UNIVERSITY OF NAIROBI

# INCIDENCE OF HUMAN PAPILLOMAVIRUS INFECTION IN ESOPHAGEAL SQUAMOUS CELL CARCINOMA AMONG YOUNG INDIVIDUALS IN KENYA: A RETROSPECTIVE LOOK

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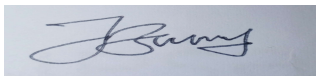
**A DISSERTATION SUBMITTED IN PART FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF MEDICINE IN RADIATION ONCOLOGY AT THE UNIVERSITY OF NAIROBI**

**DECLARATION**

I declare that this dissertation is my original work and has never been published or presented for a degree in any other University.

Signed:  Date: 14/08/2023

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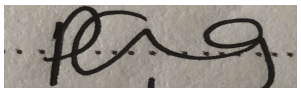
Signed:  Date: 14/08/2023

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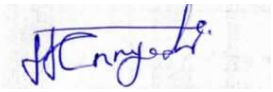
Signed:  Date: 14/08/2023

**DEDICATION**

This study is dedicated to my lovely wife Meg Night, my children Limma and Liam Raduma, my mother Grace Akinyi, my research assistant Nina Okumu, my sister Petty Otieno and my entire family, my friends and colleagues for their continuous support and encouragement since starting this Master's program as well as during this study. God bless you all.

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| | |
|---|---|
| cDNA | Circular Deoxyribonucleic Acid |
| CSFIR | Colony stimulating factor-1 receptor |
| DNA | Deoxyribonucleic acid |
| EC | Esophageal Carcinoma |
| EGFR | Epidermal growth factor receptor |
| ESCC | Esophageal Squamous Cell Carcinoma |
| GERD | Gastro-esophageal reflux disease |
| GLOBOCAN | Global Cancer Incidence, Mortality and Prevalence |
| HPV | Human Papillomavirus |
| HR-HPV | High risk Human Papillomavirus |
| KNH | Kenyatta National Hospital |
| LR-HPV | Low risk Human Papillomavirus |
| MTRH | Moi Teaching and Referral Hospital |
| OGD | Oesophago-Gastro-Duodenoscopy |
| OSCC | Oropharyngeal Squamous Cell Carcinoma |
| PCR | Polymerase Chain Reaction |
| PDGFßR | Platelet derived growth factor-ß receptor |
| PVs | Papilloma viruses |
| Rb | Retinoblastoma tumor suppressor gene |
| SPSS | Statistical package for the social sciences |
| STD | Sexually transmitted disease |

**ABSTRACT**

**Background:** Esophageal cancer is the fourth most common cancer and the third leading cause of cancer mortality in Kenya. Human papillomavirus (HPV) types 16 and 18 are known to cause majority of squamous cell carcinomas of the cervix, vulva, anus, and oropharynx. East Africa has a high incidence of squamous cell carcinoma of the esophagus similar to the Asian esophageal belt without clear etiology. There is limited information on the association of HPV and esophageal carcinoma in Kenya and the topic remains controversial.

**Broad objective**: To determine the frequency of HPV infection in ESCC in young individuals through p16 staining.

**Methods:** This was a retrospective, cross-sectional study conducted on patients with biopsy proven squamous cell carcinoma of the esophagus at Kenyatta National Hospital. After IRB approval, we reviewed the charts of a young population, 20-50 years old, who received therapy for esophagus cancer at the cancer treatment center from 2016 to 2020. Patients with non-squamous histology, incomplete medical records, or those without available tissue specimen were omitted. P16 immunohistochemistry staining was performed on slides created from paraffin embedded tissue blocks obtained from tissue repository. Statistical analysis was done in SPSS version 21.0. Chi square and T-test were used to assess statistical significance of distribution by tumor site and other factors in demographics. Incidence presented as percentage and baseline characteristics and distribution of tumor by location and geography presented using tables, pie charts and bar graphs.

**Results:** A total of 51 participants met inclusion with available specimen for review. The majority of patients were from Eastern and Central Provinces of Kenya with the majority of patients not having a history of tobacco use (60.8%) or alcohol consumption (66.7%). The median age was 46 years (range, 20-50) and male to female ratio of 1.7:1. Most patients had tumors located in the middle thoracic esophagus on endoscopy (37.3%). All patients had T3 (46.5%) or T4(53.5%) disease. The node positive rate was 55.8%. 9.8% of the participants stained positive for P16 on immunohistochemistry. There was no statistical difference in patient and tumor characteristics for patients with p16 positive vs. negative tumors.

**Conclusion:** While the availability of biospecimens was limited, we observed 9.8% incidence of positive P16 esophagus squamous cell carcinoma from a young patient population in Kenya. Our report is the first that shows positive staining for p16 amongst esophagus cancer patients in Kenya.

# CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

## 1.0 Introduction

Cancer of the esophagus is the seventh most common cancer globally with approximately 604,000 new cases and 544,000 deaths in 2020 (Hyuna Sung PHD, 2021).Approximately 70% of esophageal cancer cases and deaths occur in men which translates to approximately two to three-fold difference in incidence and mortality between men and women (Chun Quan Liu, 2022). Highest rates of esophageal cancer have been reported in Eastern Asia, in the so-called Asian esophagus belt. Additionally, high rates are observed in Eastern region of Sub-Saharan Africa (Hyuna Sung PHD, 2021).

In Kenya, esophagus cancer made up approximately 7.1% of new cancer cases in both sexes in 2020 and was responsible for 10.1% of all cancer deaths (Hyuna Sung PHD, 2021). Esophageal cancer is the fourth most common cancer in Kenya after cancers of the breast, the cervix, and the prostate. Esophageal squamous cell carcinoma (ESCC) is the most common histologic type of esophageal cancer globally (Douglas A Corley, 2001)and it accounts for more than 90% of esophageal cancer cases in Kenya (Michael Cheng, 2015). ESCC has been strongly linked with tobacco use and excessive alcohol consumption (Chung S. Yang, 2016), which is associated with approximately 90% of ESCC cases globally. Other risk factors postulated to be associated with this type of cancer include dietary factors especially low intake of fruits and vegetables, deficiency of micro-nutrients, and physical factors such as consumption of beverages at high temperatures (Joab Otieno Odera, 2017).

Emerging data also suggest involvement of certain microorganisms in the development of cancer of the esophagus. Certain viruses, such as Epstein-Barr virus and human papillomavirus, have been demonstrated to play a role in the onset of a variety of human cancers. These viruses are known to infect the esophageal epithelium (Fuju Chang, 1992). High risk subtypes of human papillomaviruses are known to be responsible for the development of squamous cell carcinomas of the cervix (Nubia Munoz, 2003). More recently, evidence shows a strong link with cancers of the head and neck, in particular the cancer of the oropharynx (Chiocca, 2019).

In terms of pathogenesis, HPV oncogene products, that is the early proteins E6 and E7 have been studied extensively to establish their role in HPV-associated carcinogenesis. These two proteins

negate the functions of p53 and retinoblastoma tumor suppressor gene, respectively (Hausen, 2002). The binding of E7 protein to Rb causes its degradation leading to the release of E2F, which translates to $p16^{INK4A}$ overexpression, also denoted as p16.Consequently, tumors which are HPV-positive are permeated by high expression of p16. This makes p16 to be widely considered as a surrogate marker for HPV infection (Serge J. Smeets, 2007)

With a median age of 56 years, the age at diagnosis in Kenya is significantly younger than that reported in other parts of the world, such as North America, Europe, Australia, and Asia, where the median age is greater than 65 years (Machoki MS, 2015) (Qiao-Li Wang, 2018) (Zhong Ying Shen S. P., 2002) (Fangli Cao H. H., 2014). Additionally, the use of tobacco and alcohol is infrequent in the Kenyan culture. This study aimed to explore the possibility of human papillomavirus as a risk factor in ESCC in young patients in Kenya

### 1.1 Biology of Human papillomavirus

Human papillomaviruses belong to the family Papillomaviridae. This family consist of non-enveloped small double-stranded DNA viruses. These viruses usually infect the basal layer cells of the epithelium of the mucous membrane and skin. Various subtypes of human papillomaviruses have been identified. They are associated with the development of both benign and malignant growths.

Papillomaviruses are broadly grouped into three main types. The first group considered to be mostly mucosotropic are termed Alphapapillomaviruses. The second category are viruses which are mostly cutaneous referred to as Betapapillomaviruses. The last group are found in both cutaneous and mucosal sites and are known as Gammapapillomaviruses (Christensen, 2016). HPV associated with human tumors can be considered as either low-risk types or high-risk types 16, 18, 31, 33, 34, 35, 39, 45, 51 among others. The high-risk subtype 16 has been identified in many studies as the most prevalent subtype (Nwokolo NC, 2009).

The HPV genome is made up of a double-stranded circular DNA which encodes the DNA sequences for both early and late proteins. There are about six identified early proteins and two late proteins found on the viral capsid. The early proteins include E1, E2, E4, E5, E6, and E7 proteins with the E1 and E2 proteins being responsible for the viral replication and translation.

E2 has also been shown to be associated with the regulation of the expression of both E6 and E7 proteins. The assembly of the viral proteins and stimulation of growth is controlled by both E4 and E5 proteins (Nwokolo NC, 2009).

### 1.2 Pathogenesis

The life cycle of human papillomavirus is unique and different from that of other viral families. For replication to occur, the virus has to infect cells that are capable of proliferation, thus it commonly affects the basal layer cells found within the mucosal and epidermal epithelium. Infection by the virus requires a break in the normal epithelial lining which is normally facilitated by trauma or abrasions. Once the virus has entered the basal layer, it triggers the expression of viral genes that stimulate proliferation. There are three genes which have been identified to be stimulants of proliferative activity, they include E5, E6 and E7. Initiation of viral replication as well as translation is controlled by the proteins E1 and E2. The E6 and E7 proteins negate the functions of p53 and retinoblastoma tumor suppressor gene, respectively. Binding of E7 protein to Rb causes its degradation leading to the release of E2F, which translates to $p16^{INK4A}$ overexpression. (Hausen, 2002).

### 1.3 Mode of transmission

HPV infection is commonly transmitted via sexual contact; however, nonsexual transmission has also been documented. Strong evidence suggests both vertical and horizontal transmission of HPV in cancer of the cervix. Infants can acquire HPV infections by birth while children and adults require close contact to get infected (Sasidharanpillai Sabeena, 2017).

The factors that increase HPV infection include high risk sexual behaviors such as early sexual debut, having multiple sexual partners, practicing orogenital sex, unprotected sex, concurrent STD particularly Chlamydia infection, alcohol consumption, smoking, and oral contraception use in women (Anne M Johnson, 2012). Low socioeconomic status has also been postulated to increase the risk. Nevertheless, in most cases, subclinical infection occurs and is cleared by the host immune mechanisms. It is the persistence of HPV infection that increases the likelihood of malignant transformation (Hausen, 2002).

### 1.4 HPV Detection Methods

The difficulty in routine cultivation of HPV virus makes the establishment of infection by human papillomavirus to be hinged primarily on the detection of the genome of the virus by molecular biological methods. Because of the well outlined physical structure and organization of its genes, detection of HPV in specimens is based on nucleic probe technique (Andre L P Abreu, 2012).

The three commonly used methods are nucleic acid-hybridization assays, nucleic acid amplification assays, and signal amplification assays.

### 1.5 $P16^{INK4A}$ Immunohistochemistry

P16 protein is also referred to as multiple tumor suppressor 1 or cyclin dependent kinase inhibitor 2A. It is usually encoded by CDKN2A gene which is a tumor suppressor gene. The p16 protein routinely binds to and inhibits cyclin D dependent kinases CDK 4 and CDK 6 ergo maintains Rb in its hypophosphorylated state. This in turns leads to failure of dissociation of Rb from E2F transcription factor and halts the progression of the cell cycle. Studies have reliably shown that p16 can be used as a surrogate marker for high-risk HPV infection (MD, 2016). P16 over-expression is strongly related to active HPV infection based on the concept that functional inactivation of Rb by E7 induces p16 up-regulation.
HPV p16 immunohistochemical test has been demonstrated to have a remarkable sensitivity and specificity of 100% and 79% respectively (Serge J. Smeets, 2007). The accuracy of p16 staining was explored in a study of oropharyngeal squamous cell carcinoma. The results revealed a sensitivity of 94% and specificity 83% (Elena-Sophie Prigge, 2016)

### 1.6 Human Papillomavirus and esophageal cancer

Reports of involvement of human papillomavirus infection in the cancer of the esophagus were first suggested as early as in the 1980s (KJ., 1982). However, to date controversy still lingers as to the specific role played by the virus in the development of EC. Presence of high-risk HPV types in esophagus cancer squamous variant have been explored with reported figures of 11.67% for the high-risk subtype 16 and 1.82% for subtype 18 (Fang Yong, 2013).

X.Li et al. carried out a systematic review with meta-analysis in 2013. This analysis included a total of 8,990 ESCC patients from 67 studies. Of these, 46 studies were conducted in Asia, 19 in Europe and America, and 2 in Africa. HPV prevalence in ESCC was 22.2% for all patients. The prevalence rates were significantly higher in the studies that were conducted in Asia with a reported figure of 26.3% than those from Europe and America noted as 14.0%. Prevalence by tumor location was 18.3% for the upper part, 17.9% for the middle part, and 15.7% for the distal part of the esophagus. The conclusion from this series was that an evident heterogeneity existed between the studies that were included. The strength of the association depicted was relatively weak as compared to that seen in the cancers of the cervix and larynx. It is noteworthy that this analysis was marred by language bias as the included studies were only those published in English (X Li, 2014).

In Anyang province in China, a case control study performed by Guo et.al demonstrated a higher presence of HPV DNA in ESCC cases than controls with prevalence rates of 31% and 6.8% respectively. Their study also tested for the presence of HPV16E7 antibody in the serum with a higher positivity rate seen among the cases than the controls (17% vs 3.1%). Therefore, they concluded that the existence of serum antibody to HPV-16 E7 oncoproteins as well as HPV infection in the esophagus was associated with increased risk of cancer of the esophagus. This was especially true when infection involved ‘’oncogenic’’ types of HPV (Fangcen Guo, 2012).

Another study conducted in China attempted to investigate the relationship between infection with HPV and ESCC in the backdrop of existence of other well-known risk factors for this type of cancer. Some of the risk factors considered in this study included cigarette smoking and alcohol consumption. There was a significant increase in the development of squamous cell cancer of esophagus in patients who tested HPV positive and also consumed alcohol and smoked cigarette. The authors of this study thus concluded that HPV infection was not an independent risk factor for the development of ESCC (Z.Qi, 2013).

Swiss investigators carried out a case-control study conducted among the population in Switzerland aiming to identify high-risk HPV subtypes in EC. They included patients with both esophageal squamous cell carcinoma and adenocarcinoma of the esophagus as well as the

gastroesophageal junction. This study failed to show a positive association between HPV and either types of esophageal cancer (Jesper Lagergren, 1999).

In Africa, a prospective study conducted in Malawi by Geßner et.al in 2010 included 55 patients undergoing endoscopic biopsy for dysphagia. 40 patients had ESCC, 3 patients had dysplastic epithelium and 12 patients displayed normal epithelium. HPV DNA was detected using Multiplex Quantitative PCR assay and in situ hybridization. P16 testing was done as a surrogate marker for oncogenic activity. They reported a prevalence rate of HPV in ESCC of 15%. This study also demonstrated that positive p16 staining corresponded with the presence of HPV DNA (Anja Lidwina Gebner, 2018).

In Kenya, a study done in the Southwestern region evaluated biopsies taken from 29 patients with pathology confirmed diagnosis of esophageal squamous cell cancer. They employed the use of reverse line blot PCR to detect HPV DNA sequences. In this study, 20 subjects were male while 9 were females, with a mean age of 58years. This study failed to demonstrate any linkage between HPV and ESCC (R.E White, 2005). The result of this study could have largely been limited by the small sample size.

HPV as a significant etiologic agent in ESCC remains a topic of controversy and continues to elicit debate with attention shifting towards its role in prognostication of cancer of the esophagus. Attempts by several researchers have been documented evaluating the prognostic role of HPV-ESCC infection. One such study discovered that infection with HPV was associated with a decrease in response to treatment as well as a decrease in the overall survival. Infection with HPV has been demonstrated to be a negative prognostic factor in patients with ESCC (Laura Bognar, 2018). A contrary outcome was shown in a study conducted in northern China with HPV-positive ESCC patients doing better (Fangli Cao H. H., 2014).

### 1.7 Conclusion

Human papillomavirus has been identified as a causative agent in many human cancers such as cancer of the cervix, oropharynx, anus, vulvovaginal and penile cancers, as well as genital warts.

Its role in the etiology of cancer of the esophagus still remains a topic of controversy with the existing body of literature supporting and disputing its influence in equal measure.
Locally, limited literature exists on the prevalence of human papillomavirus in cancer of the esophagus with studies conducted having a small sample size to draw a significant conclusion from. This study therefore aims to bridge the existing literature gap as well as include a bigger sample size to effectively explore the role of HPV in squamous cell carcinoma of the esophagus.

## CHAPTER TWO: JUSTIFICATION AND OBJECTIVES

### 2.1 Study Justification

High-risk human papillomaviruses have been identified as causal agents in cancers of some subsites in the human body. These include the cancers of perineal and genital region, head, and neck region among others. (Marc Arbyn, 2012).More than 90% of cervical and anal malignancies, about 70% of female genital cancers, 70% of cancers of oropharynx as well as more than 60% of cancers of the penis have been attributed to human papillomavirus infection (Marc Arbyn, 2012).

Studies of head and neck malignancies have elicited the involvement of human papillomavirus in the pathogenesis of some oropharyngeal cancers with the prevalence ranging between 5-70% (Aimee R Kreimer, 2005).

The lining of the oral mucosal epithelium is in direct continuity with that of the esophageal mucosal epithelium and therefore it is postulated that infection of HPV may involve the esophagus via oral route. Indeed, initial descriptions of HPV involvement of oral mucosa occurred in the early 1980s (Syrjänen, 1987).Prior to that, there had been earlier suggestions that the virus was responsible for development of malignant as well as benign lesions of the esophagus (KJ., 1982) (Syrjänen K, 1982).

Diverging evidence exists in regards to the relationship between HPV and cancer of the esophagus. Example of negative relation is given of study conducted in Sweden which revealed absence of any role of high-risk HPV in the etiology of either form of cancer of the esophagus (Jesper Lagergren, 1999), and a review study that concluded lack of association of HPV with ESCC despite global HPV-ESCC rates that range between 11.7% to 38.9% (Ethan B.Ludmir, 2015).

Positive association was demonstrated by X.Li et al. in a systematic review of literature which showed a HPV prevalence in ESCC of 22.2%. HPV16 was the most frequently observed subtype (X Li, 2014) however, the study did not relate the age at diagnosis with HPV infection.

In Kenya, limited studies have been conducted on esophageal cancer and human papillomavirus infection. One such study conducted by Patel et al at MTRH failed to detect 17 types of HPV in 29 samples of EC. The same conclusion was reached in samples from Tenwek Mission Hospital (R.E White, 2005). However, the small sample sizes likely impacted determination of true prevalence of HPV infection in ESCC.

While smoking of cigarette and excessive alcohol use have been strongly shown to be risk factors of esophageal cancer, the high numbers of EC in young patients who do not drink and smoke in Kenya has prompted the need to establish the existence of another explanation for this unique epidemiologic picture. This study thus aims to explore the possibility of human papillomavirus being a risk factor for the rise of esophageal cancer among young people in Kenya.

Determination of a strong relationship between HPV infection and cancer of the esophagus could lead to the intensification of vaccination against HPV infection.

**2.2 Significance of the study**

Cervical cancer association with HPV is the most studied with the consequence being the development of immunization against this virus. This study aims to demonstrate the role of HPV infection in evolution of cancer of the esophagus and thus propose that immunization may be of benefit to more high-risk populations besides women for cervical cancer.

Concomitantly, the study aims to give an insight into the rising prevalence of cases of esophageal carcinoma among young individuals in Kenya.

Previous studies have investigated the prognostic significance of human papillomavirus infection in ESCC with HPV-positive patients responding poorly to multimodal therapy and having shorter survival compared to HPV-negative patients (Laura Bognar, 2018). This study aims to provide evidence of association between infection with HPV and ESCC locally and consequently provide a platform for future studies to explore its prognostic influence on esophageal cancer patients in Kenya.

Detection of HPV association in head and neck cancer patients have led to changes in the staging system and treatment standards as HPV–linked cancers have favorable outcomes (Liam

Masterson, 2014). For example, studies in head and neck are exploring less intensive treatment protocols for HPV-associated malignancies of the oropharynx. Evidence of HPV involvement in ESCC could lead to evaluation and revision of the treatment protocols for cancer of the esophagus.

### 2.3 Research question

What is the frequency of Human Papillomavirus infection in esophageal squamous cell carcinoma in young individuals in Kenya?

### 2.4 Research Hypothesis

There is a high number of Human Papillomavirus infection in esophageal squamous cell carcinoma in young individuals in Kenya

### 2.5 Study Objectives

#### 2.5.1 Broad objective

To determine the frequency of HPV infection in ESCC in young individuals in Kenya through p16 staining.

#### 2.5.2 Specific objectives

1. To determine the incidence of HPV-ESCC in young individuals in Kenya.
2. To determine the distribution of HPV-ESCC based on the tumor location.
3. To determine the geographical distribution of the HPV-ESCC cases.

# CHAPTER THREE: METHODOLOGY

## 3.1 Study design

This was a retrospective cross-sectional study from 2016-2020 looking at the presence of HPV infection in esophageal squamous cell carcinoma patients using a surrogate marker (p16) for HPV infection.

## 3.2 Study site

The study was conducted at the cancer treatment center and histopathology department in Kenyatta National Hospital in Nairobi Kenya with p16 immunohistochemistry test outsourced to Lancet Pathology Kenya. KNH is one of the two national referral hospitals in the country. It is also a teaching hospital of the University of Nairobi College of Health Sciences as well as the Kenya Medical Training College.

The hospital is situated in the area to the immediate west of Upper Hill in Nairobi about 3.5 kilometers to the West of the city's central business district.

## 3.3 Study population

This study included patients with pathologically confirmed esophageal squamous cell carcinoma from 2016 to 2020 meeting the inclusion criteria.

### 3.3.1 Inclusion criteria

1. Pathologically confirmed esophageal squamous cell carcinoma.
2. Age between 20 to 50 years.
3. Diagnosis made within the duration of interest (2016-2020).

### 3.3.2 Exclusion criteria

1. Patients who did not have signed consent form.
2. Non- squamous histology.
3. Incomplete medical records.

### 3.4 Sample size

The study included all the patients who met the inclusion criteria during the period starting January 2016 to December 2020.

### 3.5 Sampling Method

A census of all esophageal squamous cell carcinoma (ESCC) patients during the period of the study was conducted.

### 3.6 Study Tool

The study employed the use of a data collection tool that contained the patients' demographic data, residence, smoking, and alcohol use history, tumor location on endoscopy, and the stage and p16 status.

### 3.7 Study procedure

The principal investigator and two research assistants reviewed the health records data at the cancer treatment department from 2016 to 2020 to identify patients with a pathologically confirmed diagnosis of esophageal squamous cell carcinoma and meeting the study's inclusion criteria. A study tool containing the demographic data, residence, risk factors, and tumor location on endoscopy was then filled based on information on the patient's chart. The histology number was retrieved and a pathologist engaged to trace the tissue blocks from the histopathology laboratory at KNH as well as the pathologists at four peripheral laboratories. This was followed by screening for adequacy of tissue for staining.

#### 3.7.1 p16 immunohistochemistry procedure

Formalin fixed paraffin embedded tissue was cut at a thickness of 4 μm. Known positive control and test tissue was placed on the same immunistochemistry adhesive charged slides. The slides

were then dried on a hotplate at 60 degrees Celsius for five minutes. Labels were affixed on the slides. Tissue was deparaffinized by heating at 75 degrees for four minutes then antigen retrieval was achieved by use of cell conditioning buffer 1(CC1) at 95 degrees Celsius for sixty-four minutes. Primary antibodies, CINtec p16INK4a, clone E684 was added and ultraView Universal DAB Detection Kit polymer amplification system (Benchmark Ventana XT machine) was used according to manufacturer's instructions. Sections were washed with distilled water and counterstained with hematoxylin then rehydrated with graded alcohol. Drying was done and mounted with DPX for analysis.

### 3.7.2 P16 immunohistochemistry reporting

1. Strong and uniform p16 staining (both cytoplasmic and nuclear) in more than 50% of tumor cells was considered as positive and less than 50% expression was considered as negative for statistical analysis.
2. Absent or weak p16 staining in neoplastic cells was considered as negative

## 3.8 Data protection and management

This study involved health records review, specimen processing, and staining, and results recording.

Completed research tools were collected and stored in a locked cabinet by the principal investigator during data collection. An excel sheet was used to enter the information that was processed and cleaned from the completed data collection tools. A statistician was then given the coded data in the excel sheet for analysis.

All data was stored on password-protected drives. Only study personnel had access to the data.

<u>Statistical analysis</u>

Data was analyzed using the computer package SPSS version 22. Descriptive analyses with means for continuous variables and frequencies and percentages for categorical variables was

performed. Chi-square and t-tests was used to assess the statistical significance of differences by tumor site and other factors in demographic and clinical factors.

**Table 3.1: Showing study variables**

| **Outcome variable** | **Description** |
|---|---|
| HPV status | Positive or negative |
| **Exposure variable** | |
| Age | 20-29; 30-39; 40-50 |
| Gender | Male or Female |
| Tumor location | AJCC classification<br>Cervical esophagus (15-20cm)<br>Upper thoracic esophagus (>20-25cm)<br>Mid thoracic esophagus (>25-30cm)<br>Lower thoracic esophagus (>30-40cm) |
| Residence | Urban or rural |
| Ethnicity | Kikuyu, Luos, Kamba, Ameru, Somali, etc |
| Alcohol use | Yes/No |
| Tobacco use | Yes/No |
| HIV status | Positive, Negative, Unknown |
| Tumor stage | I, II, III or IV |

### 3.9 Ethical consideration

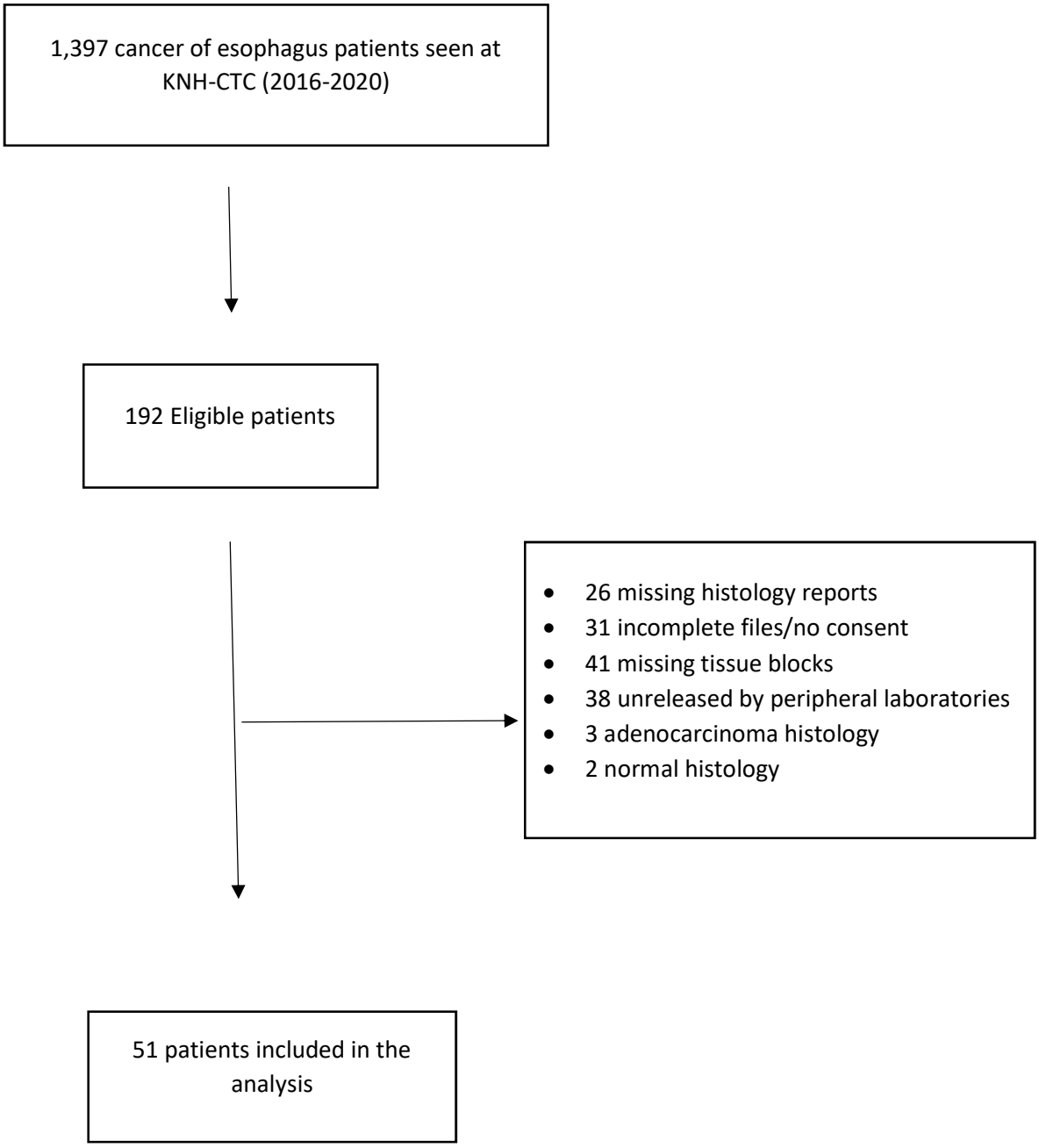
This study was conducted after a review of the study proposal and approval was done by KNH/UoN Ethical Review Committee. Only patients who had written informed consent were included in this study. Patient confidentiality and anonymity was maintained at all times with patient data only accessible to authorized personnel.

## CHAPTER FOUR: RESULTS

### 4. 1 Patients' recruitment flow chart

A total of 1,367 patients with esophageal cancer were seen at the Kenyatta National Hospital cancer treatment center between 2016 to 2020. After review of ineligible patients, a total of 51 patients were included (fig. 4.1).

**Figure 4.1 Patients' recruitment flow chart**



### 4.2 Baseline characteristics

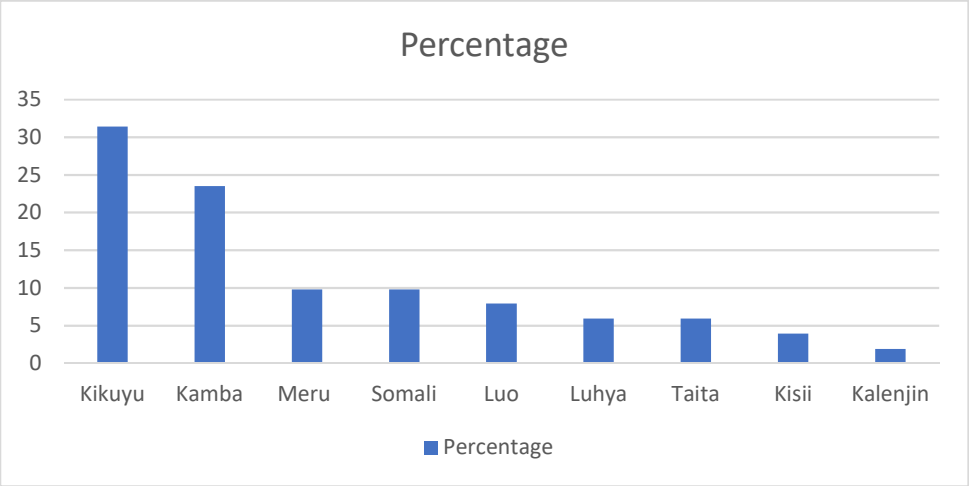
The mean age was 43.7 (SD 6.7) years with a median age of 46 (range; 20-50) years. The male to female ratio was 1.7:1. The middle thoracic esophagus was the most common site of tumor location on endoscopy. See table 4.1 below.

**Table 4.1: Baseline characteristics of the study patients and their tumor characteristics**

| Characteristics | Total (N=51) no. (%) | HPV Positive (N=5) no. (%) | HPV Negative (N=46) no. (%) | P value |
|---|---|---|---|---|
| Gender | | | | |
| Male | 32(62.7) | 5(100) | 27(58.7) | 0.143 |
| Female | 19(37.3) | 0(0) | 19(41.3) | |
| Age | | | | |
| Median (range) | 46 (20-50) | 41(35-46) | 46.5(20-50) | 0.056 |
| Tumour location | | | | |
| Cervical | 12(23.5) | 1(20) | 11(23.9) | 0.664 |
| Upper thoracic | 10(19.6) | 1(20) | 9(19.6) | |
| Middle thoracic | 19(37.3) | 1(20) | 18(39.1) | |
| Lower thoracic | 10(19.6) | 2(40) | 8(17.4) | |
| T status | | | | |
| T3 | 20(46.5) | 4(80) | 16(42.1) | 0.167 |
| T4 | 23(53.5) | 1(20) | 22(57.9) | |
| N status | | | | |
| N0 | 19(44.2) | 3(60) | 16(42.1) | 0.758 |
| N1 | 14(32.5) | 2(40) | 12(31.6) | |
| N2 | 8(18.6) | 0(0) | 8(21.1) | |
| N3 | 2(4.7) | 0(0) | 2(5.3) | |
| M status | | | | |
| M0 | 31(72.1) | 4(80) | 27(71.1) | 1.000 |
| M1 | 12(27.9) | 1(20) | 11(28.9) | |
| TNM Stage | | | | |
| II | 10(23.3) | 2(40) | 8(21.1) | 0.816 |
| III | 10(23.3) | 1(20) | 9(23.7) | |
| IV | 23(53.4) | 2(40) | 21(55.2) | |

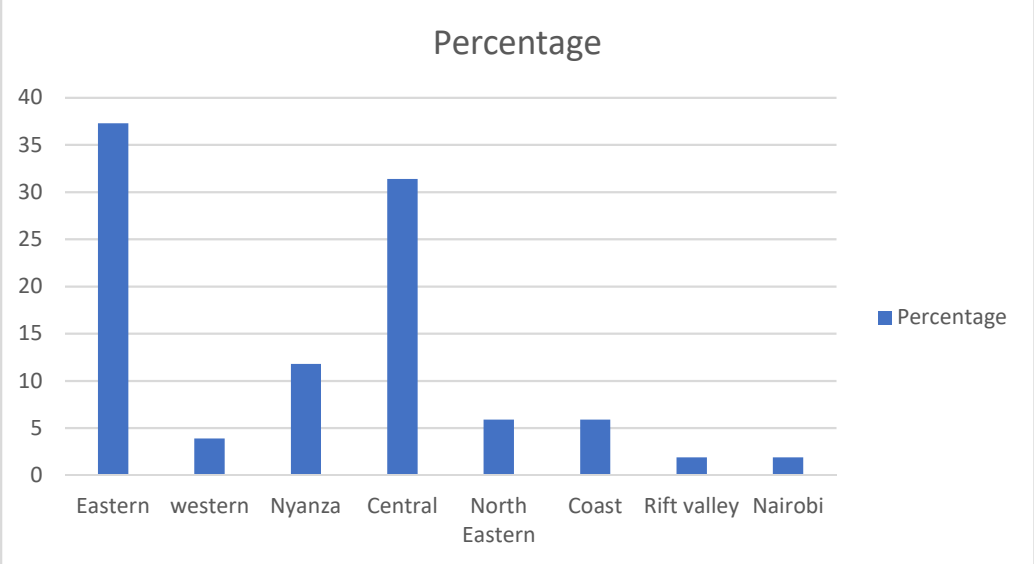
In terms of tribe distribution, majority of the participants were Kikuyu at 31.4% (16), followed by Kamba 23.5% (12) while Kalenjin at 1.9% (1) was the least represented as shown in the figure 4.2 below.

**Figure 4.2: Ethnicity distribution**




Geographical distribution analysis showed 37.3% of the patients were from formerly Eastern Province with Nairobi and Rift valley provinces having the least number of patients in the study at 1.9% respectively.

**Figure 4.3: Geographical distribution**



Majority of the patients were HIV negative (76.5%) while 17.6% had unknown status. 39.2% of them smoked tobacco while 33.3% consumed alcohol.

**Figure 4.4: HIV status**

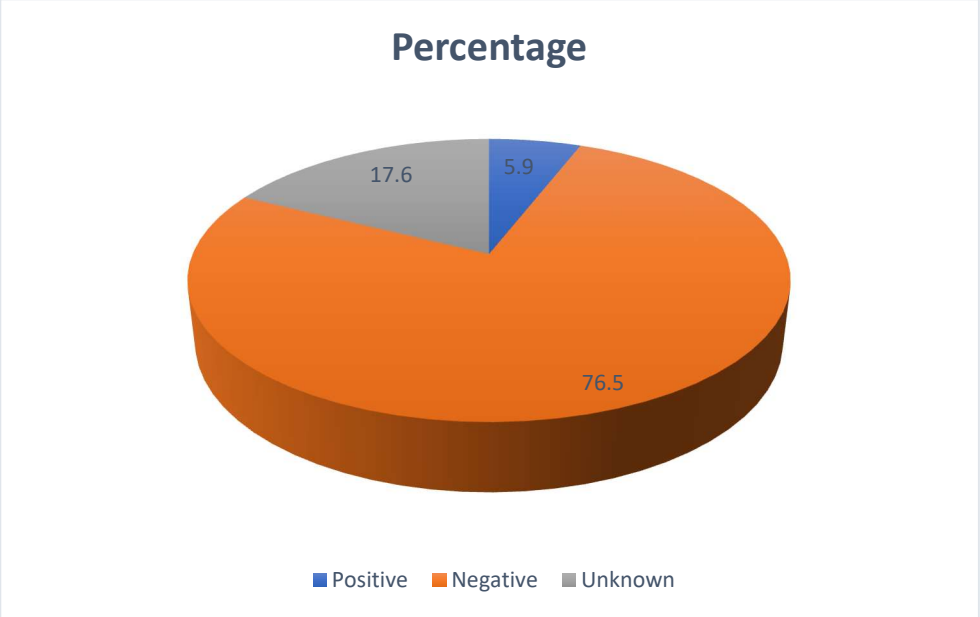
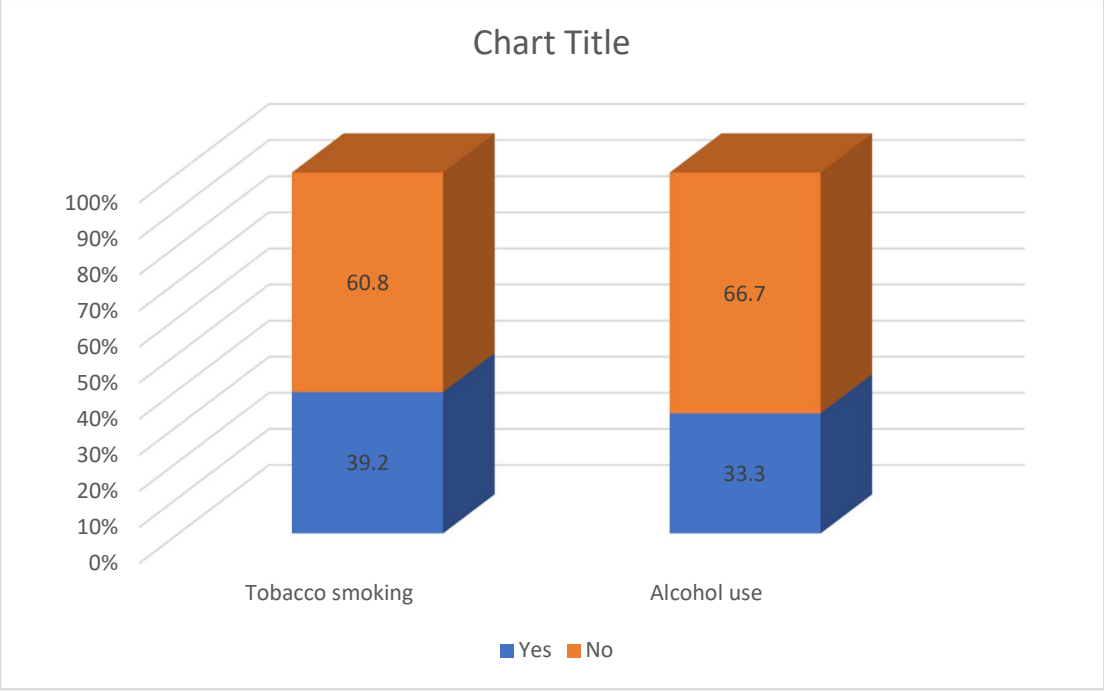



**Figure 4.5: Tobacco and alcohol use**



### 4.3: HPV analysis

A total of 5 patients (9.8%; 95% CI, 4.3-21) of the 51 patients with ESCC stained positive for p16 immunohistochemistry (fig.4.6). No difference was observed in the baseline characteristics of the HPV positive and negative groups (Table1). The median age of the HPV-positive group was 41(range; 35-46) years vs 46.5(range; 20-50) years in the HPV negative group (p=0.056). All patients with p16 positive staining were male with tumor located in the cervical esophagus (n=1), upper thoracic (n=1), middle thoracic (n=1) and lower thoracic (n=2). The geographical distribution of patients with positive p16 staining include; Central province (n=2), Eastern province(n=1), Western province (n=1), and Nyanza province (n=1). Most patients with p16 positive cancer were HIV negative (80%) or unknown status (20%) and without significant tobacco or alcohol use history (80%).

**Figure 4.6: HPV incidence**

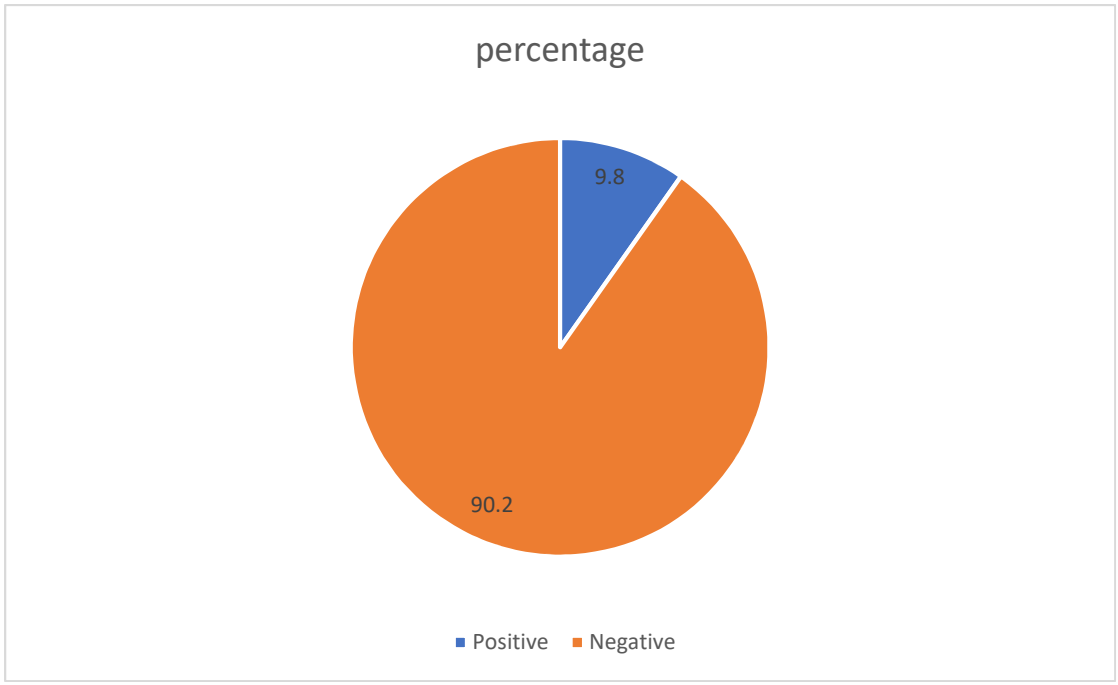


**Figure 4.7: HPV-ESCC distribution by tumor location**

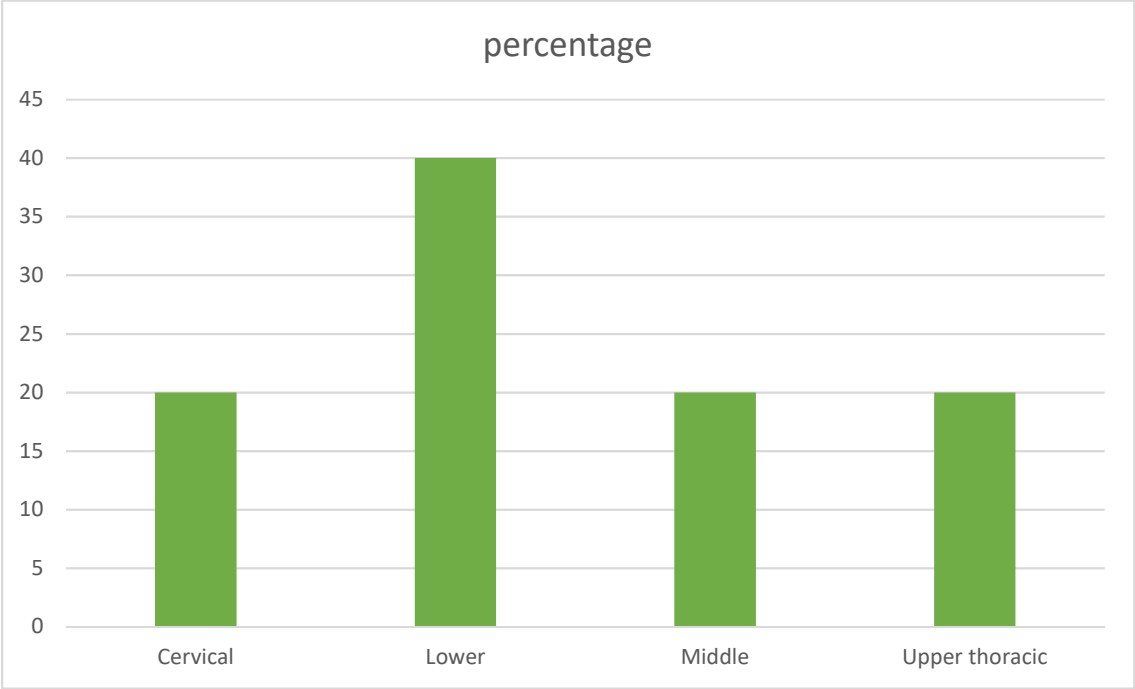



**Figure 4.8: Distribution of HPV-positive cases by geographical region**

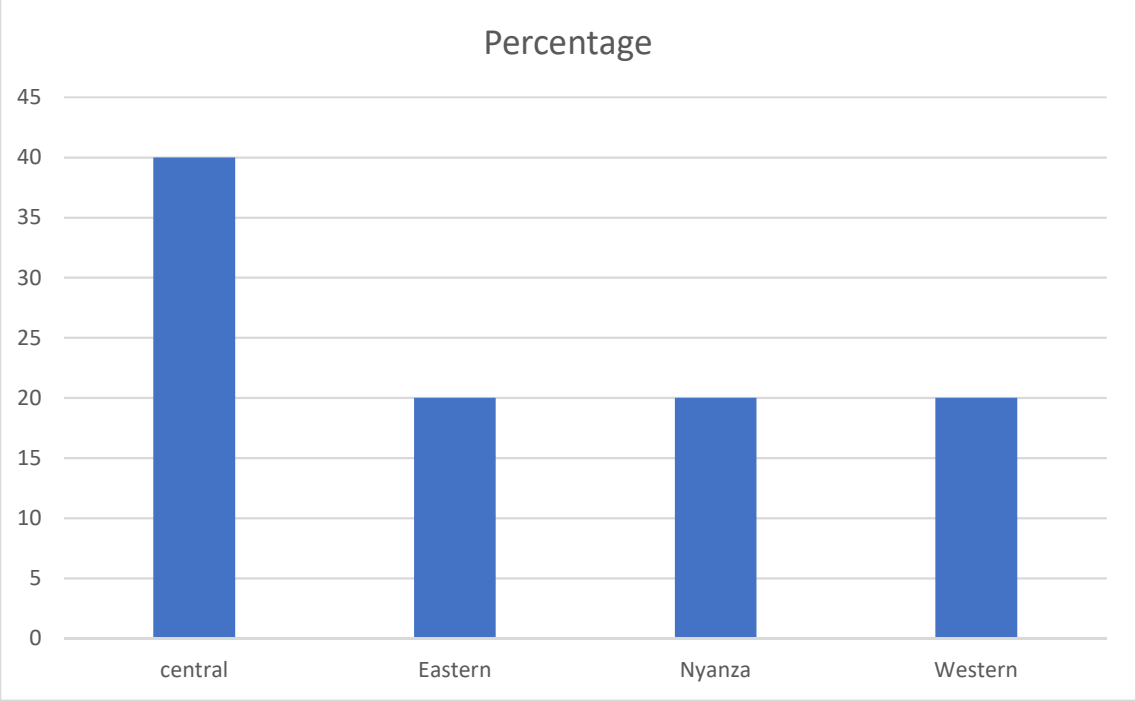
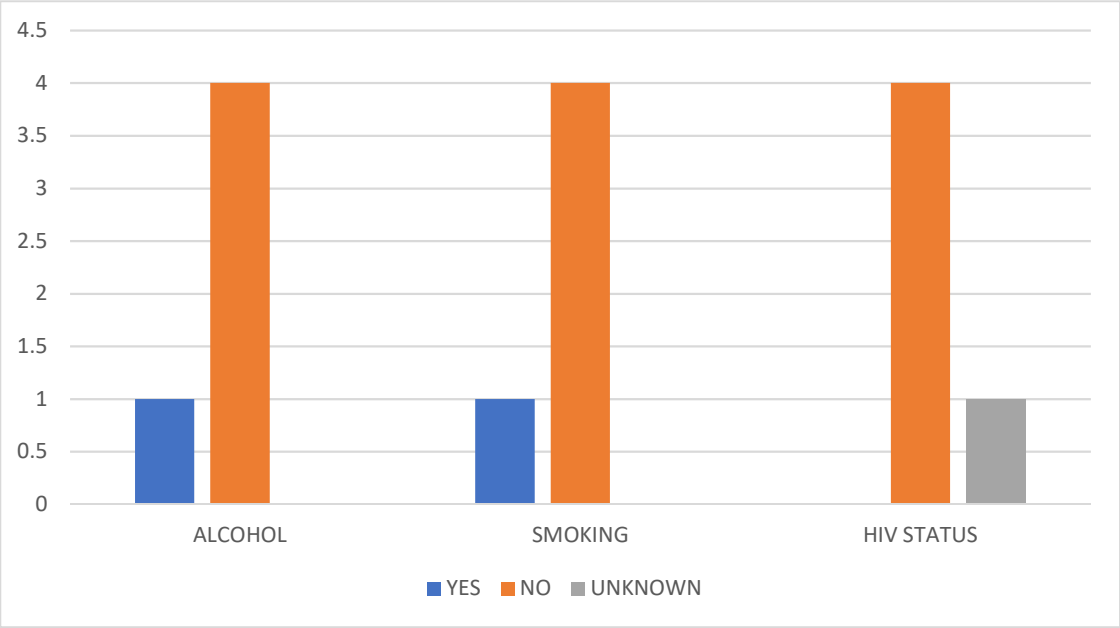


**Figure 4.9: Showing ESCC risk factors**




## 4.4: Treatment characteristics

After endoscopy, staging was most commonly performed by computed tomography of the chest, abdomen and pelvis (51%). A total of 33.3% of the patients were staged using a combination of CT scan of the chest and abdominopelvic ultrasound and 15.7% had incomplete staging. Of those with complete staging and workup,23.3% had stage II, 23.3% had stage III, and 53.4% had stage IV disease.

In total, 64.7% of the patients received some form of treatment for ESCC while the remaining 35.3% did not return for treatment or received supportive care alone. Of the patients who received cancer directed therapy, 15(45.5%) patients had definitive chemoradiotherapy. 5/15 (33.3%) patients had concurrent weekly cisplatin 40mg/m2 while 10/15(67.3%) patients had concurrent carboplatin AUC 2/Paclitaxel 50mg/m2 weekly.53.3% of patients receiving definite chemoradiotherapy completed the prescribed dose. The commonly prescribed definitive RT dose was 45Gy in 25 fractions (60%), 50.4Gy in 28 fractions (30%), and 60Gy in 30 fractions (10%). 3 patients (9.1%) had neoadjuvant chemoradiotherapy with 1 patient proceeding to surgery. 8

(24.2%) patients had palliative radiation therapy while 7(21.2%) patients had some form of chemotherapy.

Table 4.2 Showing treatment characteristics.

| **Definitive CRT n (%)** | | **Neoadjuvant CRT n (%)** | | **Palliative RT n (%)** | **Chemotherapy n (%)** |
|---|---|---|---|---|---|
| **15(45.5)** | 5 pts weekly cisplatin | 3(9.1%) | Surgery n=1 | 8(24.2) | 7(21.2) |
| | 10pts weekly carboTaxol | | No Surgery n=2 | | |

# CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATION

## 5.1 Discussion

HPV is a small double-stranded DNA virus with tropism for the squamous epithelium where it can cause hyperproliferative lesions (Chung S. Yang, 2016). The etiological role of HPV in ESCC is still not clear. The incidence of HPV in ESCC varies between different geographical areas. It is postulated that areas with high incidence of esophageal carcinoma have higher rates of HPV than areas with low incidence of esophageal carcinoma (Zhong Ying Shen S. P., 2002).

In the present study, we observed an approximate 10% of young ESCC patients with positive staining for p16. This is one of the first reports from Kenya that shows positive staining for p16, or HPV analysis, in patients with ESCC. Previous study by Patel et al, showed no association of HPV in patients with ESCC (R.E White, 2005).This could possibly be explained by the difference in the baseline characteristics of patients in their study and this. They included 29 patients while this study looked at 51 patients. The mean age was 58 years compared to 43.7 years in this study, while majority of the participants in that study were from Rift valley province unlike our study which had most participants from Central and Eastern provinces. Both studies had almost twice as many males to female participants. It is noteworthy to mention that the two studies used different methods of HPV detection with Patel et al employing the use of reverse line blot PCR to detect HPV DNA sequences while this study employed the testing of p16 immunohistochemistry.

Similar to our study, a report from Malawi showed a 15% detection of HPV association in ESCC. This study also showed that positive p16 staining corresponded with the presence of HPV DNA (Anja Lidwina Gebner, 2018). In South Africa, Matsha et al demonstrated HPV frequency of 46% in ESCC (T Matsha, 2002). Other studies from Asia have shown a larger range of positive rates ranging from 17 to 66% in ESCC (Fangli Cao H. H., 2014) (Zhong Ying Shen S. P., 2002).

The baseline characteristics of P16 positive patients were not significantly different from those with P16 negative, though the median age was less, and all 5 cases were male. Interestingly, the majority of the non-p16 positive tumors were located in the middle thoracic esophagus while

HPV positive cases showed a more heterogenous distribution in the esophagus. This is consistent with the findings of a systematic review by X. Li.et al (X Li, 2014)

Additionally, we did not observe any correlation with P16 staining and HIV infection, alcohol or tobacco use, similar to that reported in the study conducted in Malawi (Anja Lidwina Gebner, 2018). While most patients in this study were from the eastern region who are majorly of Kamba and Ameru tribes, those with P16 positive staining were from many regions across the country but ultimately too few in total to draw a particular pattern of distribution. Some studies suggest regions with high incidence of esophageal carcinoma may have higher rates of HPV than areas with low incidence, though information on HPV status and limited data in Kenya limit our ability to corroborate these findings (Zhong Ying Shen S. P., 2002).

Majority of the patients in this study presented with stage IV disease which is a late-stage disease. This is comparable to other local and regional studies which have shown that majority of patients present with late stage disease (E. Kamau, 2018).Some of the reasons for delayed diagnosis and presentation include patient outdated traditional beliefs, preference of alternative medicine, infrastructural deficiencies and financial constraints.

Patients seen between 2016 and 2018 were staged mostly using a CT scan of the chest and an ultrasound of the abdomen and pelvis. However, those seen from 2019 were staged using CT Chest, Abdomen and pelvis. The staging modality used is evolving with the availability of more infrastructure and well-trained personnel in their utilization. The evolution also coincided with the introduction of radiation oncology residents training program by University of Nairobi at KNH.

Treatment outcomes could not be statistically analyzed due to the large number of patients lost to follow up. Patients were partly lost to follow up due to the global COVID-19 pandemic which was unprecedented.

**5.2 Conclusion**

1. The incidence of HPV in young individuals with esophageal squamous cell carcinoma in Kenya determined in this study is 9.8%. This is different from previous studies done in Kenya on the same topic.

2. The distribution of the HPV positive cases in terms of anatomic location was heterogenous which is consistent with findings from other studies.
3. The HPV positive cases were scattered across the country with the number being few to draw a particular pattern of distribution.

### 5.3 Recommendation

1. A large-scale population study is necessary to determine the association of HPV and esophageal cancer and the implication on treatment outcomes.

2. This study highlighted the challenges of record keeping, with large number of histology blocks missing. We recommend more efforts to be put into documentation and storage of patients records to facilitate future audits.

### 5.4 **Limitations**

The study was limited by;

1. A small sample due to unavailability of biospecimens for processing and evaluation as many patients are initially diagnosed at hospitals outside KNH.

2. Incomplete medical records with missing charts, consent forms, histology report, risk factor history etc.
3. The unprecedented global COVID-19 pandemic greatly impacted patient follow up during this period, and many long-term cancer outcomes are unavailable for review due to this.

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**APPENDICES**

**Appendix 1: Data collection tool**

**CODE** ☐

**HUMAN PAPILLOMAVIRUS ASSOCIATION WITH ESOPHAGEAL SQUAMOUS CELL CARCINOMA DATA COLLECTION SHEET**

(Tick/mark/write *as appropriate)*

1. **DEMOGRAPHIC DATA**
   a) Birth date ☐☐☐
   b) Age at Diagnosis……..
   c) GENDER MALE ☐ FEMALE ☐
   d) LEVEL OF EDUCATION
      Primary ☐ secondary ☐ college/university ☐ other ☐

      *If other specify*…….
   e) EMPLOYMENT STATUS
      Employed ☐ Unemployed ☐

      Specify occupation……
   f) RESIDENCE/COUNTY ……...
   g) Ethnicity/Tribe…….
   h) HIV status
      Reactive ☐ Non-Reactive ☐ Unknown ☐

**2. RISK FACTOR HISTORY**

| | YES | NO | Pack years |
|---|---|---|---|
| 1. Smoking | ☐ | ☐ | ☐ |
| 2. Alcohol | ☐ | ☐ | ☐ |

**3. TUMOR LOCATION**

a) Anatomic location

Upper third ☐ Middle third ☐ Distal third ☐

b) (Tumor location in centimeters on endoscopy)

15-20 cm ☐ >20-25cm ☐ >25-30cm ☐ >30-40cm ☐

**4. TUMOR STAGE**

Overall clinical stage……...

I. T stage……
II. N stage…….
III. M stage……...

**5. P16 RESULT**

I. POSITIVE ☐
II. NEGATIVE ☐
III. INDETERMINATE ☐

**6. TREATMENT GIVEN**

a) Neoadjuvant chemoradiation (dose/fractions) …….
b) Definitive chemoradiation (dose/fractions) …….
c) Surgery…….
d) Chemotherapy……

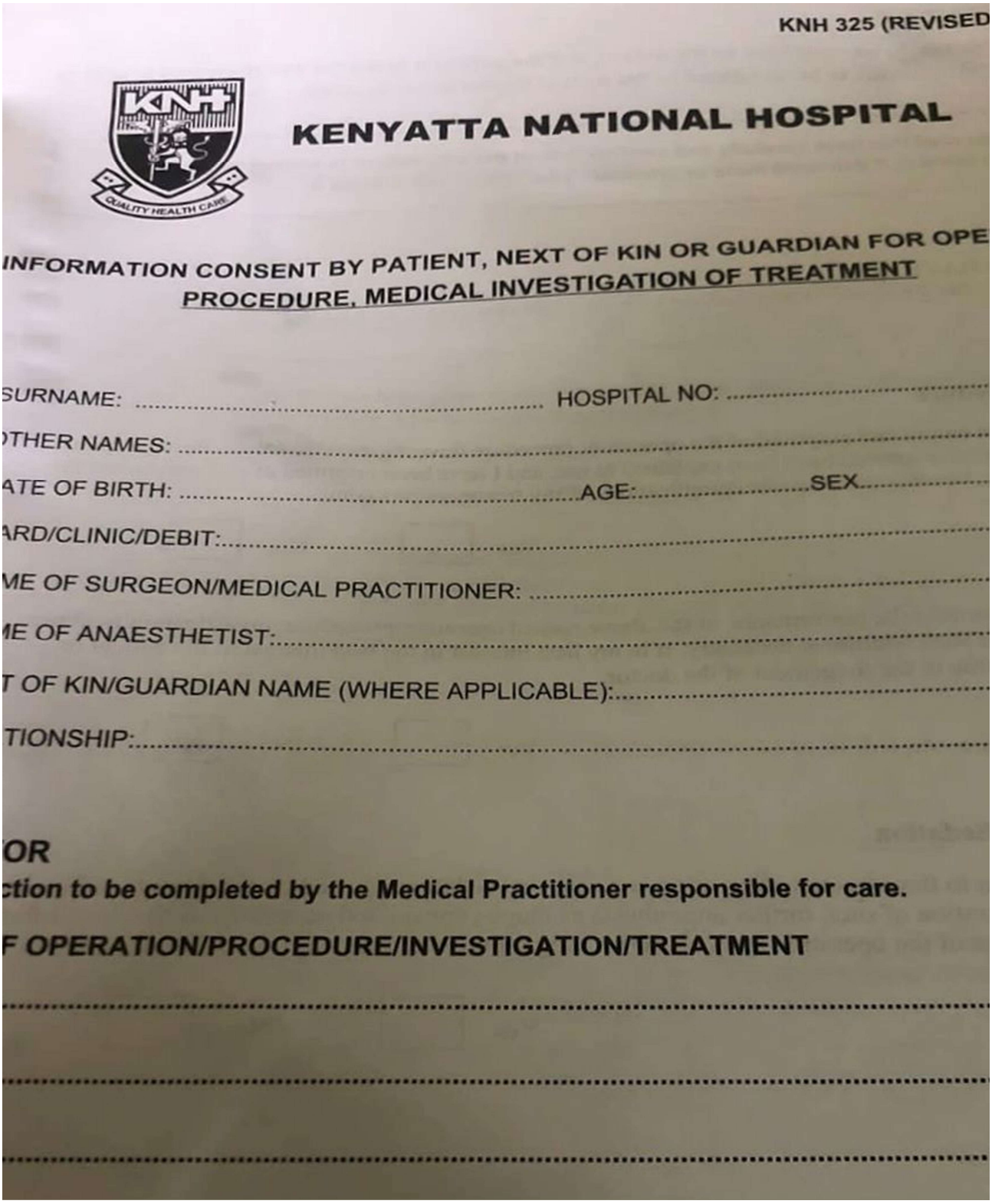
7. **RECURRENCE**

Site…… Date……

**8. DATE OF DEATH/LAST FOLLOW-UP**

Date of death…….
Date of last follow up…….

**Appendix II: Consent form**



KNH 325 (REVISED

KENYATTA NATIONAL HOSPITAL

INFORMATION CONSENT BY PATIENT, NEXT OF KIN OR GUARDIAN FOR OPE
PROCEDURE, MEDICAL INVESTIGATION OF TREATMENT

SURNAME: ..... HOSPITAL NO: .....

OTHER NAMES: .....

ATE OF BIRTH: .....AGE:.....SEX.....

ARD/CLINIC/DEBIT:.....

ME OF SURGEON/MEDICAL PRACTITIONER: .....

ME OF ANAESTHETIST:.....

T OF KIN/GUARDIAN NAME (WHERE APPLICABLE):.....

TIONSHIP:.....

OR

ction to be completed by the Medical Practitioner responsible for care.

F OPERATION/PROCEDURE/INVESTIGATION/TREATMENT

.....

.....

.....

**PATIENT**

**This section to be completed by the patient, give own consent, to be completed by the Next of Kin/Pati**

**Please read this form carefully and confirm that all the information is correct and that you understand it. If you want more information, your doctor will provide it.**

**DECLARATION**

The doctor has explained / I understand that I have the following condition / diagnosis

.....

**The procedure**

1. The nature and purposed of the operation/ procedure /investigation/treatment, the appropriate available options have been explained to me, and I have been informed of and understand the risk, benefits and possible complications of my treatment/procedure.

Yes ☐ No. ☐

I consent to the performance of the above named operation/procedure /investigation/treatment and of such additional necessary, if in my best interest/in the best interest of the patient or desirable in the judgement of the doctor.

Yes ☐ No. ☐

**esthesia/Sedation**

I consent to the administration of an anaesthesia / sedation advised by the doctor, and to the administration of such further anaesthesia as maybe considered necessary by the doctor during the course of the operation /procedure/investigation/treatment.

Yes ☐ No. ☐

**Tissue disposal**

4. I consent to the disposal by the authorities of this medical facility of any tissue or parts, which may be necessary to remove.

**Exceptions**

5 Exceptions to the proposed operation/anaesthesia/investigation/treatment, if any

.....

**Medical Training**

6. I understand that for general patients, operation/procedures/investigation/treatment will be carried out by the surgeons/anaesthetists/Medical Practitioners and other practitioners on the hospital Rota.

Assurance /No assurance has been given to me that the operation / procedure / investigation / treatment will be performed by a particular medical practitioner.

Yes ☐ No. ☐

7. I understand that for surgeon Anaesthetist/Medical Practitioner may have assistance to help him/her during the procedure(s). These assistants may include other licensed doctors in training, surgical assistant, nurses and medical student; these assistants may perform a variety of tasks generally facilitating the procedure as the need arises.

8 I am also aware that, photographs and other illustration may be taken of this procedure for continuation of medical training and education.

Yes ☐ No. ☐

**Other health information**

9 I understand that it is my duty to inform my primary care specialist/practitioner of interacti and treatment schedules that I may have with other healthcare providers.

Yes ☐ No. ☐

**od and / or products**

I consent /do not consent to the administration of blood or blood products as may be f necessary and the best interest of the patient during the course of the operatio procedure/investigation/treatment.

Yes ☐ No. ☐

**PRIVACY AND CONFIDENTIALITY**

1. Understand the laws that protect and confidentiality of medical information will be respected, and that on information obtained in the course of my treatment/procedure and caring my identity will be indiscreetly disclosed researches or other entities without my consent.

**CONSENT FOR RELEASE OF INFORMATION**

1. Understand that the Chief Executive Officer of Kenyatta National or assignee may release relevant medical information complied in my medical records during this admission or outpatient visit to any organization, which is or may be liable or responsible for payment of charges. If my inquiry is work-related, I understand the hospital may release any iformation from my records to my employer or its designee.

Yes ☐ No ☐

*Patient's Signature:* ..... *Date:*.....

*Signature of parent / Next of Kin:* ..... *Name:*..... *Date:*.....

I/we confirm that I/we have explained the above operation/procedure/Investigation/Treatment, The appropriate available options, and the type of anaesthetic proposed (general/regional/sedation) to the patient/Next of Kin/Guardian.

*Surgeon/Medical Practitioner's signature:*.....*Name:*..... *Date:*.....

*Anaesthetist's signature:*..... *Name:*..... *Date:*.....

**CONSENT IN CASE OF EMERGENCY ADMISSION OF PATIENT NOT FIT TO GIVE OWN CONSENT; IN ABSENCE SUITABLE GUARDIAN/NEXT OF KIN**

*Surgeon/Medical Practitioner's Name:*.....

*Signature:*.....

**5. Reference**

Anaesthesia - Short test bool of Anaesthesia.
Tissue disposal - Public Health Act Capt.

**6. Appendices**

**Appendix III: Quality assurance protocol**

CINtec histology kit will be used to carry out the p16 test

**Manual Procedure**

1. Formalin fixed Paraffin Embedded tissue was cut at a thickness of 4 μm
2. Known positive control and test tissue was placed on same immunohistochemistry adhesive charged slides.
3. The slides were dried on a hotplate at 60 degrees Celsius for 5 minutes,
4. Labels affixed on the slides
5. Tissue was deparaffinized by heating (75°C for 4 minutes), then antigen retrieval was achieved by use of cell conditioning buffer 1 (CC1) at 95°C for 64 minutes. Primary antibodies, CINtec p16INK4a,
   clone E684 was added and ultraView Universal DAB Detection Kit polymer amplification system (Benchmark Ventana XT machine) was used according to manufacturer's instructions.
6. Sections were washed with distilled water
7. Counterstained with haematoxylin
8. Rehydrated with graded alcohol,
9. Dried and mounted with DPX for analysis

REPORTING OF THE IMMUNOHISTOCHEMISTRY

1. Strong and uniform p16 staining (both cytoplasmic and nuclear) in more than 50% for tumour cells was considered as positive and less than 50% expression was considered as negative
   for statistical analysis.
2. Absent or weak p16 staining in neoplastic cells was considered as negative