punwaney also reported co-infection with EBV and HPV in an endemic cohort [161]. In Morocco, which is an NPC endemic area, all NPC samples were positive for EBV with 34% having HPV as well. Majority of the HPV genotypes were 31 with a few 16, 18, 33, 35 and 45 [100].

Ghana is a low incidence country with regard to NPC, and had EBV positivity of 25% and HPV positivity of 19.23% with HPV types 18 and 31. There was co-infection among 4.2% cases [86].

It is notable that HPV presence alone in HNSCC tumours in African countries has remained low. Literature has likened the EBV-HPV interaction in NPC to what has been observed between EBV and malaria within the Burkitt lymphoma zone. In the latter case, it has been demonstrated that P. falciparum acts at two points: immunosuppression with resultant high throughput of EBV-infected cells in the germinal centers, and deregulation of an enzyme responsible for alteration of immunoglobulin genes in B cells when they enter the germinal center. The result is DNA damage, translocations and lymphoma development. [162].

CHAPTER 3: STUDY METHODOLOGY

3.1 Study Design

This was a descriptive cross-sectional study.

3.2 Setting

The otolaryngologists as well as maxillo-facial surgeons at KNH manage HNSCC patients. These patients are therefore channeled to the ENT-HN and Maxillofacial clinics for work-up and management. Depending on the site of the tumour and the patient general status most of the patients complete their haematological and radiological work-up including taking of the biopsy in the clinics with only a few requiring admissions to the wards for biopsy under general anaesthesia. For this study the biopsy specimens were taken in either the ENT or maxillo-facial clinics and hospital theatres. HIV immunoassay was done at the KNH immunology laboratory as part of the standard work-up for all cancer patients in the unit. The biopsy specimens were processed, and histology reported in the KNH pathology laboratory. The HPV and EBV testing were done at the Kenya Aids Vaccine Initiative laboratory.

3.3 Study Population

This consisted of patients who presented with tumours of the head and neck defined as mucosal cancer of the oral cavity, oropharynx, nasopharynx, sinonasal, hypopharynx and larynx during the study period. Two hundred and twelve patients were screened out of whom 160 with confirmed squamous cell carcinoma were enrolled for the study.

3.4 Inclusion Criteria

1. Patients who presented with squamous cell carcinoma of the head and neck confirmed by histology
2. Patients who gave informed assent and/or consent to participate in the study

3.5 Exclusion Criteria

1. Patients with tumours of the head and neck other than squamous cell carcinoma
2. Patients with no histology report
3. Patients who were too sick to consent

3.6 Sample Size

The sample size (N) was calculated using **Fischer's** formula:

$$N = \frac{z_{\propto}{}^2\, p(1-P)}{d^2}$$

$\mathbf{Z_\alpha}$ is the standardized normal deviate corresponding to a significant level $\boldsymbol{\alpha}$

The level of significance $\boldsymbol{\alpha}$ was taken as 0.05 giving **Z** = 1.96

P was the assumed proportion of HPV prevalence in HNSCC = 50%

d was the precision of the estimated values in the study and was estimated at ±0.05.

$$n0 = \frac{1.96^2 \times 0.5\,(1 - 0.5)}{0.05^2}$$

n0 = 384

Finite correction to the formula gave Nf, the corrected sample to be:

$$Nf = \frac{n0}{1 + \frac{(n0 + 1)}{N}}$$

Where N is the expected population with HNSCC during the study period estimated as 260

Nf = 155

3.7 Procedure

3.7.1 Subject Recruitment and Management

Patients with suspected head and neck squamous cell carcinoma that assented and/or consented to participate in the study were recruited from the ENT-HN and Maxillofacial clinics and wards. 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 as per Appendix II. These included the patient's age, sex, current residence, county of birth and county of residence for most of the previous ten years. Information concerning their symptoms, smoking, alcohol and other substance use history; exposure to irritants or radiation; history in keeping with gastro-oesophageal or laryngopharyngeal reflux; and sexual orientation and practices was also sought. A complete ENT-HN examination was done. Standard haematological tests for all HNC patients, which comprised of full haemogram, renal and liver function tests as well as HIV immunoassay were ordered (the latter for 53 patients whose HIV status was unknown). One hundred and seven patients who either had an HIV test report done before presentation or were on HIV treatment were not required to repeat the HIV test. 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 site 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 histopathological analysis. The other sample was placed in a specimen bottle and frozen at -80°C. Two hundred and twelve specimens were collected and frozen from which one hundred and sixty histologically confirmed HNSCC specimens were selected for DNA extraction.

3.7.2 DNA Extraction

Approximately 25 mg of each of the frozen tissue was cut into small pieces and placed in a 1.5 ml micro-centrifuge tube. One hundred microlitres of Buffer ATL were 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. Two hundred microlitres 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. Five hundred microlitres 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 help eliminate the chance of possible Buffer AW2 carryover. The QIAamp Mini spin column was put in a clean 1.5 ml centrifuge tube and the collection tube discarded. Two hundred microlitres 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 [163].

3.7.3 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 high risk genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) of human papillomavirus" [(163)]. Reaction mix was made using Hot Start DNA Polymerase, PCR-buffer-FRT and PCR mix-1 as per manufacturer's instructions for the sample to be tested. Four tubes for each clinical sample, four tubes for K2 standards and four tubes for negative control were set up as in figure 2 below (the strip for K1was replaced with a clinical sample since K1 is only required for quantitative analysis and this study was a qualitative one). Ten microlitres of **extracted DNA** sample was added to the tubes in No. 1 to 15 as well as 17. For each panel, 10 µl of controls and standards were prepared and added to strip No. 16 and 18 respectively as shown figure 2 below [(163)]. The 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" [(163)].

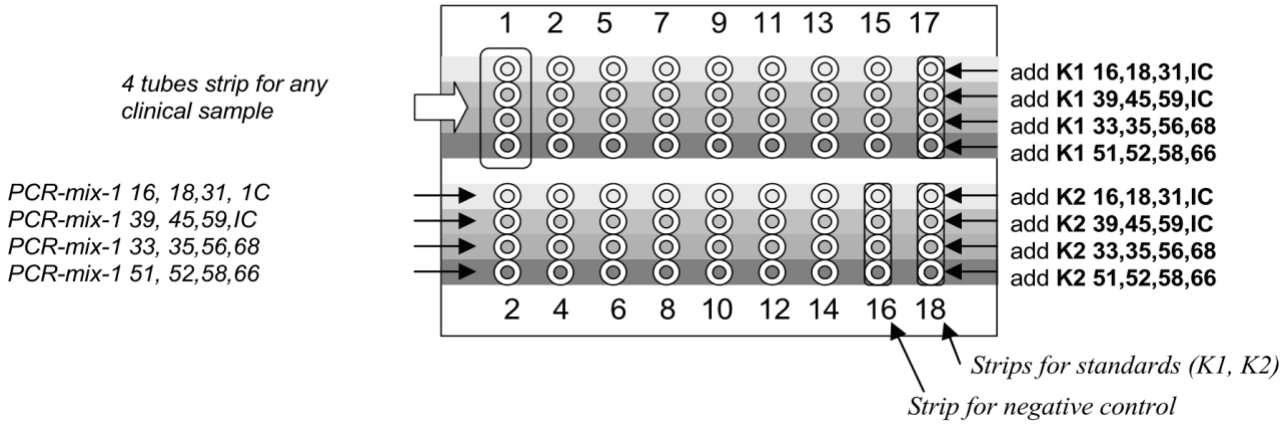


Figure 4: Sample and Reagent distribution for Real Time PCR (Adapted from "HPV Genotypes 14 Real-TM Quant HANDBOOK" by Sacace Biotechnologies) [(163)]

3.7.4 EBV PCR

The sixty-two DNA samples extracted from nasopharyngeal carcinoma tissue were subjected to EBV real time PCR. "The Reaction mix was made by mixing Hot Start Polymerase, PCR mix-1 and PCR mix-2 in the ratios provided in the manufacturer's manual. Sixty-six test tubes were prepared for the procedure. 15 µl of the Reaction mix was put in each of the 64 tubes. 10 µl of extracted DNA sample was added to 62 of the test tubes with the reaction mix. Internal control DNA was added to the 63rd test tube with the reaction mix and the negative control sample added to the 64th test tube with reaction mix. QS1 and QS2 standards were added to the 65th and 66th test tubes respectively. The tubes were closed and loaded into the Real Time Thermal cycler (Rotor Gene Q, QIAGEN). The machine was programmed as per the kit's PCR cycling conditions for EBV. PCR was performed and the results interpreted using the Rotor Gene Q software" [163].

3.7.5 HIV Immunoassay

This was done for 53 patients whose HIV status was unknown at the time of study. Their blood was drawn and processed in the immunology laboratory for ELISA using the Architect Plus i1000 SR machine. Serum from the blood sample was put in a test tube containing HIV antigen and fed into an automated machine where the resultant complex was conjugated, and a substrate added. In the presence of HIV antibodies in the serum florescence occurred which the machine read and displayed as a positive result.

3.8 Quality Control

Beta globulin gene amplification in the extracted DNA was demonstrated. Beta globulin is an indicator of the quality of the sample collected. A positive beta globulin test and a negative HPV

test indicates the sample had no HPV infection and that the negative HPV result was not due to non-amplification. Beta globulin amplification was therefore used to confirm viability of the extracted DNA. For the HIV ELISA, quality control calibration was done daily, and controls were run, with each batch of specimens.

3.9 Study Duration

The study was done from January 2015 to December 2018.

3.10 Data Management

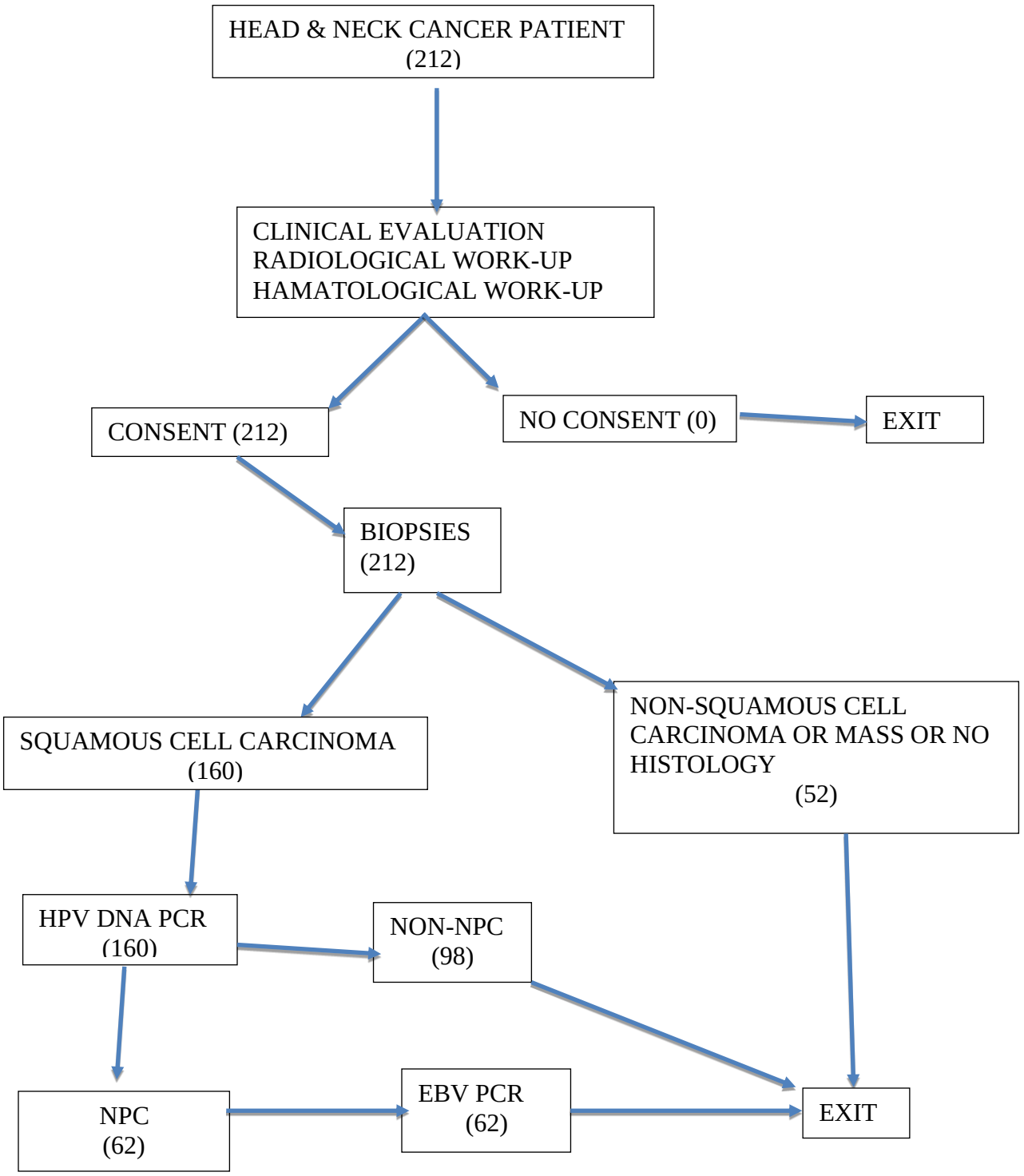
All data on patient demographics, risk factors, examination and investigation findings were collected using a questionnaire (Appendix II). It was then entered into and analyzed using SPSS version 21.0. The data was cleaned of errors, inconsistent or conflicting answers, as well as missing or duplicate entries. Descriptive statistics on socio-demographic data was used to characterize the study subjects. Summary statistics such as means, median and standard deviations were used to describe the distribution of the continuous variables. Pearson Chi square was applied to categorical data to test for association between independent and dependent variables. Fisher's exact test was employed when cell numbers were small. A p value less than 0.05 was considered significant

3.12 Ethical Considerations

1. Permission to undertake this study was sought from Kenyatta National Hospital-University of Nairobi Research and Ethics Committee. A letter of protocol approval (P402/07/2014) was obtained prior to the commencement of the study and was renewed as per Appendix III.

2. Informed consent, and assent where applicable, was obtained from the patients, parents or guardians after explaining to them the objective of the study and their role in the study and implications thereof. The consent described the purpose of the study and the procedure to be followed. The investigator conducted the consent discussion and checked that the patient/parent/guardian comprehended the information provided and answered any question about the study. Consent was voluntary and free from coercion.
3. No experimental treatments were employed in this study. Medical procedures were carried out in accordance with the KNH protocols for head and neck cancer patients.
4. Patients did not incur extra costs as a result of participating in the study. Standard tests for HNC patients that included haematological, radiological and histological services were charged by KNH as for any other hospital HNC patients. Costs for DNA extraction and genotyping were borne by the principal researcher.
5. The study participants who had any other significant findings had these communicated to them as well as ENT-HN or Maxillofacial team for the appropriate adjustment of their management.
6. Patient confidentiality was strictly held in trust by the investigator. The study protocol, documentation, data and all other information generated were held in strict confidence. No information concerning the study, or the data was released to any unauthorized third party. All evaluation forms, reports and other records that left the site were identified only by the Subject Identification Number (SIN) to maintain subject confidentiality. All raw data will, at the conclusion of the study, be destroyed. Study results will be availed to the medical fraternity through presentation in scientific conferences and publication in medical journal

Flow Chart for the Study



CHAPTER 4: RESULTS

4.1 Age and Gender Distribution

A total of one hundred and sixty patients with HNSCC, confirmed by histology, aged 16 to 87 years were enrolled in the study. The median age was 54.0 year. 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 oral cavity, oropharynx, nasopharynx, hypopharynx, larynx and 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%).

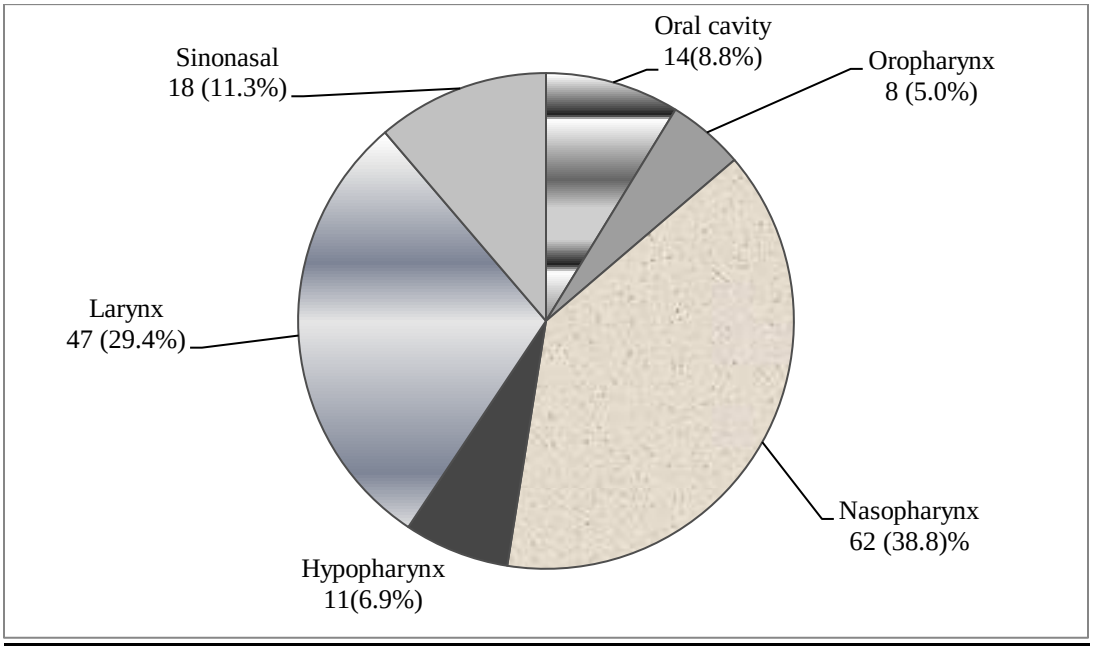


Figure 5: Distribution of tumours by site

The age and sex distribution of the patients among the various tumour sites are illustrated in Figures 6 and 7 below. Nasopharyngeal cancer had both young and old patients with a single peak in the 6th decade. Laryngeal cancer patients fell within the 4th to 9th decades with a peak in the 7th decade.

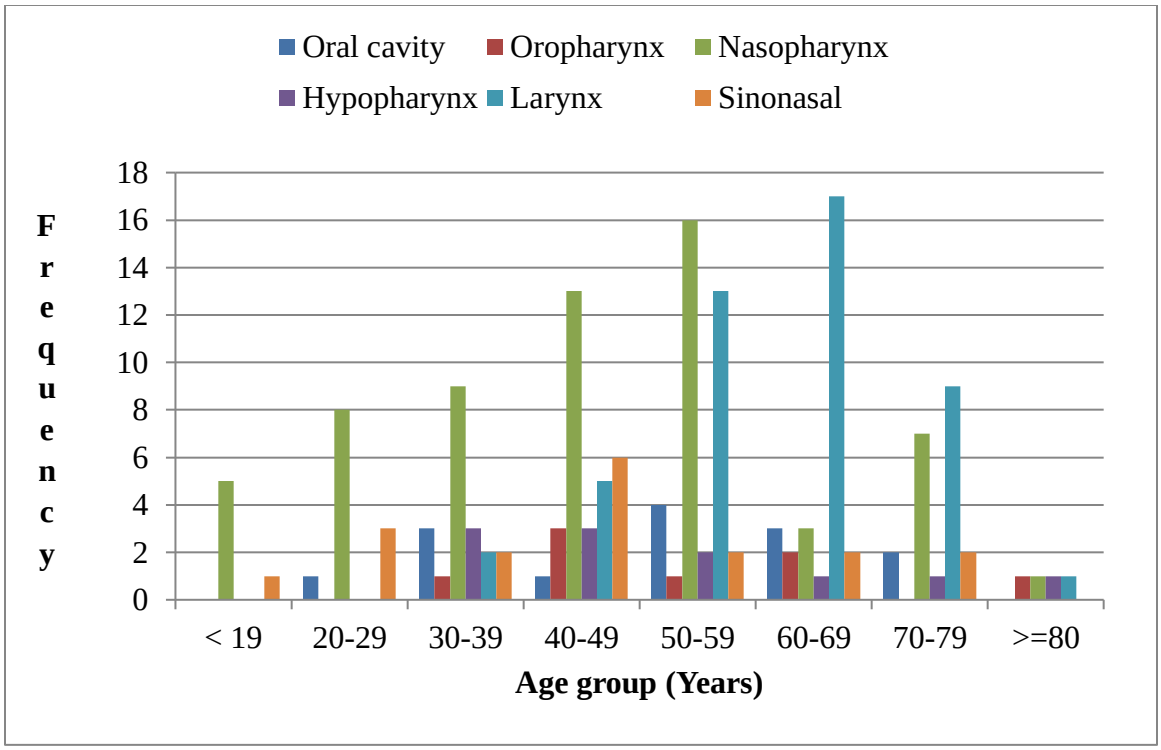


Figure 6: Age distribution by tumour site.

The male to female ratio for all the study subjects was approximately 3:1. All tumours had more males than females except for oral cavity carcinoma where the ratio was 1:1. Carcinoma of the larynx had the largest ratio of about 23:1.

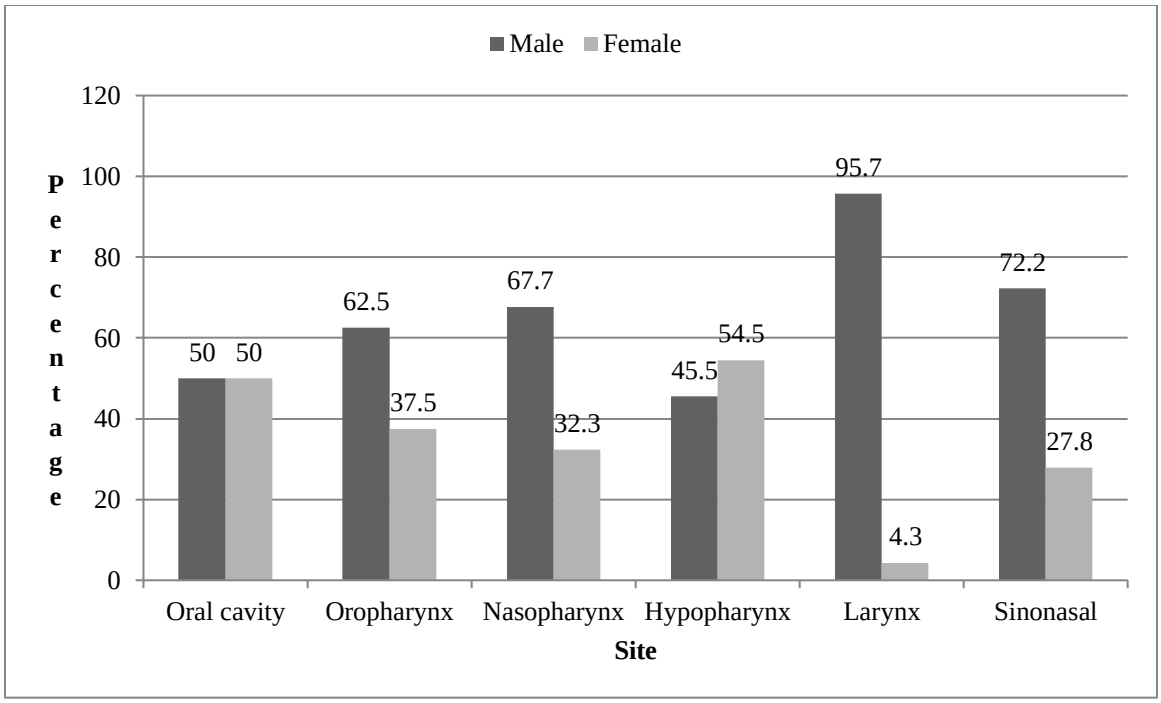
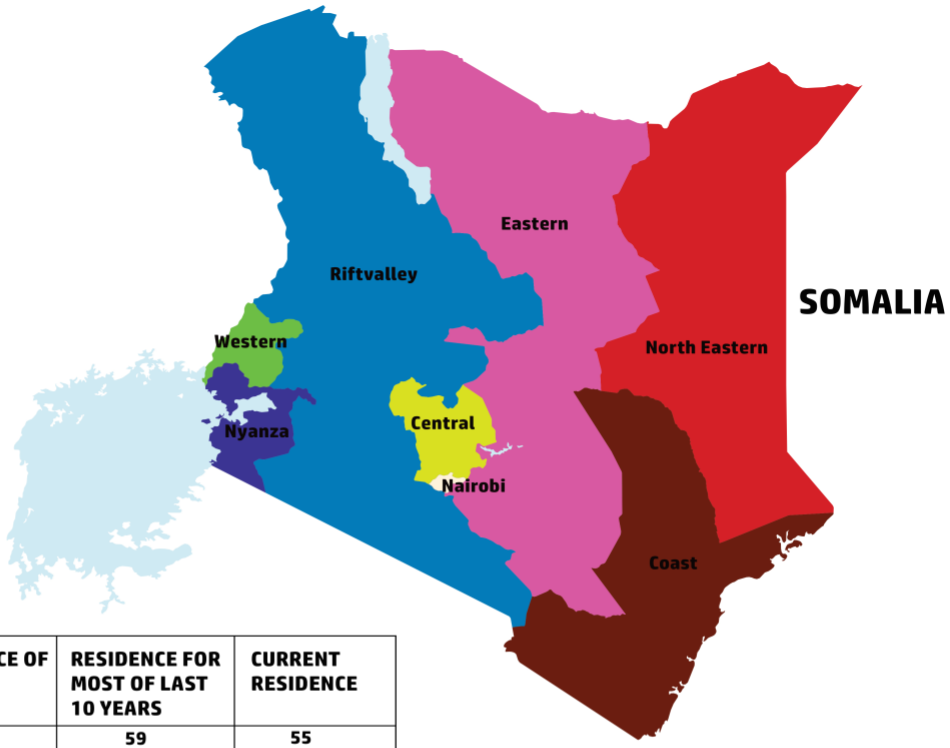


Figure 7: Gender distribution by tumour sites

4.2 Geographical Distribution

Kenya is divided into eight main geographical blocks previously referred to as provinces. The distribution of patients with regard to their province of birth, current residency and the province where they have lived for most of the previous ten years is summarized in Figure 5 below. It is important to note that majority of the patients came from within the study location (Nairobi) or from provinces neighbouring the study location. One patient came from Somalia.



KEY

PROVINCE	PROVINCE OF BIRTH	RESIDENCE FOR MOST OF LAST 10 YEARS	CURRENT RESIDENCE
CENTRAL	56	59	55
EASTERN	40	38	40
NAIROBI	31	25	31
RIFT VALLEY	11	13	10
NYANZA	11	12	11
WESTERN	6	7	7
COAST	2	3	3
NORTH EASTERN	2	2	2
SOMALIA	1	1	1
TOTAL	160	160	160

Figure 8: Distribution of Patients with regard to province of birth and residence

4.3 Referring Health Facility

Kenyatta National Hospital is the main referral Hospital with an established cancer treatment centre in Kenya. The hospital handles patients who are referred from peripheral health facilities as well as self-referrals. The levels of service delivery in Kenya from the least to the highest are the

community facilities at Level I, dispensaries at level II, health centers at level III, primary referral hospitals at level IV, secondary referral hospitals at level V and the tertiary referral hospitals at level VI. The ideal situation is for patients to be progressively referred from the lowest level of health care to the tertiary institution. A significant fraction (40%) of patients was referred to KNH from level V. Many more bypassed the middle level units to KNH with 12.5% coming as self-referrals. Mission (faith-based hospitals) and private facilities together contributed 26.9% of the patients. There were a few patients (2.5%) who were referred from within other Departments of the hospital. Table 5 shows the source of the patients.

Table 5: Referring Health Facility

Referring institution	**Frequency**	**Percent**
Internal	4	2.5
Level II	3	1.9
Level IV	26	16.3
Level V	64	40
Mission	21	13.1
Private facility	22	13.8
Self-referrals	20	12.5
Total	160	100

4.4 Clinical Presentation

Patients presented with different combinations of symptoms dictated by the primary site of the tumour as illustrated in table 6 below. At least 30% of the patients presented with nasal obstruction, hoarseness, neck swelling and difficulties in breathing.

Table 6: Presenting Symptoms

Symptom	Site						Total
	Oral cavity	Oropharynx	Nasopharynx	Hypopharynx	Larynx	Sinonasal	
Hoarseness	1 (7.1%)	1 (12.5%)	8 (12.9%)	5 (45.5%)	47 (100.0%)	1 (5.6%)	63 (39.4%)
Cough	1 (7.1%)	2 (25.0%)	2 (3.3%)	4 (36.4%)	15 (31.9%)	2 (11.1%)	26 (16.4%)
Dysphagia	2 (14.3%)	4 (50.0%)	10 (16.1%)	10 (90.9%)	14 (29.8%)	1 (5.6%)	41 (25.6%)
Neck pain	2 (14.3%)	3 (37.5%)	11 (17.7%)	3 (27.3%)	3 (6.4%)	0 (.0%)	22 (13.8%)
Ear pain	0 (.0%)	4 (50.0%)	11 (17.7%)	2 (18.2%)	3 (6.4%)	1 (5.6%)	21 (13.1%)
Breathing difficulties	0 (.0%)	3 (37.5%)	4 (6.6%)	3 (27.3%)	35 (74.5%)	3 (16.7%)	48 (30.2%)
Neck Swelling	1 (7.1%)	5 (62.5%)	35 (56.5%)	5 (45.5%)	6 (12.8%)	2 (11.1%)	54 (33.8%)
Nasal blockage	0 (.0%)	4 (50.0%)	47 (75.8%)	0 (.0%)	0 (.0%)	17 (94.4%)	68 (42.5%)
Epistaxis	0 (.0%)	2 (25.0%)	30 (48.4%)	0 (.0%)	0 (.0%)	10 (55.6%)	42 (26.3%)
Hearing loss	0 (.0%)	0 (.0%)	36 (58.1%)	0 (.0%)	0 (.0%)	2 (11.1%)	38 (23.8%)
Other symptoms	0 (.0%)	2 (25.0%)	26 (41.9%)	2 (18.2%)	2 (4.3%)	11 (61.1%)	43 (26.9%)

4.5 Exposure to Risk Factors for HNSCC

The exposure of patients to risk factors for HNSCC either in the past or current is summarized in table 7. Some patients were exposed to more than one risk factor. Cigarette smoking and drinking beer 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 initial diagnosis. 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 7: Exposure to Risk Factors

Risk factor	Frequency	Percent
Cigarette smoking	79	49.4
Tobacco chewing/sniffing	3	1.9
Khat chewing	11	6.9
Marijuana use	3	1.9
Alcohol • Beer • Spirits • Wine • *Chang'aa* • Undistilled local brews	 60 17 7 15 37	 37.7 10.7 4.4 9.4 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

4.5.1 Tobacco Use

Nearly half (51.3%) of the patients had a history of tobacco use with 49.4% having smoked cigarettes. Majority of the smokers had less than 30 pack years of smoking (Table 8). Smokers who could not give an estimate of their cigarette use were classified as indeterminate. Three patients either chewed or sniffed tobacco.

Table 8: Tobacco Use among Patients

	Smoking				
	Non-smoker	Current smoker	Ex-smoker	Current tobacco chewer/sniffer	Total
Non-smoker	78 (100%)	0 (0%)	0 (0%)	0 (0%)	78 (48.8%)
< 30 pack years	0 (0%)	37 (75.5%)	21 (70.0%)	0 (0%)	58 (36.3%)
30-39 pack years	0 (0%)	6 (12.2%)	2 (6.7%)	0 (0%)	8 (5.0%)
>=60 years	0 (0%)	2 (4.1%)	0 (0%)	0 (0%)	2 (1.3%)
Indeterminate	0 (0%)	4 (8.2%)	7 (23.3%)	3 (100%)	14 (8.8%)
Total	78 (100%)	49 (100%)	30 (100%)	3 (100%)	160 (100%)

Table 9 shows the amount of smoking among patients with relation to the type of tumour. The oral cavity and nasopharyngeal tumour patients had the least number of tobacco users (21.4% and 29% respectively) with majority of laryngeal cancer patients having the largest percentage of smokers (87.2%). Only (30) 37.0% of the tobacco users had quit smoking by the time of onset of their symptoms. Most tobacco users were light smokers, a big proportion of whom had tumours of the larynx and oropharynx.

Table 9: Tobacco Use and Tumour Site

	Site						
Pack Years	Oral cavity	Oropharynx	Nasopharynx	Hypopharynx	Larynx	Sinonasal	Total
Non-smoker	11 (78.6%)	3 (37.5%)	44 (71.0%)	6 (54.5%)	6 (12.8%)	8 (44.4%)	78 (48.8%)
< 30	0 (0%)	5 (62.5%)	14 (22.6%)	3 (27.3%)	31 (66.0%)	5 (27.8%)	58 (36.3%)
30-39	0 (0%)	0 (0%)	2 (3.2%)	1 (9.1%)	4 (8.5%)	1 (5.6%)	8 (5.0%)
>=60	1 (7.1%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5.6%)	2 (1.3%)
Indeterminate	2 (14.3%)	0 (0%)	2 (3.2%)	1 (9.1%)	6 (12.8%)	3 (16.7%)	14 (8.8%)
Total	14 (100%)	8 (100%)	62 (100%)	11 (100%)	47 (100%)	18 (100%0	160 (100%)

4.5.2 Alcohol use

Table 7 above shows the number of patients who took alcohol with beer and unprocessed brews having most users. The patients were grouped into light and heavy drinkers based on the estimated number of drinks per day or week. The National Institute on Alcohol Abuse and Alcoholism (NIAAA) guidelines were used to estimate the class of drinking among the patients (Appendix IV). The guidelines assign the group of alcohol use based on the number of drinks per day. It is important to note that in this definition one alcoholic drink-equivalent contains 14g of pure alcohol. It was not possible to retrospectively determine the drink-equivalent of the consumed alcohol in this study and therefore the drinks are an estimate. Some patients had quit alcohol use prior to onset of disease symptoms and were therefore considered past alcohol drinkers. A significant fraction of both current and past alcohol users was unable to estimate the quantity of alcohol they consumed and were classified as the indeterminate drinkers. The results are presented in table 10 below.

Table 10: Alcohol use among Study Subjects

Alcohol use	Frequency	Percent
CURRENT		
Non-drinker	77	48.1
Current Heavy drinker	12	7.5
Current light drinker	10	6.3
Indeterminate current drinker	20	12.5
PAST		
Past heavy drinker	6	3.8
Past light drinker	16	10
Indeterminate past drinker	19	11.9
Total	160	100

Nearly half (51.9%) of the study patients had a history of alcohol consumption half of whom had quit drinking before developing the cancer symptoms. Majority of alcohol users were either indeterminate or light drinkers. As with tobacco use, more alcohol consumers had laryngeal and oropharyngeal tumours as shown in Table 11 below.

Table 11: Alcohol use in relation to tumour site

	Site						
	Oral cavity	**Oropharynx**	**Nasopharynx**	**Hypopharynx**	**Larynx**	**Sinonasal**	**Total**
Non-drinker	8 (57.1%)	2(25.0%)	43 (69.4%)	7 (63.6%)	7 (14.9%)	10 (55.6%)	77(48.1%)
Current Heavy drinker	1 (7.1%)	0 (0%)	2 (3.2%)	3 (27.3%)	4(8.5%)	2 (11.1%)	12 (7.5%)
Current light drinker	0 (0%)	1 (12.5%)	3 (4.8%)	0 (0%)	3 (6.4%)	3 (16.7%)	10 (6.3%)
Undetermined current drinker	0 (0%)	2 (25.0%)	4 (6.5%)	0 (.0%)	13 (27.7%)	1 (5.6%)	20 (12.5%)
Past heavy drinker	1 (7.1%)	0 (0%)	2 (3.2%)	0 (0%)	2 (4.3%)	1 (5.6%)	6 (3.8%)
Past light drinker	4 (28.6%)	2 (25.0%)	2 (3.2%)	0 (0%)	8 (17.0%)	0 (0%)	16 (10.0%)
Undetermined past drinker	0 (0%)	1 (12.5%)	6 (9.7%)	1 (9.1%)	10 (21.3%)	1 (5.6%)	19 (11.9%)
Total	14 (100%)	8 (100%)	62 (100%)	11(100%)	47 (100%)	18 (100%)	160 (100%)

4.5.3 *Khat* Chewing

Eleven (6.9%) of the 160 patients had used *khat* for variable durations ranging from one to 20 years. The profiles of the *khat* users are summarized in table 12 below.

Table 12: Profiles of *khat* chewers

Characteristic	Frequency	Percent
▪ Age		
▪ ≤ 60 years	9	81.8
▪ >60 years	2	18.2
▪ Gender:		
▪ Male	11	100
▪ Female	0	0
▪ Alcohol use		
▪ Yes	8	72.7
▪ No	3	27.3
Cigarette smoking		
▪ Yes	9	81.8
▪ No	2	18.2
Marijuana use:		
▪ Yes	0	0
▪ No	11	100
Residence:		
▪ Nairobi	3	27.3
▪ Eastern	6	54.5
▪ Northeastern	1	9.1
▪ Central	1	9.1
Tumour site		
▪ Oral cavity	2 of 14	14.3
▪ Nasopharynx	4 of 62	6.5
▪ Larynx	2 of 47	4.3
▪ Sinonasal	1 of 18	5.6
▪ Oropharynx	1 of 8	12.5
▪ Hypopharynx	1 of 11	9.1
Histology		
▪ Grade I	2	18.2
▪ Grade II	4	36.4
▪ Grade III	0	0
▪ Grade IV	5	45.4
HIV ELISA		
▪ Positive	2	18.2
▪ Negative	9	81.8
HPV Status		
▪ Positive (HPV 56)	2	18.2
▪ Negative	9	81.8

Majority of Khat users also had smoking (81.8%) and alcohol (72.7%) use; both of which are risk factors for HNSCC.

4.5.4 Sexual Characteristics of Patients

Table 13: Age of patient versus sexual activity

	Sexually active		
Age	**No**	**Yes**	**Total**
<=19	6 (100%)	0 (0%)	6 (100%)
20-29	2 (16.7%)	10 (83.3%)	12 (100%)
30-39	0 (0%)	20 (100%)	20 (100%)
40-49	0 (0%)	31 (100%)	31 (100%)
50-59	0 (0%)	38 (100%)	38 (100%)
60-69	0 (0%)	28 (100%)	28 (100%)
70-79	1 (4.8%)	20 (95.2%)	21 (100%)
>=80	0 (0%)	4 (100%)	4 (100%)
Total	9 (5.6%)	151(94.4%)	160 (100%)

One hundred and fifty-one patients were sexually active. Eight of the 9 who were not sexually active were young patients aged less than 30 years (table 13). None of the patients was homosexual or bisexual. There was, however, only 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 [(164)]. Patients who had at least four lifetime sexual partners were classified as having had multiple sexual partners [(165)]. 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.

4.6 Tumor Staging

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 as per Appendix V. The group staging is shown in table 14 below. Only 12.6% of the patients had early tumours (stage 1 and 2). Four (2.5%) patients had recurrent tumours.

Table 14: Tumour Staging

	Site						
	Oral cavity	**Oropharynx**	**Nasopharynx**	**Hypopharynx**	**Larynx**	**Sinonasal**	**Total**
Stage 1	2 (14.3%)	0 (0.0%)	2 (3.2%)	0 (0.0%)	5 (10.6%)	1 (5.6%)	10 (6.3%)
Stage 2	0 (0.0%)	1 (12.5%)	4 (6.5%)	0 (0.0%)	3 (6.4%)	2 (11.1%)	10 (6.3%)
Stage 3	2(14.3%)	0 (0.0%)	9 (14.5%)	1 (9.1%)	17 (36.2%)	1 (5.6%)	30 (18.8%)
Stage 4A	9 (64.3%)	5 (62.5%)	30 (48.4%)	8 (72.7%)	20 (42.6%)	14 (77.8%)	86 (53.8%)
Stage 4B	0 (0.0%)	2 (25.0%)	14 (22.6%)	2 (18.2%)	2 (4.3%)	0 (0.0%)	20 (12.5%)
Recurrence	1 (7.1%)	0 (0.0%)	3 (4.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (2.5%)
Total	14 (100%)	8 (100 %)	62 (100%)	11 (100%)	47 (100%)	18 (100%)	160 (100%)

4.7 Histological Grading

All tumours were assigned the WHO histological grading I to IV (Appendix VI). The results are presented in figure 9 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 NPC WHO types as per Appendix VII. Type I NPC were 6.4%, type II were17.7% and type III 75.8%.

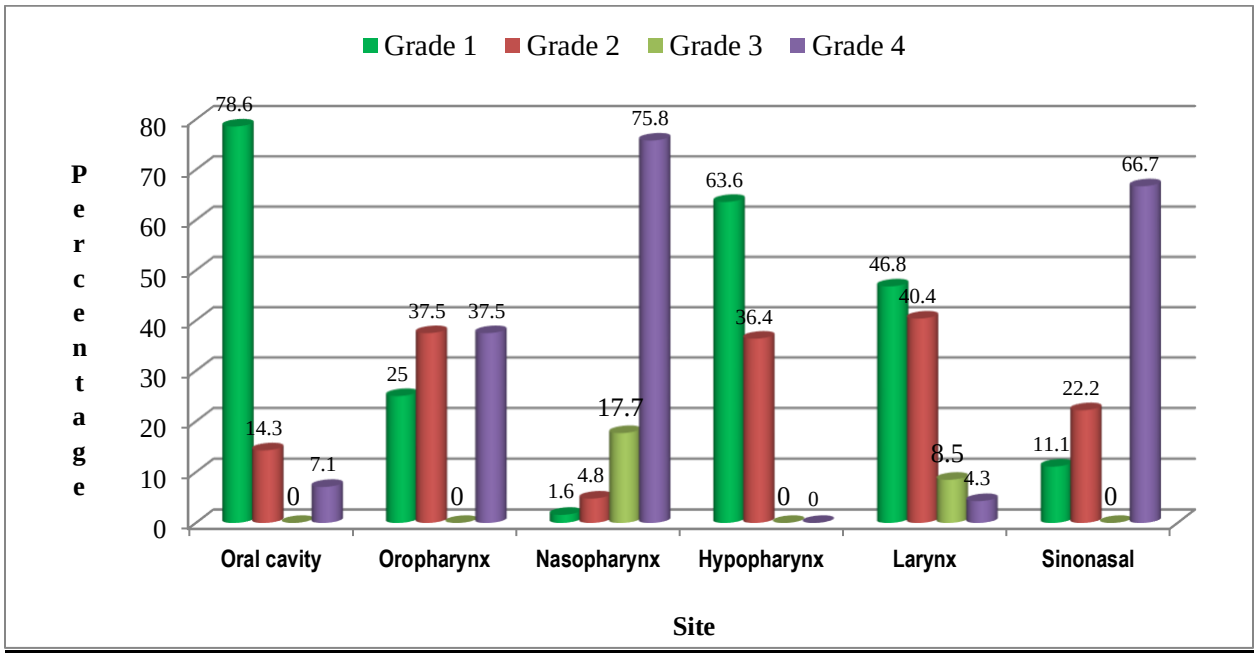


Figure 9: Histological Grading of Tumours

4.8 HIV Status

Ten (6.3%) of the patients were positive for HIV. The distribution of patients by HIV status is shown in figure 10 below. These consisted of 4 females and 6 males aged 23 to 57 with median age of 42.5 years. All the males had a history of smoking and alcohol use. The HIV positive patients had nasopharyngeal (6), oropharyngeal (2), hypopharyngeal (1) and laryngeal (1) carcinoma. Two (20%) of the HIV positive patients tested positive for HPV. Both had nasopharyngeal carcinoma and HPV genotype 56.

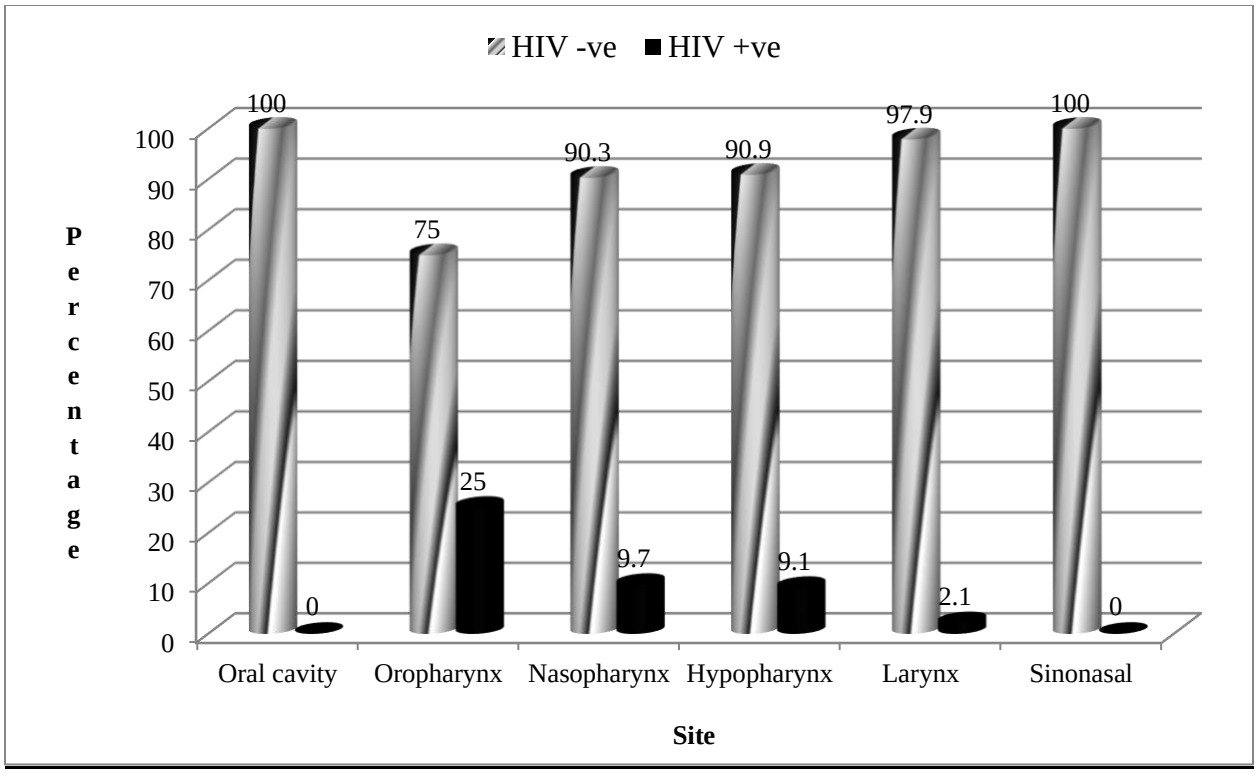


Figure 10: HIV Status among patients

4.9 HPV Status

Twelve (7.5%) of the study patients tested positive for HPV by real time PCR. The only high-risk HPV genotypes detected were 56 (10 patients) 52 and 33 (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 tables 15 and 16 below.

Table15: HPV Status among patients

	PCR		
	Positive	**Negative**	**Total**
Oral cavity	2 (14.3%)	12(85.7%)	14(100.0%)
Oropharynx	0 (0%)	8 (100.0%)	8 (100.0) %
Nasopharynx	7 (11.3%)	55 (88.7%)	62 (100.0) %
Hypopharynx	0 (0%)	11 (100.0%)	11 (100.0) %
Larynx	1 (2.1%)	46 (97.9%)	47 (100.0) %
Sinonasal	2 (11.1%)	16 (88.9%)	18 (100.0) %
Total	12 (7.5%)	148 (92.5%)	160 (100.0) %

There was an equal number of males and females among the HPV positive patients with 50% being less than 60 years of age (median age of 51.0 years). Alcohol and cigarette users in the group were few with no marijuana user. None had early sexual debut and only 25% had multiple sexual partners. Cystic lymph nodes were only found in five patients in the entire study none of whom tested positive for either HIV or HPV. Three of these patients with cystic lymph nodes had nasopharyngeal carcinoma, one sinonasal and one oral cavity carcinoma. More than half of the HPV positive patients had nasopharyngeal carcinoma with a similar number having undifferentiated histological types. Only 2 of the patients were HIV positive. Ten patients had HPV type 56 with one each of type 33 and 52.

Table 16: The profile of HPV Positive Patients(n=12)

Characteristic	Frequency	Percent
Age		
• ≤ 60 years	6	50
• >60 years	6	50
Gender:		
• Male	6	50
• Female	6	50
Alcohol use		
• Non-drinker	8	66.7
▪ Current drinker	2	16.7
▪ Past drinker	2	16.7
Cigarette smoking		
▪ Yes	2	16.7
▪ No	10	83.3
Marijuana use No	12	100
Age at sexual debut:		
▪ Non-applicable	2	16.7
▪ Indeterminate	1	8.3
▪ Early	0	0
▪ Late	9	75
Number of sexual partners	2	16.7
▪ Indeterminate	3	25.0
▪ Multiple	5	41.7
▪ Few	2	16.7
▪ Unknown		
Anogenital sex No	12	100
Tumour site		
▪ Oral cavity	2	16.7
▪ Nasopharynx	7	58.3
▪ Larynx	1	8.3
▪ Sinonasal	2	16.7
Histology		
▪ Grade I	1	8.3
▪ Grade II	3	25.0
▪ Grade III	1	8.3
▪ Grade IV	7	58.3
HIV Elisa		
▪ Negative	10	83.3
▪ Positive	2	16.7
HPV genotype		
▪ 33	1	8.3
▪ 52	1	8.3
▪ 56	10	83.3

Table 17 below shows the association between patient/tumour factors with HPV positivity. There was no patient or tumour factor that was predictive of HPV status.

Table 17: Association between Patient/Tumour characteristics and HPV status

CHARACTERISTIC		PCR		PVALUE	ODDS RATIO	CONFIDENCE INTERNALS	
		NEGATIVE	POSITIVE			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 Use	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	80	0.231	0.425	0.123	1.473
	Non-drinker	8 (10.5%)	68 (89.5%)				
Previous head & neck irradiation					4.39	0.421	45.830
	Yes	1 (25.0%)	3 (75.0%)	0.2			
	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
	None/Few	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%)				
	Hypopharynx	0 (0%)	11 (100%)				
	Larynx	1 (2.1%)	46 (97.9%)				
	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%)				

4.10 Nasopharyngeal Carcinoma Patients

There were 62 patients with nasopharyngeal carcinoma with a male to female ratio of nearly 2:1. The age range was from 16 to 80 years, with a median age of 45.5 years. Figure 12 below shows age distribution of the patients. There was a single peak between 50 and 59 years of age.

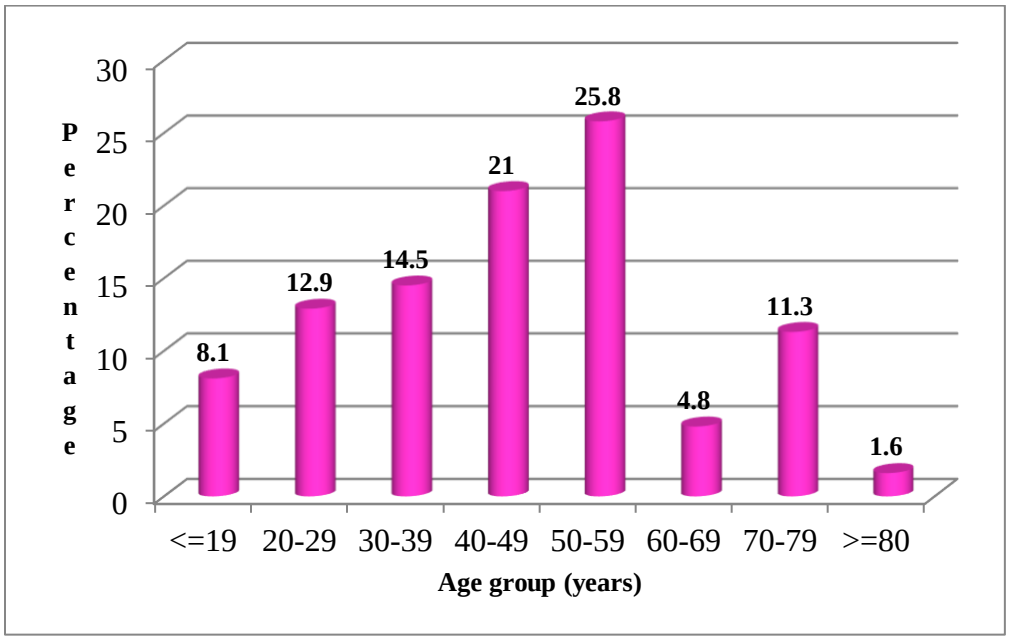


Figure 11: Age Distribution of NPC Patients

All the patients with nasopharyngeal carcinoma tested positive for EBV with 7 (11.3%) testing positive for HPV (table16). The HPV positive patients were aged 16, 37, 42, 49, 50, 54 and 80 years old. These included both the youngest and oldest patients in the study. Their mean age was 45.5 years. The HPV positive NPC patients consisted of four females and three males. All except one HPV positive patients had genotype 56. The exception was genotype 33. Only 2 of the HPV positive patients had used alcohol and only one had a smoking history. Two HPV positive patients were also HIV positive. The histological types of the HPV positive patients were WHO type III five patients and type II and I one patient each.

CHAPTER 5: DISCUSSION

5.1 Introduction

Human papillomavirus has been linked to a number of Head and Neck Cancers with incidence varying from population to population and dependent on the sub-site in question and study method used. Studies on HPV related HNSCC from Africa are scarce with majority showing low prevalence [82-84,90-95]. The incidence of cancers attributable to HPV in Kenya shows that cancer of the cervix uteri, other anogenital cancers and Head and Neck cancers stand at 33.8 in 100,000 women, 1.9 in 100,000 people and 0.2 in 10,0000 people respectively [72]. This is despite the fact that all three groups of cancer are caused by the same high-risk HPV genotypes. Worldwide HPV-related cervical cancer occurs predominantly in less developed countries while North America and Northern Europe have the bulk of HPV attributable Head and Neck cancers. [74]. More and more countries are setting up programs for HPV vaccination for both girls and boys as a measure of prevention of HPV attributable cancers. In addition, the presence of HPV has led to a revision of the TNM staging system for oropharyngeal carcinoma with adjustment in the treatment protocol for the same. Such programs and changes in management protocols can only be adopted if the target population is well defined based on reliable statistics.

Majority of HPV positive Head and Neck tumours tend to be oropharyngeal and more specifically the lymphoid tissue sub-sites. However, statistics still show a significant presence of HPV in other head and neck subsites: 45.8% in the oropharynx, 24.2% in the oral cavity and 22.1% in the larynx and hypopharynx [78]. This study, being the first of its nature in Kenya, included all HNSCC tumours of the oral cavity, oropharynx, nasopharynx, hypopharynx, larynx and sinonasal.

5.2 High Risk HPV in HNSCC Patients at KNH

In this study we found a low prevalence of HPV in HNSCC of only 7.5%. The HPV genotypes isolated were 33, 52 and 56. There was no HPV 16 or 18. The profile of the HPV positive patients in this study is presented in table 16 above. None of the patients had the typical characteristics of HPV patients as described in literature.

The low prevalence of HPV in this study is consistent with findings from most of the African continent countries, which have recorded a low prevalence of HPV in HNSCC [82-84]. Studies employing p16 immunohistochemistry, however, tend to produce higher prevalence rates of HPV in HNSCC [89, 102]. This may arise from the fact that p16 IHC has a high sensitivity (100%) but low specificity (79%) [36]. Whereas p16 IHC has shown good correlation with other HPV detection methods, there still exist concerns over the subjectivity of the test, lack of scoring or interpretation criteria and the variability of p16 expression in HNSCC [166]. A study done at KNH to determine P16 expression by IHC in 103 formalin-fixed paraffin embedded (FFPE) HNC blocks between 2008 and 2013 revealed an overall prevalence of 14.6% with majority being reported in specimens from the oral cavity (46.67%) followed by the larynx (26.67%) and pharynx (26.67%) [102]. 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 [89]. PCR studies have often produced low prevalence rates of HPV in most of Africa. 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 tested specimens [90]. 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 $P16^{INK4a}$ over-expression [91]. The mismatch between HPV DNA presence and lack of $P16^{INK4a}$ over-expression may suggest either $P16^{INK4a}$ inactivation in the presence of functional inactivation of Rb by E7 or false positives arising from sample contamination by HPV virions. In Mozambique no HPV was found among the patients with HNC [94]. Cameroon recorded low (5%) presence of HPV in oral swabs and rinses from HIV patients but high (28.6%) HPV from OPSCC by p16 IHC and ISH [93]. Central African Republic yielded only 0.74% HPV by PCR on 25 HNC FFPE specimens [92]. Two of three studies from Ghana have shown consistent findings for HPV in HNSCC (19.23% and 18%) with predominantly HPV 16 genotype but low positivity in OSCC (3.4%) [86,87,95]. It is noteworthy that the two similar reports were from the same locality different from the one with the lower prevalence. This supports the observation that there is a geographical/environmental influence on the distribution of HPV in HNSCC.

Sudan has, in the latter years, reported unique results some of which are at variance with our findings. Some of the reports support HPV as an important factor in oral cancer causation alongside *toombak* use [103]. A study of 150 HNSCC patients showed an overall positivity of 4% predominantly HPV 16 and with more presence in oral cavity and pharynx [88]. In yet another study, HPV was found by PCR sequencing in 39 (27%) of 145 FFPE oral cavity samples from *toombak* users with non-users of *toombak* having 7% HPV positivity. Oral brushings from *toombak* users without oral cancer or dysplasia tested positive for HPV in 40%. The authors concluded that HPV infections are common and may influence cancer development [99]. It is worth noting the high presence of HPV in *toombak* users only. T*oombak* has high levels of nicotine which is an independent risk factor for HNC. Additionally, the *toombak,* through its effect on mucosa may also facilitate entry of HPV by causing alkaline burns [104]. In another case 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 and 0% among controls leading to the conclusion that HPV 18 and 16 may have a causal role in oral SCC in Sudan [98]. 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 [100]. 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%) [96, 101]. The low prevalence in laryngeal lesions may relate to the site which is not usually associated with significant HPV associated tumours. What seems to emerge is that in North Africa and the greater Middle East there are generally higher HPV prevalence rates; a pattern that differs from the consistently low prevalence in the sub-Saharan countries. This has sometimes been attributed to limited study numbers and the varied scope of work covered. Studies on HPV in cancer of the cervix have shown similarities between the North African countries and Europe attributing the similarities to geographical proximity [105]. 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 global 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 [7]. From the foregoing, it is clear that HPV-related HNSCC has glaring disparities in its distribution.

The low prevalence of HPV in head and neck cancer in our study and among Africans may be influenced by an interaction of several factors that have not been verified but can be extrapolated from other disease processes. Among these are socio-cultural, genetic and environmental factors.

This study had subjects of one racial background – black. Racial disparity with regard to HR-HPV-positive HNSCC has been reported in America. The differences have been attributed to various factors that differ between the African Americans and White Americans. A number of studies have revealed that the prevalence of HR-HPV-related HNC is lower among African Americans compared to white Americans [107, 167, 168]. A multi-institutional retrospective cohort study among black and white HNSCC which 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%) [115]. These observations further confirm that other than environment, race may be crucial in as far as HPV-driven HNC is concerned. Among factors associated with this discrepancy are differences in marital status, smoking prevalence, marijuana use, adenotonsillar diseases, sexual practices, genetic and epigenetic issues [166,167,168]. It has been observed that more African Americans are unmarried, smoke and use alcohol more but engage less in oral sex; all factors that do not favour HPV -related HNSCC. It is also known that adenotonsillar hypertrophy is more in blacks than whites; the former tend to have chronic inflammation in the tissues resulting in sustained secretion of inflammatory mediators some of which have antiviral properties. Further to this, many of those who get the tonsils and adenoids removed may be protected by the fact that the tissues are not available for HPV replication. Additionally, expression of host molecules necessary for HPV entry into epithelial cells are controlled by genetics and these vary among different racial groups [167, 168,169]. While these arguments may apply for Kenya, the explanation fails to hold for the reports from Ghana. Interestingly, a meta-analysis of fifteen publications on the subject found a similar HPV16 or HPV18 positive disease in whites (61%) and blacks (58%) compared to Asians (25%); with HPV18 being detected fifteen times more frequently in black oropharyngeal cancer patients [170]. The same authors attributed the differences in HNC incidence rates to behaviour and

environmental risks rather than race [171]. The later argument may explain the different HPV positivity rates in HNSCC among people of the black race from different countries.

Literature offers more explanations for the disparities between black and white Americans; these include differences in sexual behavior, level of marijuana use, genetic differences, differences in host response to HPV infection and intratypic variation of HPV 16 within geographical areas [107]. Classification of human papillomavirus types, subtypes and variants are based on the L gene sequence similarities [108]. Several phylogenetic variants of HPV 16 have been isolated in cervical cancer patients namely the European, North-American-1, Asian, European-Asian, Asian-American, African-1, African-2, etc. [109]. Different biological properties of HPV 16 variants have been demonstrated in vitro and are thought to be responsible, in part, for variations in persistence, pathogenicity, carcinogenic risk and immunogenicity of the virus among different populations [115]. 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 [110]. 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 [105]. Based on these observations, molecular sub-typing of high-risk HPV types may provide useful information with regard to geographical and ethnic disparity in HPV-related HNSCC. Determination of the HPV variants of the isolated genotypes was, however, beyond the scope of this study.

The origin of HR-HPV in head and neck sites is linked to a sexually acquired oral HPV infection that is not cleared by the immune system. Sexual behaviour varies widely across ethnic groups and the globe at large. This has been fronted as one of the reasons for the disparity in prevalence of HPV-associated HNSCC. Risky sexual behavior has been linked to oral HPV acquisition. These

include early sexual debut, multiple sexual partners and prior sexual practice of the partner. Whereas marital status may give a better picture of an individual's sexual character, this is an area that is shrouded in secrecy to the extent that the truth is difficult to bring out in a study such as this. Sexual practices and orientation are generally divergent across the globe. In America, 78% of men were reported to have engaged in oral sex compared to only 9% of Indian men [107]. Another study from America demonstrated that oral sexual behavior is the primary predictor of oral HPV infection and that differences in gender, age-cohort and race are responsible for the varied prevalence of oral HPV infection [75]. This trend is likely to be reflected among whites and blacks on the African continent. It is noteworthy in this Kenyan study that all the patients were black and there was only one subject out of 160 patients who had admitted to having engaged in orogenital sex. Oral sex has been reported in Kenyan groups despite the secrecy [76]. There are scarce statistics on these practices in Kenya and most of Africa. Observations are that the indulgence on oral sex may be less compared to the developed world and this may contribute to the low prevalence of HPV in HNSCC in Kenya. The differences may also exist within different African countries and communities and be responsible for the observed differences in HR-HPV prevalence among the African nations.

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 an immune response may be evoked by acquisition of a genital HPV infection before HPV exposure through oral sex and this may decrease the risk of oral HPV and therefore HPV-related HNSCC [112]. Considering negligible oral sex among the subjects of our study, this argument may apply.

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* [113]. It is, therefore possible that perhaps, an equivalent immunological protection against HPV-related HNSCC by some yet to be determined factor may exist in certain geographical regions to explain the high prevalence of HPV in cervical cancer but not in HNSCC in the same population. 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 [114]. Based on these observations, it is postulated that different populations respond differently to given disease entities and this might be the case with HPV in HNC in some African populations including Kenya.

The most common HPV genotype associated with HNSCC is 16 followed by 18 [10, 80]. 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 [100]. 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 $P16^{NK4a}$ overexpression [91]. 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[86]. Two studies from South Africa, though with very few positive cases, had HPV types 18, 16 and 11 [82, 84]. 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% with HPV types 16 and 18 being found in two men with a history of oral sex. There was an association between HPV and the number of sexual partners [85].

HPV related HNSCC is associated with particular characteristics usually not present in HPV negative cancers (table 4). These clinicopathological profiles that are characteristic of HPV positive tumours include male gender, younger age, higher socioeconomic status, marijuana use, minimal tobacco and/or alcohol consumption, orogenital sex, multiple sex partners, HIV co-infection, early primary tumour stage and advanced nodal stage with the nodes being cystic. The histology for the HPV-related HNC 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 [118-122]. The twelve (7.5%) patients in this study who tested positive for HPV have their profiles shown in table 16 above. None of the patients had the typical characteristics of HPV patients as described in literature. This might be explained by the small proportion of patients who tested positive for the disease in addition to the possibility that tobacco-related effects may have obscured the HPV influence. There were equal numbers of males and females unlike most studies which show males to be at higher risk than females. This has been attributed to protective factors unique to females like hormonal protection, sex-driven dimorphism in immune system that allows faster clearance of pathogens and higher HPV transmission for vaginal-oral than penile-oral sex. Similarly, there were equal distribution of patients above and equal to or less than 60 years unlike the expected younger age-group reported in many studies. It may be argued that with the detected HR-HPV genotypes, the characteristics may be different from HPV 16 and 18.

All characteristics considered, there was no patient or tumour characteristic from the current study predictive of HPV positivity (table 17).

5.3 HIV in HNSCC at KNH

Ten (6.3%) of the 160 patients in this study tested positive for HIV. There was no distinction between the HIV-positive only and the AIDS patients among them. There were 4 females and 6 males with an age range of 23 to 57 years and a mean of 42.8. The mean age for all the HNSCC was 51.6 ± 16.6 years. All except the female patients were either past or current cigarette smokers and alcohol consumers supporting the notion that HIV positive patients tend to engage in high-risk behavior [(128)]. The patients consisted of two with oropharyngeal, six nasopharyngeal, and one each with laryngeal and hypopharyngeal tumours. Only one patient had stage II disease with the rest having advanced tumours. Two (20%) of the ten HIV positive patients tested positive for HPV type 56. Both had nasopharyngeal carcinoma.

Both HPV and HIV have been classified by IARC as carcinogens. Much of the interaction between HIV and HPV is extrapolated from observations among patients with cervical cancer. It has been reported that infection with multiple high-risk HPV make women more susceptible to HIV-1 infection and that HIV-infected persons are more likely to have HPV. The explanation for this is that HPV infection increases the susceptibility to HIV-1 acquisition through the immune response of mucosal tissue to HPV, which tends to increase the number of cells susceptible to HIV-1 infection. HIV-1, on the other hand, has been shown to increase the prevalence and persistence of HPV while at the same time reducing the clearance of HPV. At the molecular level, HIV tat and gp120 proteins together with cytokines produced by HIV-infected cells promote HPV infection by disrupting the epithelial tight junctions and potentiating penetration of HPV into the basal epithelial cells in addition to upregulating HPV E6 and downregulating p53 protein. Both viruses tend to evade immune surveillance by modification of the host immune system through tumour-driven

alterations in macrophage differentiation, cellular immune response compromise, imbalance between type 1 and type 2 T helper cells, and downregulation of dendritic cell activation and maturation [43-44].

This study had a 6.3% prevalence of HIV among the HNSCC patients. In 2017, there was a reported 4.8% adult (15-49 years) HIV prevalence in Kenya [129]. This would suggest a higher prevalence in the study population (HNSCC) perhaps due to the factors alluded to above. The association between HIV and HNSCC is unclear with higher prevalence of HNSCC in HIV positive patients being reported. Studies have attributed the higher prevalence of HNSCC in HIV positive patients to immunosuppression, opportunistic infections, increased tobacco use and infection with high-risk HPV subtypes [40-42]. AIDS patients tend to suffer more from AIDS-defining tumours most of which are not SCC. With more HIV-positive patients using HAART, there is reported increase in non-AIDS defining cancers including HNSCC among HIV patients. This study did not determine how many of the HIV positive patients were on HAART. Susceptibility to HNSCC among these patients is attributed to mild to moderate immunosuppression and higher cigarette use and other risky behavior among HIV patients [128]. Studies from renal transplant patients have also shown that prolonged immunosuppression, even when only modest, is a risk factor for HPV-related carcinomas [130]. In cervical cancer, detection of cervical intra-epithelial lesions is directly proportional to the severity of HIV-induced immunosuppression as demonstrated by the level of CD4 counts and HIV viral load [134].

The median age of the HIV positive HNSCC patients was 42.5 years compared to the full study median of 54.0 years. This is in keeping with the observation that HIV-positive HNSCC patients

tend to be young (mean age of 33 for females and 37 years for males) with advanced disease and poor prognosis [132]. All our patients had advanced stage disease except one. Both immunosuppression and opportunistic infections may explain the advanced disease at presentation. Stigma arising from HIV status may also account for late presentation to the health facility.

Head and neck squamous cell carcinoma is the third commonest HNC among HIV patients after Kaposi's sarcoma and non-Hodgkin's lymphoma [38, 39]. This study only considered HNSCC but an earlier study done at the institution reported a prevalence of head and neck neoplastic lesions of 27% among HIV-infected patients distributed as 68% Kaposi's sarcoma, 17% SCC, 13% non-Hodgkin's lymphoma and 2% Burkitt's lymphoma [131]. In Uganda, HIV prevalence among head and neck patients excluding Kaposi's sarcoma, lymphomas and thyroid neoplasms was 15% against the national prevalence of 7.3% [132]. In the USA, HIV infection has been reported to increase the risk of HNSCC by approximately two to three times [109].

The head and neck regions at greatest risk for malignancy in HIV-positive patients are the larynx, oral cavity, oropharynx, lips, salivary glands and conjunctiva [134]. An increased risk for NPC in HIV positive patients has been reported with the highest risk being associated with the non-keratinizing histological type [136]. In Kenya, a prevalence of conjunctival SCC of 7.8% has been reported among HIV positive patients [137]. The patients in the current study consisted of two with oropharyngeal, six nasopharyngeal, and one each with laryngeal and hypopharyngeal tumours. In literature, the larynx is the commonest site for HIV-positive HNSCC followed by oral and pharyngeal cancers [57]. This kind of site distribution may not be obvious with small numbers as in this study. Perhaps, a larger sample would give a more representative picture.

Two (20%) of the ten HIV positive patients tested positive for HPV type 56. Both had nasopharyngeal carcinoma. A study done among Kenyan women with invasive cervical cancer found more multiple HPV-type infections in HIV positive women than in the HIV negative women (37. 2% versus 13.7%) but similar HPV type distribution. The only difference of statistical significance was an excess of HPV 52 among the HIV positive women (19.6% versus 5.2%) and low HPV 45 (7.8% versus 17.0%) [145]. The only HPV type 52 detected in the current study was from an HIV negative patient and there was no HPV type 45 detected in the entire study. There were no multiple HPV types in any one patient. Again, the issue with small numbers may be the reason for the limited types of HPV isolated.

The oral cavity and oropharynx are important with regards to HPV and EBV persistence and transmission. HPV and EBV detection in the oral cavity of HIV-positive patients is higher compared to HIV-negative patients (13.7% versus 4.5%) [144]. McLemore et al found 24% (6 out of 25) of HIV-positive HNSCC to be positive for HPV 16, 26 and 29. There were 5 HPV 16 genotypes with one HPV 26/29. It is notable that the later are not high risk. Despite 65% of the subjects in McLemore's study having tumours of the larynx, 5 (83%) of the HPV positive ones were from the oropharynx with one from the nose [140]. This is in keeping with other authors who found no or minimal HPV in laryngeal tumours of HIV positive patients [141, 142].

Overall, HPV positivity was 20% in the HIV-positive HNSCC patients only and 6.7% among the HIV-negative patients in this study. The difference is big although the numbers involved are too small and may not permit any statistical inference to be made. Perhaps this begs for further studies

to determine the relationship to be done. In the meantime, HIV patients are one group of patients in whom HPV determination can be considered especially where the other features that characterize HPV positive HNSCC are present.

5.4 HPV and EBV Co-infection in Nasopharyngeal Carcinoma Patients at KNH

Sixty-two nasopharyngeal specimens were tested for EBV by PCR and all tested positive for EBV. Seven (11.3%) were positive for HPV 56 (six) and HPV 33 (one). These patients were aged 16 to 80 years with a single age peak in the 6th decade. Five (8.1%) patients were below 20 years of age (figure 11). The males were more than the females with a ratio of 2:1. The commonest WHO histological type was III (75.8%).

Kenya has been classified as an intermediate incidence region with relation to nasopharyngeal carcinoma. Low incidence areas tend to have two age peaks; one in the late childhood and the other in the 6th decades. The first peak is attributed to germline alterations while the second one is related to lifestyle and environmental influences [146]. This study had a single peak in the 6th decade that is in keeping with high incidence areas. There were, however, five patients below 20 years of age. Male patients were more than females as observed in most studies on nasopharyngeal carcinoma. Two earlier studies done at KNH had shown peaks at 31-40 and 41-50 years with a male: female ratio of 2.2:1 and 1:1 respectively [148, 149]. It would appear that the peak age for NPC in Kenya has been increasing progressively with time. This might suggest a new factor in the pathogenesis of the disease that either requires more time to induce carcinogenesis or affects more of the older generation. Nasal, aural and neck complaints were, as expected, the most frequent symptoms at presentation.

The histological types of the nasopharyngeal specimens were type I 6.4%, type II 17.7% and type III 75.8%. The predominance of WHO type II and III histological types in the NPC specimens in this study mirrors more of what is seen in endemic areas. Low incidence areas tend to have more of the differentiated types in association with smoking [151]. All the specimens tested positive for EBV by PCR. Over a decade ago, a determination of EBV viral capsid antigen specific immunoglobulin A serum titres in NPC patients at KNH yielded a rise in 35% against 22.5% among controls [149]. A Sudanese study on 43 nasopharyngeal carcinoma patients and 10 controls had no WHO type I histology with all 43 patients testing positive for EBV by ISH and all the controls testing negative [172]. It is estimated that nearly all NPC cases in endemic areas have EBV while the WHO type I associated with non-endemic areas is usually negative for EBV but positive for HPV [150-151]. Association of EBV with the differentiated NPC types has, however, been reported in geographical regions with high incidence of undifferentiated NPC [151, 153]. This finding of 100% EBV presence in the specimens is suggestive of endemic status for Kenya.

The role of HPV in the pathogenesis of NPC is not as clear as EBV. What is known is that both viruses suppress the p53 and retinoblastoma protein in similar ways. Studies comparing endemic and non-endemic area findings seem to associate EBV positivity with endemic status and HPV positivity with non-endemic status. In America, a cohort study comparing EBV and HPV presence in endemic (Southern China) and non-endemic (USA) cohorts with NPC showed no HPV among the Southern China cases or Chinese American patients. All except 3 cases from the endemic cohort were EBV positive. All EBV negative cases were white Americans who also had high risk HPV and smoking association. There was no co-infection with both viruses [53]. This is similar to another

study where HPV was detected in non-endemic (Danish) cohort but not in the endemic (Inuit) cohort [159]. A low incidence area in America posted similar results where HPV positivity was reported in four of five subjects all of whom were EBV-negative and white Americans. The only EBV positive patient in the said study was HPV-negative and Korean [158]. In Greece, there was EBV presence in 32% and HPV in 19% of 63 FFPE NPC tissues with no co-infection [157].

Our study had 100% EBV presence with co-infection with HPV in 11.3%. This is not an isolated case of co-infection with HPV and EBV. In Morocco, there was 100% EBV presence in 70 NPC specimens with 34% HPV co-infection while Taiwan had 51% HPV and 83% EBV positivity with co-infection in 42%. In Iran 95% of 20 NPC patients tested positive for EBV, 20% for HPV 11,16 and 18; with three (15%) patients having both EBV and HPV. Ghana posted lower figures of 25% EBV, 19.23% HPV and a co-infection rate of 4.2% [66, 86, 100, 160]. Jordan posted EBV/HPV results in nasopharyngeal tumours very similar to our study except for the HPV genotypes: 95% EBV, 10% HPV 6/16 and 10% HPV 16/18 and co-infection in 15% [173]. The co-infection aspect is the one interaction that seems to cut across continents and might be significant with regard to the role of HPV in NPC in Kenya. It is notable that HPV presence alone in HNSCC tumours in African countries has remained low. Whether EBV-HPV interaction is a similar situation to what has been observed between EBV and malaria within the Burkitt lymphoma belt remains to be proven. In the latter case, it has been demonstrated that P. falciparum acts at two points: immunosuppression with resultant high throughput of EBV-infected cells in the germinal centers, and deregulation of an enzyme responsible for alteration in the immunoglobulin genes in B cells when they enter the germinal center. The result is DNA damage, translocations and lymphoma development. [162].

These findings, definitely, call for further research in the area of EBV-HPV co-infection in NPC. Based on the findings, adoption of EBV serology in management of NPC patients in Kenya may be considered.

5.5 Other aspects of HNSCC Patients at KNH

5.4.1 Demographics

One hundred and sixty patients aged 16 to 87 years with a mean age of 51.6 years were recruited into the study. There was a wide age range attributed to the nasopharyngeal tumour patients, 21% of whom were below 30 years. It is a well-established fact that in low-risk populations, NPC has a bimodal age peak with one in early adolescence (15-24 years) and the second one in the 6th decade [(146)]. Overall, there were more males than females (male: female = 3:1) with the biggest ratio being 23:1 for laryngeal cancer. The oral cavity male to female ratio was 1:1 while hypopharyngeal male to female ratio was 5:6. Two earlier studies conducted at KNH on laryngeal cancer found a male: female ratio of 24:1 [(64, 65)]. This is not unexpected considering the prevalence of alcohol and tobacco use among Kenyan patients is 80.8% and 56.4% respectively for males compared to 30.6% and 5.6% respectively for females [(66)]. In 2004 statistics from a KNH study showed a male: female ratio of 1.3: 1 among oral carcinoma patients with a peak in the 7th decade [(174)]. The hypopharyngeal tumours were the only ones with a slight female preponderance (male: female ratio of 5:6). An unpublished study on hypopharyngeal carcinoma at KNH reported a slight male preponderance [(175)]. Literature has supported higher prevalence of hypopharyngeal carcinoma in females for the post-cricoid subsite only in association with Plummer-Vinson syndrome but an overall male preponderance. This study did not sub-classify the tumours with regard to the subsites involved. It is, therefore, not clear whether this ratio is influenced by the subsites involved.

The distribution of tumours for the study subjects in descending order from nasopharynx (38.8%), larynx (29.4%), sinonasal (11.3%), oral cavity (8.8%), hypopharynx (6.9%) and oropharynx (5.0%) reflects the recruitment process rather than the true prevalence of the tumours in Kenya. Based on the Nairobi cancer registry, which uses statistics from hospitals within Nairobi (Capital of Kenya), the commonest head and neck cancers in Nairobi in decreasing order are oral cavity, larynx, and nasopharynx [(1)]. A hospital-based study done at Kenyatta National Hospital showed the order from the most common head and neck malignancies by site to be laryngeal cancer followed by the tongue, mouth and nasopharynx [(60)]. In the current study it is possible that a significant fraction of oral cavity and oropharyngeal tumour patients were attending the University of Nairobi Dental School Hospital facility, which is in the neighbourhood of KNH, and were therefore missed out from this study. This is mainly because of the overlap in specialty areas where in Kenya most of the oral cavity disorders get referred to the dentist and/or maxillofacial surgeon rather than the ENT surgeon.

Kenya is divided into eight geographical blocks previously referred to as provinces. These have different terrains and are occupied by people of different ethnic backgrounds and culture. Kenyatta National Hospital is located in Nairobi Province, which houses the capital city of the country as well as the economic hub and headquarters of all government ministries. An analysis of the province of current residence, birth, and where each patient had lived most of the last ten years revealed that most of the patients were born, resided and lived for most of the last 10 years in Nairobi and its environs. There were minimal differences in statistics relating to the province of birth, previous and current residence. Central province topped the list with at least 34.3% of the patients having been drawn from there followed by Eastern and Nairobi provinces which

contributed 25% and 19% respectively. A study on the four-year trend of Head and Cancers at the Nairobi Cancer Registry showed similar distribution of the patients' residence [59]. The pattern was not much different in the 1960s when Clifford reported that most of the NPC patients came from the highland areas particularly Central Kenya [147]. Unlike Clifford who attributed the presence of nasopharyngeal carcinoma to exposure to smoke emitted from burning wood in small poorly ventilated huts, there were no patterns in this study to suggest that local factors at the province of residence influenced the disease distribution among the study subjects apart from proximity and therefore access to Kenyatta National Hospital. *Khat* chewing, which has been associated with oral cavity cancer in Saudi Arabia is largely grown in Eastern province and consumed within Nairobi, Eastern, Northeastern and Central regions in addition to overseas [181]. Despite this observation, *khat* chewing was only reported in 6.9% of the patients, majority of whom hailed from the *khat*-growing provinces.

5.4.2 The Referral System

Kenyatta National Hospital is the only public health facility with an established cancer treatment unit in Kenya. Majority of patients with cancer get treated at KNH. The health referral system in Kenya involves six levels of healthcare starting with the community health services (level 1), primary care services (level 2 and 3), primary level hospitals (level 4), secondary level hospitals (level 5) and tertiary level hospital (level 6). The referral system, however, has four levels of service through which referred patients can go except in emergency situations. These are community health services (level 1), primary care facilities (level 2 and 3), county referral facilities (level 4 and 5) and national referral health facilities (level6). Kenyatta National Hospital is one of the two tertiary referral hospitals in the country and should therefore receive referrals from the county facilities.

This only happened in 56.3% of the patients in this study. Inter-departmental referrals contributed 2.5% of the patients. The rest of the patients by-passed the laid down referral system to get to KNH. Lack of adequate facilities and cancer specialists at most hospitals other than KNH and a few private hospitals is responsible for this practice. Patients prefer to go directly to KNH to avoid loss of time and the inconvenience of making unnecessary trips to middle-level institutions, only to be referred to KNH eventually. A study from a county in the neighbourhood of KNH identified poor infrastructure, inadequate capacity of healthcare staff, lack of effective information transfer, financial constraints and patient non-compliance as challenges hindering the implementation of the recommended referral system [(174)]. These are factors that, if addressed adequately, can streamline the referral system not only for HNC patients but all patients and improve the overall performance of the health system. Onyango et al while studying factors that contributed to delays in management of head and neck cancer patients at KNH found the referral system to be wanting. Majority of the patients went through multiple referrals and only reached KNH on the fourth referral [(68)]. This would suggest that patients faithfully went through the levels of referral without getting a service because there were neither informed personnel nor facilities to offer the required service at the lower levels. This points to a referral system that is not functional. It would be sensible for each level of service to be aware of what service is available at every level and perhaps even confirm before drawing a referral letter. In Ghana, it was found that in addition to adequate health personnel, transportation, communication infrastructure, finance and social capital played an important role in encouraging or dissuading referred patients into or against complying with the referral process [(176)]. Although the association of inefficient referral system to late presentation of HNSCC in this study could not be determined, it is possible that the referral system may have an impact on the disease progression and staging.

5.4.3 Clinical Presentation

The primary site of tumour largely dictated the patients' symptoms at presentation (table 5). Tumours in the upper aspect of the UADT tended to have more of nasal and oral symptoms while those in lower anatomical sites had more of swallowing and respiratory symptoms. The large number of patients presenting with nasal symptoms and hoarseness reflects the large number of nasopharyngeal/sinonasal and laryngeal tumours in the study respectively. Advanced sinonasal and nasopharyngeal tumour patients had a number of central nervous system symptoms like headaches, visual disturbance and other cranial nerve palsies arising from their close relation to the skull base. These were responsible for the high percentage (41.9% and 61.1% for nasopharyngeal and sinonasal respectively) of the "other symptoms" category. An overall presence of neck swelling of 33.8% represents high levels of regional nodal involvement at presentation. The actual nodal involvement as confirmed at staging by physical and radiological examination was 87.4% indicating late presentation of HNC patients. This impacts negatively on survival as presence of cervical nodal metastases in head and neck cancer is an important prognostic factor. Breathing difficulties was the most frequent symptom (74.5%) among patients with cancer of the larynx. This is in keeping with the observed high tracheostomy rate of 81% among patients with laryngeal cancer at KNH attributed to late presentation [48]. Several factors have been identified as responsible for late presentation of HNC at KNH with the cumbersome referral system topping the list. Other factors included patient unawareness, non-specific symptoms for HNC that mimic other disorders, and missed diagnosis at primary care health facilities [52].

5.4.4 Risk factors for head and neck cancer

Tobacco and alcohol use were the most common risk factors for cancer among the study patients. At least half (51.3%) of the patients had a history of tobacco use with 49.4% having smoked

cigarettes. Fifty-eight (36.3%) of all the patients had less than30 pack years of smoking. Carcinoma of the larynx had the highest percentage (87.2%) of smokers of all the tumour sites followed by oropharynx and sinonasal. This confirms findings of another study from KNH that showed that both current and past smoking conferred a positive risk for carcinoma of the larynx [49]. An unpublished study at KNH has also shown a five-fold and three-fold risk for oropharyngeal SCC for current and past smokers respectively [87]. The association of smoking and nasopharyngeal carcinoma is not as strong as for the other UADT sites. Furthermore, a significant fraction of the patients who had nasopharyngeal carcinoma were relatively young and may not have started smoking or smoked for long. Alcohol has a synergistic effect with smoking in causation of cancer of the UADT. Laryngeal and oropharyngeal SCC patients had the most alcohol and tobacco users. The most common alcoholic drinks taken were beer (37.7%), unprocessed brews (23.1%), spirits (10.7%) and *chang'aa* (9.4%). The latter is a traditional home-distilled and unpurified spirit. The relatively low percentage of tobacco and alcohol use among oral cavity and hypopharyngeal cancer patients may be due to higher proportions of females in the groups and overall relatively fewer patients in the groups. Again, it has to be remembered that alcohol and cigarette use among Kenyan female patients has been shown to be much lower compared to their male counterparts [50].

K*hat* (Catha edulis) chewing was reported in only (11) 6.9% of the patients. The *khat* users in this study (table 12) were all males aged between 21 and 64 years old who had chewed *khat* for periods ranging from one to twenty year. Majority (81.8%) of the *khat* users were less than 60 years of age. Their primary tumours were four nasopharyngeal, two each oral and laryngeal cancer; and one each oropharyngeal, hypopharyngeal and sinonasal carcinoma. Considered as a percentage of chewers for each tumour site, the oral cavity and oropharyngeal tumour patients who chewed *khat* were

14.3% and 12.5% with the other sites having less than 10%. Cigarette smoking and alcohol use among the *khat* chewers was 81.8% and 72.7% respectively.

Khat being a new entrant among factors suspected to have an association with some head and neck cancers specifically oral cancer calls for a deeper analysis of the characteristics of the *khat* chewers in this study. This is particularly necessary because Kenya is one of the African countries that grow *khat* with some of it being consumed locally. The active chemical in *khat* is cathinone. *Khat* leaves and stalks are chewed for several hours with the residues being retained in the cheeks for long hours. This process allows most of the chemical contents to be in contact with and to be absorbed through the oral mucosa. Additionally, the chewing process causes both mechanical and chemical irritation resulting in thickening and keratinization of the mucosa. *Khat* has also been shown to have cytotoxic effects on oral mucosa cells. A systematic review article of 2015, despite concluding that *khat* chewing has health hazards and may be associated with potentially malignant oral disorders at the site of the chewing did not find conclusive evidence for *khat* being a risk factor for development of potentially malignant or malignant disorders of the oral cavity. This was partly related to the study designs of the reviewed publications, subject selection, reliability of studies originating from countries where *khat* is prohibited, and failure to control for confounders like smoking, alcohol consumption and use of pesticides on the *khat* [(177)]. An updated review on the same subject concluded that the evidence that chewing *khat* is a risk factor for oral cancer is still weak for similar reasons [(178)]. As stated above, cigarette smoking and alcohol use among the *khat c*hewers was 81.8% and 72.7% respectively. It is an established fact that smoking and alcohol are independent risk factors for head and neck cancer. In the absence of control for known risk factors for HNC, it is impossible to apportion a role to *khat* in the carcinogenic process in this study. Apparently *khat*

chewers tend to smoke and use alcohol as well, thereby making it difficult to draw any conclusions on the role of *khat* in HNC where all three factors are in play [179,180]. A survey carried out in Saudi Arabia demonstrated a strong correlation between *khat* chewing and oral cancer after demonstrating that 10 of 28 HNC patients who had used *khat* for at least 25 years and had never smoked had oral cancer (8), parotid tumour (1) and a metastatic node (1) [181]. In Kenya, a case of verrucous carcinoma in a patient who was a tobacco chewer, snuff taker and *khat* chewer has been described but with no evidence for a causal relationship between the tumour and *khat* chewing [174]. Buccal smears of habitual *khat* chewers in Meru, Kenya were examined for cytological changes and only yielded 0.5% atypia of undetermined significance with the rest having inflammatory lesions [0]. Despite the varied observations regarding *khat* use and oral cancer, this is an area that requires focus in future studies.

Previous exposure to radiation in this study was not considered significant as a risk factor since all the patients who reported exposure were being followed up for either residual or recurrence of the same cancer that they had been irradiated for. Although 6.9% of patients reported symptoms suggestive of GERD or LPR, the actual presence or absence of these conditions in these patients and in those who had no symptoms could not be verified. Similarly, the irritants that the patients were exposed to could neither be identified nor quantified.

Oral human papillomavirus infection is considered a sexually transmitted disease. Certain genotypes of the virus are associated with HNSCC especially in the oropharyngeal subsites. Early sexual debut, multiple sexual partners and oral sexual contact have been linked to HPV-associated HNSCC [80]. One hundred and fifty-one (94.4%) of the patients in this study were sexually active.

Six of the nine sexually inactive patients were high school students aged less than 19 years, with the other two in the third and one in the eighth decades. None of the sexually active patients was homosexual or bisexual. Only 2 patients had an early sexual debut and only one patient admitted to having engaged in anogenital sex. None of the patients had practiced orogenital sex. These findings are not unexpected in a largely conservative country like Kenya where same-sex relationships are shunned and often criminalized. Homosexuality in Kenya is illegal and would attract a prison sentence of several years. This makes Kenyan homosexuals unlikely to own up to it in an interview for fear of their privacy being breached. In the African setting, matters regarding sexuality are taboo and are not openly shared, especially with strangers. It is, therefore, difficult to ascertain whether the responses given in this study with regard to the age at sexual debut and number of sexual partners are reliable considering the circumstances of the interview. Self-administered questionnaires are more likely to yield reliable responses than face-to-face interview when dealing with sensitive issues. Disclosure of the number of sexual partners in an African setting can be manipulated as the numbers may be misinterpreted to suggest either promiscuity or naivety based on the circumstances in question. For this study, 29.8% of the sexually active patients had multiple lifetime sexual partners, 9.9% could not remember the numbers, with 60.3% having had less than four lifetime sexual partners. Again, these are statistics that have to be interpreted with caution given the circumstances of the interview. A recent systematic review of the prevalence of oral and anal sex among adolescent and adult heterosexuals in sub-Saharan Africa included statistics from Kenya showing a prevalence of oral sex among 18-34-year-old Kenyan men of 29.0% and Kenyan women of 21.0%. The prevalence of anal sex among Kenyan women working in food and recreational facilities was, however, low (0.4%) [76]. These reports support the practice of oral and anal sex among Kenyans.

5.4.5 Disease stage and Histological grading

The AJCC 2010 TNM staging was used to stage the tumours and later assign them group staging. Only 12.6% of the patients had stage I and II disease. This can be attributed to late presentation of patients to the institution. A study on laryngeal cancer done at KNH nearly a decade ago revealed that the duration of delay between onset of symptoms and presentation to the hospital was 4 to 300 weeks with a mean of 50 weeks [64]. With regard to HNC, the time lapse from the first symptom to consultation was zero to eight months and from referral to presentation at KNH was zero to thirteen weeks. These delays may be related to lack of cancer awareness by the public, poor transport infrastructure and access to health services, cumbersome referral system and in some instances inadequate clinician knowledge of early cancer symptoms and signs. Many head and neck cancers present with symptoms that mimic benign diseases resulting in inappropriate initial management including tooth extractions, nebulization, flu treatment, etc. [68]. It is notable that even after 43.7% of the patients bypassed the established referral system, patients presented with advanced disease.

All tumours were assigned WHO histological grades I to IV. Majority of the tumors of the oral cavity (92.9%), oropharynx (62.5%), hypopharynx (100%), and larynx (87.2%) were well and moderately differentiated while majority of sinonasal (66.7%) tumours were poorly differentiated or undifferentiated. Nasopharyngeal tumours were largely poorly differentiated and undifferentiated. The NPC specimens were separately reclassified according to the NPC WHO classification as keratinizing SCC (Type I), non-keratinizing carcinoma (Type II), and undifferentiated carcinoma (Type III). Type I NPC specimens were 6.4%, type II were17.7% and type III 75.8%. Type I NPC is more prevalent in low incidence areas often in association with smoking and HPV. Type III NPC, on the other hand is more prevalent in NPC endemic areas in association with EBV [151-153]. These

observations would cast doubts on the classification of Kenya among intermediate incidence zones with regard to NPC.

6.0 CONCLUSION

The prevalence of high-risk HPV at KNH is low (7.5%) among HNSCC patients. Only three high risk types 33, 52 and 56 were isolated. There were no clinical or pathological predictors for HPV associated HNSCC. In contrast, there is a high (20%) prevalence of HPV among HIV positive HNSCC and all the HPV positive cases had HPV 56. There is 100% EBV and 11.3% HPV presence in NPC patients at KNH with features consistent with high NPC incidence status.

7.0 RECOMMENDATIONS

Given the low prevalence and the absence of HPV 16 and 18 in HNSCC patients at KNH, the role of HR-HPV in HNSCC in this population is insignificant and does not warrant routine testing for HPV. Additionally, the current treatment protocols for HNSCC that were drawn without consideration of HPV status remain applicable. The relatively higher prevalence of HR-HPV among HIV associated HNSCC may be useful in directing the focus of future studies on HR-HPV to these specific populations. The developed world is investing heavily in HR-HPV related HNSCC control among boys and girls through vaccination; the priority for Kenya with regard to HNSCC should be controlling the established risk factors like smoking and alcohol and directing the available resources to HPV vaccination of girls only.

Considering the high prevalence of EBV in NPC in this study, it may be prudent to adopt EBV serology in screening and follow up of NPC patients as this may facilitate early diagnosis of new cases and recurrences with resultant better prognosis. The predominance of WHO type III in association with EBV presence in NPC patients calls for re-classification of Kenya into endemic group with regard to NPC.

8.0 STUDY LIMITATIONS

Recall of information, especially, regarding smoking and alcohol use can be a challenge as these are consumed as and when circumstances allow. The alcoholic drinks are packaged or dispensed in varied concentrations and volumes, which cannot be accurately determined by the consumer.

In the African setting, matters related to sexuality are not readily shared with strangers or younger persons. It is, therefore, possible that some patients did not correctly disclose their true sexual orientation and practices.

The findings of this study would have been strengthened by doing multivariate analyses for the variables presented. However, this was not possible due to the small numbers of patients.

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APPENDIX I:
CONSENT/ASSENT EXPLANATION AND FORM

My name is Dr. Joyce Aswani. I am the principal researcher in this study, which has been approved by the KNH/UON Ethical and Research Committee. Am assisted by Dr…….... (Research assistant). We are conducting a study amongst patients with cancer of the mouth, throat and voice box to find out if any of them have human papillomavirus (HPV) in the cancer tissue and what features are common among the patients that have the virus. This is the same virus that causes cancer of the cervix in women. In developed countries where this test is routinely done, the results are used to select the best type of treatment shown to give good outcomes. In our set-up we have not been doing the test because of the cost implications. If, from this study, we can determine the specific features of patients with the virus in their cancer, we can use the features to select the best treatment option for each patient. We may also be able to recommend the use of the vaccine against the virus for prevention as is being done for cancer of the cervix. You/your child's/dependant's inclusion in this study will be voluntary.

What is involved in this study?

Once you consent for your/your child's/your dependant's participation, we will handle you/your child/your dependant the way we normally do for all patients with cancer of the mouth, throat and voice box. The standard procedure for all patients with cancer of the mouth, throat or voice box includes asking questions about the illness with regard to the symptoms and exposure, presence or use of substances that could have contributed to the symptoms. An examination of the affected and related areas is done. Blood samples are taken for checking haemoglobin, as well as the function of the liver and kidneys. You also undergo pre-test counseling for HIV and get tested for HIV

thereafter. Post-test counseling is done before your HIV status is disclosed to you. To confirm the cancer, a small piece of tissue has to be taken from the cancer growth. If the growth is in a place where it can be reached easily in an adult patient, an anaesthetic (medicine to remove sensation) will be applied or injected to the site and the specimen removed with the patient awake. If the cancer growth is in a hidden site or in a child, arrangements are made for the specimen to be removed in theatre while the patient is asleep. The specimen taken from the cancer growth is processed in the laboratory to determine the kind of cancer so that the appropriate treatment is planned. For the purpose of this study, a part of the specimen taken will be subjected to further tests to confirm whether or not it has HPV. Should we find results that require adjustment of your/your child's/your dependant's treatment, your/your child's/your dependant's doctor will be advised to consider initiating the change as appropriate.

Are there any risks involved?

There are no known risks arising from the extra tests to be done on the specimen.

Is there a penalty for declining to participate in the study?

No, all patients will receive the same attention and treatment irrespective of whether they participate in the study or not.

What benefits will I get if I participate?

You/your child/your dependant may not get any immediate direct benefits by participating though the choice of treatment you receive may change depending on your test result. The study results will also help doctors and the hospital to come up with the best method of treatment for this condition.

What about confidentiality?

All the information we obtain will be kept confidential.

How much will it cost me?

You will not incur any extra cost.

Are you satisfied with the information given?

In case of any other questions, feel free to contact the following for clarification:

1. Principal Investigator:

   Dr. Joyce Aswani
   Department of Surgery,
   College of Health Sciences,
   P.o. Box 19676 – 00202, Nairobi.
   Tel: 0722814483

2. Supervisors:

   I. Prof. Nimrod Mwang'ombe.
   Professor & Chairman of Neurosurgery Unit,
   College of Health Sciences, University of Nairobi.

   II. Prof. Omu Anzala,
   Programme Director, KAVI
   Professor, Department of Microbiology, University of Nairobi.

3. The Chairman,
   KNH/UON ERC,
   Kenyatta National Hospital.

If you are satisfied with the explanation, kindly complete and sign the consent form below:

CONSENT/ASSENT FOR THE STUDY

I……..…….. ID No……..of …… do hereby consent Mr/Mrs/Master/Miss/Self …….to be included in this study on "High risk human papillomavirus subtypes in head and neck cancer". The nature of the study has been fully explained to me by Dr. ……... I have not been promised any material gain to participate.

Signed (Patient) …… Date…….

Signed (Doctor) ……. Date ……...

KIAMBATISHO I:

MAELEZO YA UTAFITI NA KIBALI CHA KUSHIRIKI

Jina langu ni Daktari Joyce Aswani. Mimi ni mtafiti mkuu katika utafiti huu, ambao umepewa idhini na kamati inayosimamia utafiti katika hospitali kuu ya Kenyatta na chuo kikuu cha Nairobi.

Nasaidiwa na Daktari .....(mtafitimsaidizi).Sisi tunafanya utafiti miongoni mwa wagonjwa wa saratani ya mdomo, koo na kipaza sauti ili kujua kama yeyote kati yao ana virusi vya Human Papillomavirus (HPV) katika chemichemi za saratani na ni sifa zipi zinapatikana kati ya wagonjwa walio na virusi hivyo. Hivi virusi ni sawa na virusi vinavyo sababisha saratani ya kizazi katika wanawake. Katika nchi zilizoendelea ambapo vipimo hivi hufanywa mara kwa mara, matokeo hutumiwa kuchagua aina bora ya matibabu yanayotoa matokeo mazuri. Katika mipangilio yetu, sisi hatufanyi vipimo hivi kwa sababu ya gharama ghali. Kama, kutokana na utafiti huu, tunaweza kuamua sifa maalum za wagonjwa walio na virusi kwa saratani yao, tunaweza kutumia sifa hizi kuchagua matibabu bora kwa kila mgonjwa. Tunaweza pia kuwa na uwezo wakupendekeza matumizi ya chanjo dhidi ya virusi kwa ajili ya kuzuia kama inavyofanyika kwa saratani ya kizazi. Wewe/mtoto wako/mtegemezi wako kuingizwa katika utafiti huu itakuwa kwa hiari.

Ni nini kinashirikishwa katika utafiti huu?

Punde baada ya kukubali kushiriki kwako/mtoto wako/mtegemezi wako, sisi tutashughulikia wewe /mtoto wako/ mtegemezi wako kwa njia ya kawaida tunavyofanya kwa wagonjwa wote wa saratani ya mdomo, koo nakipaza sauti. Taratibu za kawaida kwa wagonjwa wote wa saratani ya mdomo, koo au kipaza sauti ni pamoja na kuwauliza maswali kuhusu ugonjwa na dalili zake, kuwepo au matumizi ya vitu ambavyo vinaweza kuchangia kuwepo kwa ugonjwa wa saratani. Vipimo kwa walioathirika katika maeneo husika hufanywa. Sampuli za damu huchukuliwa kwa ajili ya kuangalia viini vya damu, pia kazi yaini na figo. Unaweza pia kupitia ushauri na saha kabla yakupima virusi vya ukimwi. Ushauri na saha baada ya kupima hufanyika kabla ya hali yako ya HIV kuwekwa wazi kwako. Kuthibitisha saratani, kipande kidogo cha nyama kinakuchukuliwa kutoka mahali pa saratani. Kama saratani ipo katika mahali ambapo panaweza kufikiwa kwa urahisi

kwa mgonjwa ambaye ni mtu mzima, dawa ya kugandisha mahali hapo inawekelewa au kudungwa na sampuli kuchukuliwa bila mgonjwa kulalishwa. Kama saratani iko mahali ambapo haiwezi kufikiwa kwa rahisi au mgonjwa anayeugua ni mtoto, mipango hufanywa kwa ajili ya sampuli kuchukuliwa katika chumba cha upasuaji wakati mgonjwa amelala usingizi. Sampuli huchukuliwa kutoka mahali pa saratani na kupelekwa maabara ili kuamua aina ya saratani kuwezesha matibabu sahihi kuanzishwa. Kwa madhumuni ya utafiti huu, sehemu ndogo ya sampuli itachukuliwa ili kufanyiwa vipimo zaidi ili kuthibitisha kama iko na virusi vya HPV. Sisi tukipata matokeo ambayo yanahitaji marekebisho ya matibabu yako /mtoto wako/mtegemezi wako, daktari watakushauri kuhusu mabadiliko kama inavyofaa.

Je, kuna hatari yoyote kushiriki?

Hakuna hatari inajulikana kutokana na vipimo vya ziada vinavyofanyika juu ya sampuli.

Je, kuna adhabu kwa kutoshiriki katika utafiti huu?

La, wagonjwa wote watapata matibabu sawa bila kujali kama wao walishiriki katika utafiti au la.

Faida gani mimi nitapata iwapo nitashiriki?

Wewe /mtoto wako/ mtegemezi wako huenda msiweze kupata faida yoyote moja kwa moja kwa kushiriki ingawa uchaguzi wa matibabu mtapokea inaweza kubadilika kulingana na matokeo ya vipimo vyenu. Matokeo ya utafiti pia itasaidia madaktari na hospitali kujua njia bora za matibabu kwa hali hii.

Je kuhusu usiri?

Taarifa zote tunapata tutaziweka kuwa siri.

Kiasi gani itanigharimu mimi?

Hakuna gharama yoyote ya ziada itahusishwa ila ile ya kawaida kwa wagonjwa wa saratani.

Je, umeridhika na maelezo yaliyotolewa?

Iwapo unayo maswali mengine yoyote, uko huru kuwasiliana na wafuatao kwa ajili ya ufafanuzi:

1. **Mpelelezi Mkuu:**
   Dk. Joyce Aswani,
   Idara ya upasuaji, Chuo Kikuu cha Nairobi,
   PoBox19676-00,202, Nairobi.
   Nambari ya simu:0722814483

2. **Wasimamizi:**
   I. Prof. Nimrod Mwang'ombe.
   Profesa na Mwenyekiti wa Neurosurgery,
   Chuo Kikuu cha Nairobi.

   II. Prof. Omu Anzala,
   Mkurugenzi Kavi, Profesa, Idara ya Microbiologia,
   Chuo Kikuu cha Nairobi.

3. **Mwenyekiti.,**
   KNH/UON ERC,
   HOSPITALI KUU YA KENYATTA

Kama umeridhika, jaza na kuweka sahihi makubaliano yanayofuata:

IDHINI YA KUSHIRIKI KWA UTAFITI

Mimi ..... Nambari ya kitambulisho.....

kutoka .....nakubali mimi/mtoto wangu/mtegemezi wangu

kushiriki katika utafiti huu ambao nimeelezewa kikamilifu na Daktari

.....

Mimi sijaahidiwa chochote cha kunifaidi kwa kushiriki.

Sahihi.....

Tarehe .....

APPENDIX II:
QUESTIONNAIRE

PATIENT DEMOGRAPHIC DATA

Study Identification No……

IP No…….

Age……Sex…….

Current County of residence …….

County where he/she has lived most of the last 10 years……

County of birth…….

Date first seen in ENT-HN / Maxillofacial clinic……

Referred ……. Self-referral……

Referring Health facility……

Date of biopsy…….....…….

SYMPTOMS

Symptom	Yes (1)	No (0)	Duration
Oral/oropharyngeal ulcer			
Oral /oropharyngeal mass			
Oral/oropharyngeal pain			
Oral/oropharyngeal leukoplakia			
Oral/oropharyngeal erythroplakia			
Hoarseness			
Cough			
Dysphagia			
Neck pain			
Ear pain			
Breathing difficulties			
Neck swelling			
Nasal blockage			
Epistaxis			
Hearing loss			
Others (specify)			

RISK FACTORS

Risk Factor	**Yes (1)**	**NO (0)**	**Quantity per week**	**From – till**
Cigarette smoking				
Reverse smoking				
Tobacco chewing				
Khat chewing				
Marijuana use				
Alcohol - beer				
Alcohol – spirits				
Alcohol – wine				
Alcohol – *chang'aa*				
Alcohol – unprocessed local brews				
Previous head & neck radiation				
Exposure to irritants (specify)				
GERD/LPR				
Others (specify)				

Sexual Characteristics

Characteristic	Yes (1)	No (0)
Sexually active		
Age at sexual debut		
Heterosexual		
Homosexual		
Bisexual		
Orogenital sex		
Anogenital sex		
Number of sex partners		

SIGNS

Primary Tumour

Tumour Site	**Subsite**	**T stage** **(clinical&/ radiological)**
Oral cavity	Lips	
	Palate	
	Buccal	
	Floor of mouth	
	Tongue	
Oropharynx	Tonsil	
	Base of tongue	
	Pharyngeal wall	
Nasopharynx	Lateral wall	
	Roof	
	Posterior wall	
Hypopharynx	Piriform sinus	
	Post-cricoid area	
	Pharyngeal wall	
Larynx	Supraglottis	
	Glottis	
	Subglottis	

Regional Nodes (Clinical & radiological)

Laterality	**Number**	**Size (Largest)**	**Levels**	**Cystic/Solid**	**N Stage**
Left					
Right					

HIV ELISA: Positive ……. Negative ……

Histology:

…….....

.....

.....

HPV PCR: …….

EBV PCR: …….

HPV TYPE: ……

APPENDIX III:
ETHICAL APPROVAL AND RENEWAL

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

10 SEP 2014

Ref: KNH-ERC/A/300 Link:www.uonbi.ac.ke/activities/KNHUoN 10th September 2014

Dr. Joyce Mmbone Aswani
Dept.of Surgery
School of Medicine
University of Nairobi

Dear Dr. Aswani

RESEARCH PROPOSAL: HIGH RISK HUMAN PAPILLOMAVIRUS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA PATIENTS AT KENYATTA NATIONAL HOSPITAL (P402/07/2014)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 10th September 2014 to 9th September 2015.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **www.uonbi.ac.ke/activities/KNHUoN.**

Protect to Discover

Yours sincerely

PROF.M.L. CHINDIA
SECRETARY, KNH/UON-ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Chair, KNH/UoN-ERC
The Assistant Director, Health Information, KNH
The Dean, School of Medicine, UoN
The Chairman, Dept.of Surgery, UoN
Supervisors: Prof. Omu Anzala, Prof. N. Mwang'ombe, Prof. Isaac Macharia

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Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

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P O BOX 20723 Code 00202
Tel: 726300-9
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Telegrams: MEDSUP, Nairobi

Ref. No.KNH/ERC/R/25

29th February, 2016

Dr. Joyce Mmbone Aswani
Dept.of Surgery
School of Medicine
University of Nairobi

Dear Dr. Aswani

Re: Approval of annual renewal –High Risk Human Papillomavirus in Head and Neck Squamous Cell Carcinoma patients at KNH (P402/07/2014)

Your communication of 11th February, 2016 refers.

This is to acknowledge receipt of the study progress report and hereby grant you annual extension approval for ethical research protocol P402/07/2014

The study renewal dates are 10th September 2015 –9th September 2016.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study.
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M.L. CHINDIA
SECRETARY, KNH-UON ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director CS, KNH
The Chair, KNH- UoN ERC

APPENDIX IV:
NIAAA GUIDELINES ON ALCOHOL CONSUMPTION

Class of drinking	**Drinks/week (Female)**	**Drinks/week (Male)**
Non-drinker	None	None
Light drinker	≤ 3	≤ 3
Moderate drinker	3 – 7	3 -14
Heavy drinker	7 – 14	14 – 21
Very heavy drinker	> 14	> 21

APPENDIX V:
STAGE GROUPING FOR HNC

Stage grouping	Primary tumour (T)	Regional nodes (N)	Distant metastases (M)
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T3	N0	M0
	T1	N1	M0
	T2	N1	M0
	T3	N1	M0
Stage IVa	T4a	N0	M0
	T4a	N1	M0
	T1	N2	M0
	T2	N2	M0
	T3	N2	M0
	T4a	N2	M0
Stage IVb	T4b	Any N	M0
	Any T	Any 3	M0
Stage IVc	Any t	Any N	M1

APPENDIX VI:
WHO HISTOLOGICAL GRADING FOR SQUAMOUS CELL CARCINOMA

GRADE I: Well-differentiated squamous cell carcinoma

GRADE II: Moderately differentiated squamous cell carcinoma

GRADE III: Poorly differentiated squamous cell carcinoma

GRADE IV: Undifferentiated squamous cell carcinoma

APPENDIX VII:
NASOPHARYNGEAL CARCINOMA WHO HISTOLOGICAL TYPES

TYPE I: Keratinizing

TYPE II: Non-keratinizing

TYPE III: Undifferentiated