GENOTYPES AND PREVALENCE OF HIGH-RISK HUMAN PAPILLOMAVIRUS AMONG PATIENTS DIAGNOSED WITH HEAD AND NECK CANCERS AT ALEXANDRIA CANCER CENTRE, ELDORET

BY

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A THESIS REPORT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN MEDICAL BIOCHEMISTRY TO THE DEPARTMENT OF BIOCHEMISTRY AND CLINICAL CHEMISTRY, SCHOOL OF MEDICINE, COLLEGE OF HEALTH SCIENCES, MOI UNIVERSITY

DECLARATION

I declare that the work contained in this thesis report is my original work and that I have not previously submitted it, in its entirety or in part to Moi University or in any other institution for any degree.

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DEDICATION

This thesis is dedicated to Yuniah Kemunto and Nyambane Ombiro who've been my biggest support throughout my academic journey.

ABSTRACT

Background: American Joint Committee on Cancer defines Head and neck oncology as malignancies arising from epithelial lining of: oral cavity, nasal cavity, pharynx, larynx, paranasal sinuses and salivary glands. Global Cancer Statistics 2018 data, shows proportion of head and neck cancer to all cancers being 4.9% globally, while in Kenya it is 6%. Mortality rates globally are at 51%, while in Kenya it is at 73.4%. Research has shown the role of various high risk - Human papillomavirus (hr-HPV) genotypes in the rising incidence of head and neck cases globally. Several trials have shown that patients with Human papillomavirus positive head and neck cancer have better overall survival compared to the Human papillomavirus negative patients attributed to increased sensitivity to chemo-radiation therapy. Alexandria Cancer Centre and Palliative Care Hospital is one of the major cancer facilities in Western Kenya Region that is accredited by National Hospital Insurance Fund (NHIF) thus, making it accessible to people of all walks of life. Fortunately, samples are processed at Pathologist Lancet Laboratories which is a well-established institution with equipment and protocols in place for use in the present study.

Objectives: The broad objective was to assess the genotypes and occurrence of high-risk Human papillomavirus among patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital, Eldoret between January 2017 and December 2018.

Methods: This was a retrospective study with laboratory analysis between January 2017 and December 2018. Patient files were reviewed. Stored tissues were retrieved and: repeat microscopy, p16 Immunohistochemistry and Human papillomavirus Polymerase Chain Reaction were carried out. Data was analyzed by use of Microsoft Excel (2016).

Results: Head and neck cancer accounted for 8.8% (89/1016) of all malignancies at Alexandria Cancer Centre and Palliative Care Hospital. There were more males (64%) than females (36%), with a mean age of 50.9 ± 17.6 years. Most patients came from Western Kenya Region. About one third (35.7%) were farmers and students combined. A total of 16 samples (16/29) (55.2%) tested p16 positive, of these 2 (12.5%) tested positive with HPV PCR. One of these was from an 87-year-old female diagnosed with tongue cancer. Molecular analysis revealed the genotype to be HPV 52. The other one was from a 49-year-old male with cancer of the post nasal space who had multiple co-infections with HPV: 35, 52 and 59 genotypes. It is important to note that these genotypes have been rarely isolated elsewhere.

Conclusion: The prevalence of head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital is above both the global and national average pointing to a likely higher prevalence in this region. The potential role of the unique Human papillomavirus genotypes identified in this study require further investigations.

Recommendations: Further prospective studies with a larger sample size in order to try and gain a better understanding of the prevalence of Human papillomavirus and high-risk genotypes associated with head and neck cancer in Kenya. Additional data will allow to close the gap of knowledge between Western countries and the sub-Saharan African region, especially in Kenya which has a high burden for head and neck cancer. This could play a big role in assisting health authorities in implementing public health strategies like vaccinations and will aid in allocating necessary resources.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACCPCH	Alexandria Cancer Centre and Palliative Care Hospital
GLOBOCAN	Global Cancer Statistics Database
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
IHC	Immunohistochemistry
NHIF	National Hospital Insurance Fund
OPSS	Oropharyngeal Squamous Cell Carcinoma
PCR	Polymerase Chain Reaction
TNM	Tumor, Lymph Node, Metastasis
UADTC	Upper Aerodigestive Tract Cancer
WHO	World Health Organization

DEFINITION OF TERMS

Human papillomavirus (HPV) – It is a group of more than 300 related Deoxyribonucleic acid papillomaviruses named for the papillomas (genital and plantar warts) that some types cause on the skin and mucous membranes in humans; sexually transmitted by either direct or indirect contact.

HPV Genotypes – There are five major known Human papillomavirus genera: α, β, γ, mu and nu- papillomavirus. Categorization is dependent on the nucleotide sequence of the Open Reading Frame (ORF) coding for the capsid protein L1. There are many strains/species that are designated as "types". They can be differentiated by specific numbers that are assigned to them for instance: HPV16, HPV 18 and so on.

Head and Neck Cancer – Refers to any malignant neoplasms of the upper aero digestive tract, facial features and structures in the neck which appear as masses, ulcerations or flat lesions.

Squamous Cell Carcinoma (SCC) – Cancer that begins in squamous cells that are found in the tissue that forms the surface of the skin, the epithelial lining of the hollow organs of the body and the epithelial lining of the respiratory and digestive tracts. It is usually marked by keratinization that begins at one point and usually on the surface. The wound may later grow and spread close to the initial injury or to parts further away.

Immunohistochemistry (IHC) – Entails making use of monoclonal or polyclonal antibodies as histological tools for determining patterns of antigen distribution within a tissue. The procedure requires detecting antigens in biopsy tissue through the use of visual markers, including: enzymes, fluorescent dye, colloidal gold suspension and radioactive elements. It is presumed that antibody distribution mirrors antigen distribution. It is generally applied in investigation and interpretation of cancers because definite tumor antigens are manifested *de novo* or elevated in specific cancers.

Polymerase Chain Reaction (PCR) – It is a procedure in molecular genetics that allows the investigation of any short arrangement of Ribonucleic acid or Deoxyribonucleic acid. It is very convenient even in specimens consisting of only minimal genetic material. It is utilized to multiply chosen regions of Ribonucleic acid or Deoxyribonucleic acid for investigation and interpretation. It is used in several cases for instance: to assist in the determination of microorganisms present during an infection, revealing the absence or presence of a gene, the earlier steps of preparing Deoxyribonucleic acid for sequencing, for detecting the presence or absence of a gene and when generating forensic profiles from small specimens of Deoxyribonucleic acid usually used in criminal investigations.

Oral Cavity – It is composed of two sections, the vestibule that is the area between the lips, cheeks and teeth and the oral cavity proper that is the region enclosed by the alveolar bone comprising the teeth and the upper part hanging at the opening at the back of the throat and the floor of the mouth. There is a coating that covers the entire surface that is made up of epithelial cells. The salivary glands produce fluids that ensure that the oral cavity is kept constantly wet.

Tongue – It is a movable muscle that is attached to the floor of the mouth. blood is supplied by the use of the lingual artery. The ability to perceive taste and other sensations are made possible by the use of special structures on the covering of the tongue and the lingual nerve. There are four main tastes, bitter, salty, sour and sweet. It plays some very important roles such as talking and the ability to assist in moving food around in the mouth as its chewed before it is pushed ahead and eventually swallowed. It is made up of four muscles, genioglossus muscles which provide a way to attach it to the lower jaw, hyoglossus muscle is the means by which it attaches to the hyoid bone, extrinsic muscles allow it to move and longitudinal muscles that are interconnected with the transverse muscles to provide structure.

Pharynx – It is the part of the throat that is above the esophagus and larynx and under the nasal cavity and mouth. It measures about thirteen centimeters long and is made up of several sections an upper portion, the nasopharynx, and a lower portion consisting of the oropharynx and hypopharynx. The upper part of the pharynx is located above the soft palate and communicates with the nasal passages. It is the pathway where air moves along the nose and finally into our respiratory linings. The oropharynx extends from the soft palate and the velum palatinum to the epiglottis, which is the high-rising "flap-like" tissue found posterior to our tongue. The hypopharynx extends from the tip of the epiglottis to the lower margin of the cricoid cartilage and ends at our esophagus. The term Waldeyer's ring refers to the ring of lymphoid tissues extending throughout the nasopharynx and oropharynx and includes the palatine, pharyngeal, lingual and tubal tonsils.

Larynx – It is located within the anterior aspect of the neck, anterior to the inferior portion of the pharynx and superior to the trachea. The main purpose is to protect the airway by suddenly closing and in so doing stopping respiration and blocking the entryway of unfamiliar substances into the airway. Additional purposes of the larynx involve making of sound, coughing, the Valsalva maneuver, and control of ventilation, and acting as a sensory organ. The larynx is composed of a variety of inbuilt muscles, three pairs of slightly smaller cartilages (arytenoids, corniculate, cuneiform), 3 big, unpaired cartilages (cricoid, thyroid, epiglottis). Strictly speaking, the hyoid bone is not part of the larynx, however, it makes it possible for muscles to attach themselves that help in laryngeal motion.

Nasal cavity and paranasal sinuses – The nasal cavity and paranasal sinuses are covered by tissue that is capable of producing mucus. The nose opens into the nasal passageway, this hollow space is present along the topmost part of the roof of the mouth which divides the nose from the mouth, it then rotates downwards and connects the passageway from the mouth to the throat. Sinuses are small hollow spaces. They are referred to as paranasal due to their proximity to the nose. The nasal cavity spreads out into a meshwork of sinuses: those found on top of the nose and between the eyes are referred to as ethmoid sinuses, those that are found at the top of the eyebrow area and the inner eye are referred to as frontal sinuses, those that are located between the eyes and found deep behind the nose are referred to as sphenoid sinuses and maxillary sinuses are located below the eyes on both sides of the nose in the cheek area. The nasal cavity and paranasal sinuses play many roles for instance:

help reduce the heaviness of the skull, provide support for the face and eyes, help purify, moisten and make the air that you breath warm and help in providing the quality of sound.

Salivary glands – They are glands that produce saliva and empty it into your mouth through openings called ducts. Salivary glands can be categorized as mucous, serous or a mixture of both referred to as seromucous. Humans have many small salivary glands numbering in several hundred that are found all over the mouth and the entire lining of the digestive system that are too small to be seen without a microscope. There are also three main salivary glands. The largest being parotid glands that are found in the area in front of the ears. The saliva that is made in these glands is released into the mouth from an opening near the second molar of the upper jaw. Each of the parotid glands has two sections one located closer to the surface and another deeper. The facial nerve that supplies the tongue and muscles on the face passes in the space between these two sections. This nerve is particular importance as determines one's ability to smile, raise eyebrows and the capability to close the eyes. The major blood vessel that brings blood to the region of the head and neck, the carotid artery is also located close to the parotid gland. Also close to the parotid gland is a retromandibular vein which is a section of the veins that take deoxygenated blood from the head. Second in size are submandibular glands are found under the jaw. Saliva that is made in these glands is released into the mouth from below the tongue. They also have two sections. There are also several important parts for instance: the lingual nerve which makes it possible to have feeling in the tongue, the hypoglossal nerve which makes it possible to be able to move the part of the tongue that aids in swallowing and talking, the marginal mandibular nerve which enables one to smile and the platysma muscle, which enables you to move your lower lip. The smallest of the three are the sublingual glands. These are found on both sides of the tongue and beneath the floor of the mouth.

ACKNOWLEDGEMENT

First and foremost, I would like to thank God for giving me life and energy which enables me to study and for His daily provisions.

I wish to sincerely thank my family for their unrelenting support and encouragement throughout my academic journey.

I sincerely thank my supervisors; Dr. Geoffrey Maiyoh and Prof. Arthur Kwena for their continued support and guidance.

I wish to also thank lecturers at Moi University, College of Health Sciences, School Medicine, Department of Biochemistry and Clinical Chemistry for their guidance throughout my academic journey.

I also wish to thank Dr. Melly, Tabitha Kamau and other Staff at Alexandria Cancer Centre for allowing me access to the hospital as my research station and providing information that I needed.

I would also like to thank Dr. Rabia Mukadam, Dr. Jesca Kirimi, Robert Oyugi among others at Pathologist Lancet Kenya for their assistance and support in carrying out laboratory assays.

CHAPTER ONE: INTRODUCTION

1.1 Background Information

Cancer is a term that refers to a large group of diseases that are characterized by the uncontrolled growth and spread of abnormal cells beyond their usual boundaries that can then invade adjoining parts of the body and/or spread to other organs via lymph or blood. Cancer emerges as a result of the alteration of normal cells into tumor cells in a multistep process that generally advances from a pre-cancerous lesion to a malignant tumor. Altered cells divide uncontrollably to form lumps/masses called tumors in the case of solid tumors but some, like leukemia, do not and are referred to as non-solid tumors. Cancers are named after the part of the part of the body where the tumor originates (American Cancer Society, 2017).

Cancer incidence and mortality rates continue to rise globally exerting significant strain on populations and health systems at all income levels. The International Agency for Research on Cancer, a World Health Organization (WHO) agency reported that an estimated 18 million cases of cancer were diagnosed around the world with 9.5 million cancer deaths in 2018. Over half the cases and nearly two-thirds of deaths occurred in Africa and other low- and middle-income countries (Bray, 2018). Estimates suggest a rise in annual cancer cases to 22 million by 2030 with 13 million deaths and projections pointing to a rise to 70% in low- and middle-income countries by 2030 (Maruthappu, 2016)

The incidence and mortality for various types of cancer, substantially vary across countries and within each country. This is dependent on the degree of economic progression, development of healthcare, interrelated setting and surroundings,

sociocultural components and the way of life among others (Ministry of Health, 2018).

Extensive data on cancer incidence and mortality in Africa are not available. It is estimated that only 2% of the continent's population is covered by International Agency for Research on Cancer (IARC)-compliant cancer registries as of 2014 and there is evidence that some cancers are under-reported. Dr. Funmi Olopade from the University of Chicago points out that, 'The situation in Africa is much more dire than what the figures and information that is currently available could like to indicate. In a high percentage of the situation, once diagnosed, most patients and their families usually prepare psychologically for eventual demise with hardly any expectation of regaining a healthy status again" (Parkin, 2018).

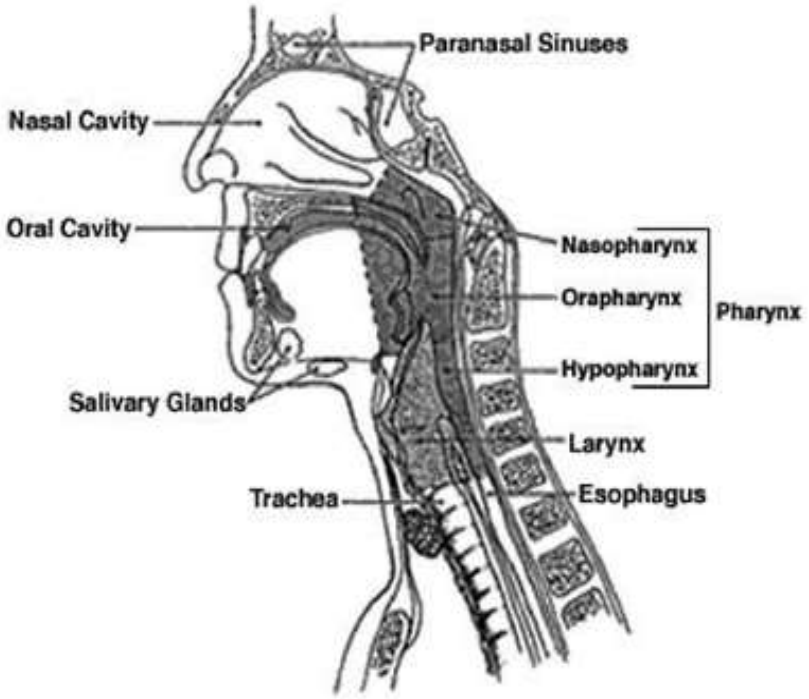

Figure 1: Anatomical Distribution of Head and Neck Cancer Malignancies

According to the definition from the American Joint Committee on Cancer (AJCC), head and neck cancer (HNC) refers to a heterogeneous entity that encompasses malignancies arising from the epithelial lining of the mucosal surfaces of upper aero digestive tract (UADT) including the oral cavity (lip, mouth and tongue), nasal cavity, pharynx (nasopharynx, hypopharynx and oropharynx), larynx, paranasal sinuses and cancers originating from major and minor salivary glands (excluding those of the brain, spinal cord (Central Nervous System), base of the skull and skin) (Cohen, 2018).

Head and neck cancer constitute a major public health concern throughout the world and is an important cause of morbidity and mortality. The disease is associated with a poor prognosis, with mortality rate of 40% to 50% (Keck, 2015) and a 5-year survival rate less than 50% (Zaravinos, 2014). This is of particular concern because, there is interruption of important functions that are crucial for life for instance: speaking, breathing, hearing, swallowing, taste, smell and vision (Gilyoma et al., 2015).

Before widespread use of tobacco prior to the 16th century, head and neck cancers were hardly ever included in medical reports. In 1650 Hayes Martin is recorded to have written the first report describing treatment of oral cancer. At around 1790, Onuigbo wrote about the recognition of lymph node metastasis. In late 1800s, diagnosis through biopsy procedures was introduced. In 1887, three biopsies from the larynx of Germany's Crown Prince Frederick were performed by Morell Mackenzi and the tissues were examined by Rudolph Virchow who reported a negative result for cancer. Unfortunately, succumbed after a short period of time. Within the time period between 1900 and 1960 several histology reports were made both on benign and malignant tumors. Later, John Batsakis published a paper titled, "Tumors of the Head and Neck" in 1974 (Alfouzan, 2019).

The head and neck region contains a number of complex structures that are essential for normal functioning of the body. Due to their location, they also play an important role in the looks of an individual and this affects one's social interaction and expression. Important biological functions can be compromised if the cancer affects the important corresponding areas of the human body. Head and neck cancers may lead to a variety of distortion and malformation depending on the particular structure affected and the extent affected. This may affect the functioning of these organs and may also severely affect one's self-esteem. The quality of life can be further worsened by treatment of head and neck tumors which can induce additional mutilation (Davidson & Williams, 2019).

Abnormalities that raise concern depend highly on the site of the tumor and the progression of disease. At the initial stages, the signs and symptoms are in most cases general in nature which makes diagnosis difficult until later stages of the disease. Rough and uneven voice may be an indicator of laryngeal cancer. Blockage of nasal passages, nose bleeding and fluid in the ears may point to nasopharyngeal cancer. Inflammation of sinuses and one-sided blockage of the nostrils maybe suggest nasal cavity and paranasal sinus cancer. Wounds that have difficulty in healing, pain and problems with gums may indicate cancer of the oral cavity. Pain in the ears and consistent one-sided pain on the throat could point out likelihood of cancers of the supraglottic region, hypopharynx and oropharynx (Alfouzan, 2019).

Human papillomavirus (HPV) is an umbrella designation that refers to a grouping of related papillomavirus strains. It is the most common sexually transmitted Deoxyribonucleic acid (DNA) virus (Wang, 2018). These small Deoxyribonucleic acid (DNA) viruses are extensively rife and can infect both females and males (Sano & Oridate, 2016). Infection with one or more Human papillomavirus strains is nearly

ubiquitous in sexually active persons with approximately 75% of humans being affected during their life time (Pizzol, 2016).

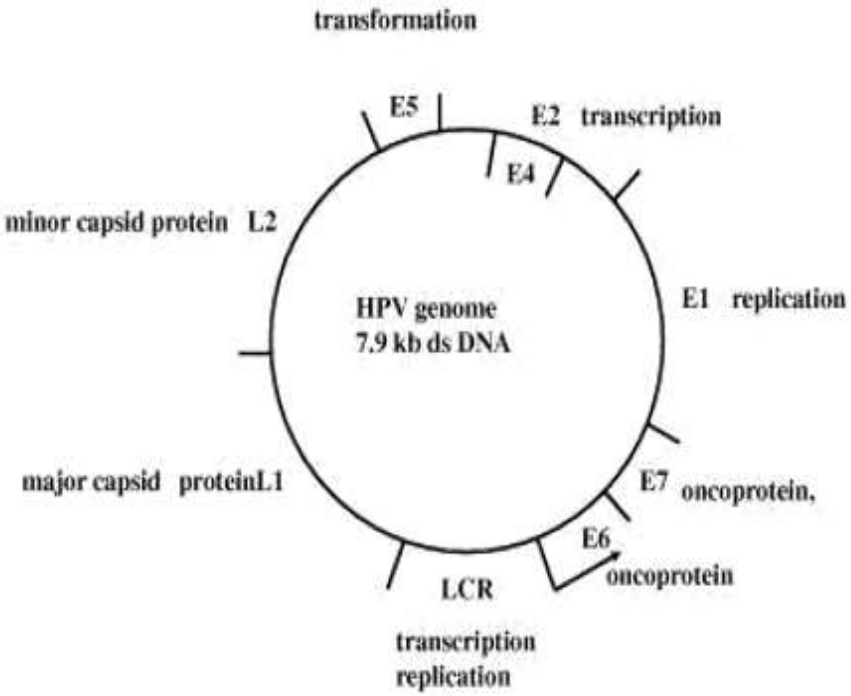

Figure 2: Structure of Human papillomavirus (HPV) genome

It is most commonly asymptomatic with the vast majority of Human papillomavirus types causing warts and benign lesions of the skin or genital region. The natural history is characterized by spontaneous regression in 70% to 93% of cases over 1 to 2 years (Cohen, 2018). Findings from Center for Disease Control and Prevention (CDC) indicate that persistent infections may lead to pre-cancers and cancers of the Cervix, Anus, Penis, Vulva, Vagina and Head and Neck (Pinatti et al., 2018).

High-risk Human papillomavirus has been on the spotlight. This is because it has been linked to about 5% of all human cancers. Looking in depth at such viruses aids in understanding the causes of a number of cancers affecting human beings. Viruses can

only reproduce with the cells of the host that they infect they then incorporate their own Ribonucleic acid or Deoxyribonucleic acid material and take over the cell cycle (Soto, 2017).

Currently, in the United States, there are three different commercial Human papillomavirus vaccines licensed for use: bivalent (2vHPV) Cervarix from GlaxoSmithKline, 9‑ valent (9vHPV) Gardasil 9 and quadrivalent (4vHPV) Gardasil from Merck. In the United States in 2015, it was proposed that Human papillomavirus vaccines be used routinely among females from the age of 11 or 12 sometimes beginning from 9 to 26 and for males from the age of 11 to 21 by the advisory committee on immunization practices in the United States. Human papillomavirus vaccination was first administered to girls and young women to prevent cervical cancer. However, with an increased number of Human papillomavirus ‑ induced head and neck carcinomas that are not gender specific, vaccination was recommended for both girls and boys before the onset of sexual activity (Alfouzan, 2019).

The American Joint Committee on Cancer and the Union for International Cancer Control eighth edition Tumor, Node, Metastasis (TNM) staging guidelines (TNM8), effective January 2018, recommend stratification of Oropharyngeal Squamous Cell Carcinoma by Human papillomavirus status to improve staging and clinical management (Craig, 2019). However, there is currently no evidence that the new staging of Human papillomavirus Oropharyngeal cancer should drive clinical decision-making. It is of global concern that there is need for de-escalation of treatment with of particular emphasis on maintaining the tumor while reducing toxicity as a major side effect. At the moment the main mode of treatment in head and neck cancers is surgery combined with chemoradiation (CRT). As such most of the studies in this field are focused on trying to reduce radiation dose, replacing and

cutting back cisplatin given since it is destructive to the kidneys and coming up with surgical methods which are less invasive to try and reduce integrity of the tissue (Durkova, 2019).

The HPV genome does not code for enzymes that are necessary for viral replication; instead Human papillomavirus makes use of the host cell proteins to replicate viral Deoxyribonucleic acid. The host cells for Human papillomavirus infection are keratinocyte progenitors located in the basal layer of stratified squamous epithelia and adhered to the epithelial basement membrane. Human papillomavirus gains access to basal cells via micro-abrasions that occur during sexual or other direct physical contact. Upon reaching the basal keratinocyte, Human papillomavirus preferentially binds components of the extracellular matrix. These triggers conformational changes in L1 and L2 that transfer virion particles to host cellular uptake receptor(s) necessary for viral internalization. The HPV genome can exist either in an episomal form within some cells of the basal layer or become integrated into the host genome. Integration into the host genome was thought to favor common fragile sites, but can occur randomly (Olivero, 2018).

The p16 gene is found on chromosome 9p21 (Galera, 2017) belongs to *INK4* family of genes and is made up of four members: $p16^{INK4A}$, $p15^{INK4B}$, $p18^{INK4C}$and $p19^{INK4D}$, all of which have similar biological properties, specifically; hindrance of cell growth and tumor suppression. It is the second most common tumor suppressor gene after p53 (Dreyer et al., 2017). The $p16^{INK4a}$ protein is part of the retinoblastoma protein (pRB)-mediated control of the G1-S-phase transition and triggers cell cycle arrest in the course of the cellular differentiation process. The p16 protein prevents progression of the cell by attaching to cyclin-dependent kinases 4 (cdk4/cyclin D) and

cyclin-dependent kinases 6 (cdk6/cyclin D) and maintains the retinoblastoma gene product (pRb) in its hypophosphorylated state, which in turn sticks to E2F transcription factor and prevents cell cycle progression (Serrano, 2018).

Under normal circumstances, the level of expression of $p16^{INK4a}$ is small and as such it is unnoticeable when tested by immunohistochemistry. The E7 from high-risk Human papillomavirus attaches to the retinoblastoma protein that is phosphorylated to a less than full extent, this attachment takes place in regions that conceal E2F transcription factors. As a result, when retinoblastoma is not able to attach to these E2F transcription factors, its role is diminished and as such the cell cycle proceeds unchecked (Ralli, 2016). Due to this, the negative feedback loop that the prevents p16 transcription is affected (Hübbers & Akgül, 2015) and therefore, leads to over production of p16 (Dreyer et al., 2017). Thus, it leads to the transformed cells with elevated levels of p16 being able to detour and avoid the intervention of the cell cycle. It has been noted the elevated production of p16 is in an ineffective endeavor to prevent uncontrolled cell growth (Serrano et al., 2018).

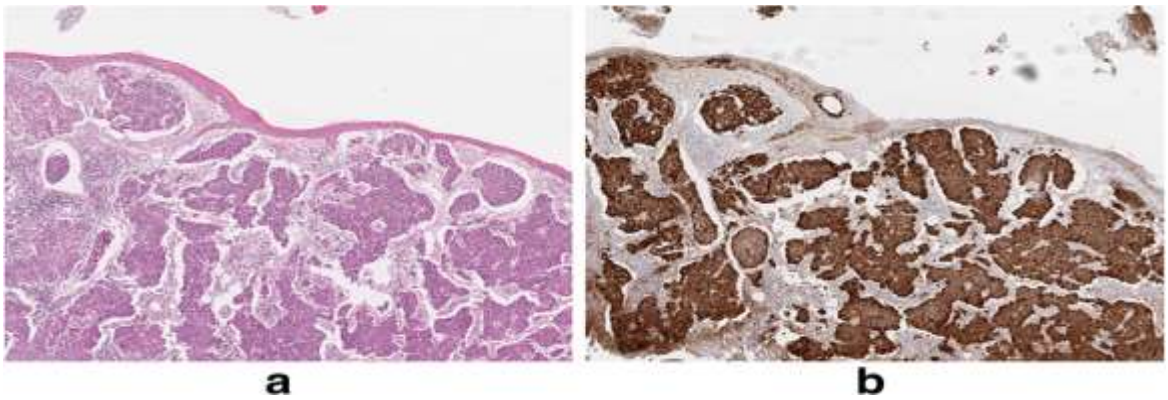

Figure 3: (a) Squamous cell carcinoma of the tonsil that is related with Human papillomavirus that is morphologically poorly differentiated and stained with Hematoxylin and Eosin (b) The same tumor stained for p16. Original image amplified 20 times.
Image obtained from (Shaikh et al., 2017).

The presence of p16 overexpression in head and neck cancer especially, oropharyngeal squamous cell carcinomas, is biological evidence of Human papillomavirus tumorigenic effect with its resultant prognostic benefits. This is usually detected by immunohistochemistry assay (p16 IHC) (Aldalwg & Brestovac, 2017) and has been largely considered as a diagnostic surrogate marker for high risk Human papillomavirus (hr-HPV) infection in formalin fixed, paraffin-embedded (FFPE) tissues (Serrano et al., 2018). To be indicative of oncogenic Human papillomavirus infection immunohistochemistry should show nuclear and cytoplasmic reaction to p16 by staining in ≥ 70% or more of the cancer cells (Stevens & Bishop, 2017). This p16 immunohistochemistry assay has advantages such as wide availability, high sensitivity, and low cost. However, it shows reduced specificity (~80–85%) as compared to Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA) based methods of Human papillomavirus detection (Shah et al., 2017).

Approximately one-third of the total HNC cases in the world have been shown to be associated with high-risk Human papillomavirus infection, but wide geographic variation exists (Shaikh et al., 2017).

It is not possible to cultivate Human papillomavirus in artificial media in the laboratory. As such, the most reliable means of distinguishing is highly based on molecular biology techniques. At the moment there are several tests that can be used the first ones entail techniques that increase the levels of genetic material: Human papillomavirus Deoxyribonucleic acid polymerase chain reaction (HPV DNA PCR), E6/E7 Human papillomavirus real time polymerase chain reaction (HPV RT-PCR), Human papillomavirus Deoxyribonucleic acid (HPV DNA) in situ hybridization (ISH). There are also techniques that focus on hybridization: in-situ hybridization, dot blot hybridization and southern blotting. There are also techniques that focus on

increasing the signal Cervista® HPV HR assay and Digene® HPV test using Hybrid Capture® 2 (hc2) technology (Economopoulou, 2020).

1.2 Problem Statement

It is estimated that of all cancers worldwide, 20–25% have a viral etiology, with a higher percentage in developing countries. In Sub-Saharan Africa (SSA), nearly one-third (~ 31%) of cancers are estimated to be caused by infectious agents. Viruses have been strongly indicated in the development of a sub-group of upper aero-digestive tract cancers (UADTC). Human papillomavirus (HPV) has been of particular interest, especially high risk – Human papillomavirus (hr-HPV) which has been shown to be carcinogenic with regard to epithelial cells.

Human papillomavirus-positive (HPV +ve) cancers are now thought to account for 25–50% of all head and neck cancers, now representing a growing public health concern that is projected to become the primary cause of head and neck cancers in the coming decades. Data from Global Cancer Statistics (Globocan 2018) shows that the proportion of head and neck cancer to all cancers is 4.9% globally, 4.0% in Africa while in Kenya it is 6%. Mortality rates globally are at 51% whereas in Africa the rate is at 68.4% while in Kenya it is 73.4%. However, although the contribution of high risk – Human papillomavirus to the head and neck cancer burden has been determined in other parts of the world, such data is not available in Kenya.

1.3 Justification of the Study

According to the Kenya National Cancer Treatment Protocols (2019), head and neck cancer constitute 5.7 % of the cancer burden in Kenya. Preliminary findings from Western Kenya indicate a high prevalence of head and neck cancer (6.5%), however, the contribution of high risk - Human papillomavirus and its genotypes to these HNC

cases remains to be determined in this region. The prevalence of Human papillomavirus, distribution of Human papillomavirus genotypes, proportion of head and neck cancer caused by high risk – Human papillomavirus varies depending on population studied, location of tumor and method of Human papillomavirus detection. Alexandria Cancer Centre and Palliative Care Hospital (ACCPCH) is one of the major cancer facilities in the Western Kenya Region that is National Hospital Insurance Fund (NHIF) accredited making it accessible to people of all walks of life. Fortunately, all samples are processed at Lancet Laboratories which is a well-established institution with equipment and the protocols in place together with competent staff to guide and assist in carrying out the study. Thus, making the institution ideal for such a study.

1.4 Objectives

1.4.1 Broad Objective

To assess the genotypes and occurrence of high-risk Human papillomavirus among patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital, Eldoret between January 2017 and December 2018.

1.4.2 Specific Objectives

1. To describe demographic characteristics of patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital, Eldoret between January 2017 and December 2018.
2. To determine the prevalence of head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital, Eldoret between January 2017 and December 2018.

3. To determine the prevalence of head and neck cancer associated with high risk-Human papillomavirus at Alexandria Cancer Centre and Palliative Care Hospital between January 2017 and December 2018
4. To perform molecular characterization of high risk-Human papillomavirus associated with various head and neck cancer sub-sites at Alexandria Cancer Centre and Palliative Care Hospital, Eldoret between January 2017 and December 2018.

1.5 Research Questions

1. What were the demographic characteristics of patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital between January 2017 and December 2018?
2. What was the prevalence of head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital between January 2017 and December 2018?
3. What was the prevalence of head and neck cancer associated with high risk-Human papillomavirus at Alexandria Cancer Centre and Palliative Care Hospital between January 2017 and December 2018?
4. Which high risk-Human papillomavirus genotypes were associated with the various head and neck cancer sub-sites at Alexandria Cancer Centre and Palliative Care Hospital between January 2017 and December 2018?

CHAPTER TWO: LITERATURE REVIEW

2.1 Head and Neck Cancer (HNC)

The occurrence and progress of head and neck is dependent on several factors that might be due to the genetic makeup, diet, routine, exposure to sunlight, geographic location among others. Tobacco use and alcohol use are the main principle factors for the development of head and neck cancer and their joint effect can be synergistic. Their risks are associated with the intensity and duration of use (Alfouzan, 2019). Other risk factors are infection by oncogenic viruses including Human papillomavirus (HPV); chewing (smokeless) tobacco and other products, chewing of betel nut, occupational exposure to toxins, radiation; diet, oral hygiene and genetic factors (Alsbeih, 2019).

In 2018, Head and neck cancer was the seventh most common cancer worldwide with 890,000 new cases and 450,000 deaths, accounting for 3% of all cancers and just over 1.5% of all cancer deaths in the United States (Chow, 2020). In Africa, new cases were 42, 521 and deaths were 29, 074 (Globocan, 2018) and in Kenya, new cases were 2,886 and deaths were 2,119 (Kenya Globocan, 2018).

In Kenya, cancer a major non communicable disease , is the third leading cause of death after infectious and cardiovascular diseases estimated to cause 7% of total national mortality every year (Kalebi. A et al., 2019). Presently, it is problematic to get definite national data on the prevalence of head and neck cancer in Kenya due to the fact that there are a few Cancer Registries with the two largest being: Nairobi Cancer Registry [NCR] and Eldoret Cancer Registry [ECR] (Kigen, 2017). However, the distribution of head and neck cancer varies around the world, accounting for 5% – 50% of all cancer globally (Gilyoma, 2015).

In a study carried out by reviewing Globocan findings 2008 and 2012, the researchers found out that: head and neck cancer ranked sixth in the world, third in Africa and fourth in the Sub-Saharan region. In Kenya, head and neck cancer ranked third; including lip, oral cavity, nasopharynx, other pharynx and larynx excluding esophagus. When esophagus was included, it ranked first (Adeola, 2018).

The contribution of Human papillomavirus to head and neck cancer in sub-Saharan Africa is not well described (Faggons, 2015) but a recent study recorded the presence of Human papillomavirus-positive Deoxyribonucleic acid (HPV DNA) in 15 (19.23%) out of 79 cases of head and neck squamous cell carcinoma (Aboagye, 2019).

Most of the structures that are affected with head and neck cancer are visible and this negatively impacts ones looks. This might lead to feelings of low self-esteem because of the way in which one gets looked at by people around them. Apart from this, since these are important organs in the body, it might affect one's life especially it affects processes such as speaking, eating, breathing among others. As a result, on top of this, the mental well-being of the patients is also affected leading to the tendency to isolate oneself and may even develop depression. It is paramount to look into how well the general population is are about the relationship between Human papillomavirus and head and neck cancer. This can used to guide treatment protocols and involve counselling (Dodd, 2016).

2.2 Head and Neck Cancer and Human papillomavirus

An association between Human papillomavirus and head and neck squamous cell carcinoma was initially suspected during the 1960s when there were reports of conversion of recalcitrant laryngeal papillomas to malignancy after radiation therapy (Shah, 2017). Syrjanen et al. in 1983 were historically, the first to evoke an association between head and neck squamous cell carcinoma and Human papillomavirus. This was based on observations on histopathological features occurring specifically in the oropharynx (middle part of the throat, including the soft palate, the base of the tongue, tonsil, uvula, and Waldeyer's ring) and immunohistochemical properties followed by confirmation of Human papillomavirus Deoxyribonucleic acid (HPV DNA) presence in a subset of Oropharyngeal Cancers (Sano & Oridate, 2016). Since that time, Human papillomavirus has been clearly implicated as the causative agent in a subset of head and neck cancer (Spence T, Bruce J, Yip KW, 2016) but its role in the initiation and maintenance of malignancy was not decisively established until 2000 when evidence began to mount confirming its nature as a causal agent (Westra, 2015).

In the last 20 years, smoking and alcohol use has declined considerably in the developed world, which contributed to a significant decrease in head and neck squamous cell carcinoma cases related to smoking and alcohol (Aldalwg & Brestovac, 2017). However, head and neck squamous cell carcinoma burden has not significantly declined, partly explained by the emergence of Human papillomavirus as an etiologic agent (Eid, 2019). It is suggested that this is related to changing sexual behavior, with an increase in oral-genital contact, sexual debut at early age and a high number of lifetime sex partners (Shaikh, 2017).

Human papillomavirus-associated head and neck squamous cell carcinoma have different risk factors, clinical characteristics and tumor biology when compared to tobacco/alcohol-associated head and neck squamous cell carcinoma. It has become apparent that there are two definite classes of head and neck squamous cell carcinoma depending on the presence or absence of Human papillomavirus. With this in mind, Human papillomavirus positive (HPV +ve) and Human papillomavirus negative (HPV –ve) tumors are now regarded as independent diseases (Aldalwg & Brestovac, 2017).

The epidemiology of Human papillomavirus infection is known to have wide variations in human populations, remnant of socioeconomic, ethnic and genetic predisposing factors that is why it is important to determine the actual prevalence of high-risk human papillomavirus in head and neck cancer squamous cell carcinoma (Alfouzan, 2019).

Nearly 90% of head and neck cancer originate from squamous cells and hence are usually referred to as Head and Neck Squamous Cell Carcinoma (HNSCC) (Iglesias Docampo, 2018). Other histological types include lymphomas, blastomas, sarcomas and neuroendocrine tumors (Gilyoma et al., 2015).

The reported prevalence of oral Human papillomavirus infection from individual studies is highly variable as it is influenced by the sampling technique and the population studied. Oral Human papillomavirus infection is rare and significantly differs by gender, with considerably higher prevalence observed in men. Data from two large studies having sample sizes of 4581 and 3762 reported figures of 4.5% and 7.5% as the oral Human papillomavirus respectively. The largest population-based study carried out in USA, as part of the National Health and Nutrition Examination

Survey, reported an oral Human papillomavirus prevalence of 6.9%, with a significant difference between men (10.1%) and women (3.6%) (Taberna, 2017).

Little is known about the natural history of oral HPV infection, peak in prevalence is around ages 30 to 34 years and 60 to 64 years, following a bimodal pattern (Pinatti, 2018). Some studies indicate that the most likely explanation for the origin of this distinct form of head and neck cancers associated with Human papillomavirus is a sexually acquired subclinical oral infection that is not cleared and persists and evolves into a neoplastic lesion (Bruni, 2019).

A recent study by Casellsagu´e et al., 2016 which included 1374 pharyngeal cancers, 1264 oral cavity cancers, and 1042 laryngeal cancers from 29 countries, reported the presence of Human papillomavirus Deoxyribonucleic acid (HPV-DNA) in 22.4% of oropharyngeal cancers, 4.4% in oral cavity cancers, and 3.5% in laryngeal cancers. A recent meta-analysis of 148 studies involving 12,163 head and neck squamous cell carcinoma cases showed the existence of Human papillomavirus Deoxyribonucleic acid (HPV-DNA) in 31.5% of tumors with greater prevalence in the oropharyngeal squamous cell carcinomas (OSCC) (45.8%), followed by oral squamous cell carcinomas (24.2%) and laryngeal squamous cell carcinomas (22.1%) (Aboagye et al., 2019).

The more prominent turning point is that Human papillomavirus positive head and neck squamous cell carcinoma especially oropharyngeal cancer, appear to form a distinct tumor entity with distinguished epidemiology, genetics, characteristic histopathology, therapeutic response and predictive clinical outcome to chemo-radiation treatment (Alsbeih et al., 2019). The patients tend to be younger at the time of diagnosis who tend to be non-smokers, non- or light- drinkers and many have relatively high socioeconomic status (Shaikh et al., 2017). Human papillomavirus

positive head and neck squamous cell carcinoma are more likely to be smaller and poorly differentiated (with basaloid features) (Spence T, Bruce J, Yip KW, 2016). They tend to have a higher incidence of advanced lymph node (LN) metastasis (Dok & Nuyts, 2016) and present with later-stage tumors (Dreyer, 2017) than those with tumors not associated with Human papillomavirus infection (Tsai, 2019). They rarely metastasize to distant organs or have second primaries (Shah et al., 2017).

Interestingly, several retrospective and prospective trials have shown that patients with Human papillomavirus positive head and neck squamous cell carcinoma, especially oropharyngeal cancer have better overall survival (OS), disease free survival (DFS) and locoregional control (LRC) compared to the Human papillomavirus negative patients and this is independent of the treatment modality (Dok & Nuyts, 2016). The 5-year survival rate for Human papillomavirus positive head and neck squamous cell carcinoma is 60% - 90% as compared with 20% - 70% in Human papillomavirus negative head and neck squamous cell carcinoma. The 3-year overall survival rates are 82.4% for Human papillomavirus positive head and neck squamous cell carcinoma versus 57.1% for Human papillomavirus negative head and neck squamous cell carcinoma irrespective of age, gender or tumor stage (Shaikh et al., 2017).

Better survival is believed to be as a result of better response to the treatment used, chemotherapy and radiotherapy. A better outcome is also thought to be due to lack of field cancerization, immune surveillance and reduced occurrence of chromosome mutations (Shah et al., 2017). Less frequent chromosome mutation, immune surveillance of viral antigens, and absence of field cancerization may also contribute to a better prognosis (Shah et al., 2017).

Viruses are obligatory intracellular parasites and encode proteins that reprogram the regulatory networks governing host cellular signaling pathways that control recognition by the immune system, proliferation, differentiation, genomic integrity, and cell death. The study of oncogenic viruses, as well as the manner in which they target regulatory nodes, has been key to the understanding of the etiology of several human cancers. More recently, tumor viruses, in particular HPV, have proven themselves invaluable in the study of the cancer epigenome (Soto et al., 2017).

There are many risk factors that are associated with development of cancer including infectious agents. For cancers that are associated with Human papillomavirus, there is a percentage that is calculated based on the cancer cases that might not have come about if it did not exist within the population. This proportion that is referred to ad attributable fraction differs across geographic regions with no figures ascertained in most parts while a figure of about 40% has been pointed out for Korea, Japan, New Zealand, Australia, Northern America and Europe. In most parts of the world, it has been pointed out that approximately 85% of head and neck cancers are associated with HPV 16 and HPV 18. The input of the other genotypes: 58,52,45,33,31,18,16,11,6 has been found to differ (Martel, 2017).

The literature on Human papillomavirus in non-Oropharyngeal Squamous Cell Carcinoma subsites is controversial. Studies revealed that Human papillomavirus is present in these subsites, albeit estimated to be 5 times less prevalent in non-Oropharyngeal Squamous Cell Carcinoma than Oropharyngeal Squamous Cell Carcinoma. A 2016 study that compared the gene expression and Deoxyribonucleic acid methylation profiles of Human papillomavirus in non- Oropharyngeal Squamous Cell Carcinoma subsites with those in Oropharyngeal Squamous Cell Carcinoma subsites found them to be identical, leading to the conclusion that Human

papillomavirus can drive carcinogenesis in non-Oropharyngeal Squamous Cell Carcinoma. The same study concluded that Human papillomavirus-driven non-Oropharyngeal Squamous Cell Carcinoma has a distinct tumor microenvironment compared with HPV-driven Oropharyngeal Squamous Cell Carcinoma. Few studies have looked at the role of Human papillomavirus at each individual non-Oropharyngeal Squamous Cell Carcinoma subsite. Tumors of some subsites, are rare; thus, accurately characterizing the prognostic role of Human papillomavirus has been difficult (Li, 2018).

2.3 Subtypes of Human papillomavirus

To date, 202 different Human papillomavirus types have been isolated (International Human papillomavirus Reference Center (Hübbers & Akgül, 2015). A phylogenetic tree construction based on the nucleotide sequence of gene coding for the capsid L1 protein provides the basis for classification of the Human papillomavirus into five genera – alpha, beta, gamma, mu and nu (Tsakogiannis, 2017).

Genus alpha-papillomavirus are also referred to as “Mucosal Human papillomavirus types” because they can infect the mucosa of the anogenital and oral tract (Sano & Oridate, 2016). They are categorized as high-risk or low-risk according to the likelihood that an infection by the Human papillomavirus type can lead to the development of cancer (Burd, 2016).

A subset of these mucosal Human papillomavirus types considered high-risk Human papillomavirus (hr-HPV) are frequently found in association with various cancers and has been shown to have transforming ability in model systems. Another subset considered potentially high risk has been found in association with cancers, but evidence demonstrating carcinogenicity is lacking (Pinatti et al., 2018).

The high-risk HPV types may be classified as shown by Table 1.1 below (Burd, 2016).

Table 1: Further Classification of High-Risk Human papillomavirus (HPV) Types

High-Risk HPV Types	
Highest risk	16,18,31,45
Other high risk	33,35,39,51,52,56,58,59
Probably high risk	26,53,66,68,73,82

The World Health Organization (WHO) and the International Agency for Research on Cancer (IARC) categorized 12 Human papillomavirus types as group 1 carcinogens in humans: HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 (Taberna et al., 2017). Group 2A and group 2B are considered probably carcinogenic to humans (Burd, 2016). At least 10 of these oncogenic Human papillomavirus types (HPV16, 18, 31, 33, 45, 51, 52, 56, 58, and 59), as well as 6 low-risk Human papillomavirus types (11, 32, 44, 53, 57 and 81), have been isolated from head and neck squamous cell carcinomas (Faraji, 2017) and ensued by a minority of other sporadic genotypes (Alsbeih et al., 2019).

2.4 Molecular Biology of Human papillomavirus (HPV)

Human papillomavirus is classified under family Papovaviridae. The genome is round with a diameter of between 50 to 60 nm and made up of about 8kb (8000) base pairs. The genome is contained in two intertwined Deoxyribonucleic acid strands that are enclosed within a protein shell that has an icosahedral shape but there is no outer lipid covering. Generally, the genetic makeup is sub-divided into three sections: early, late and a regulative sector that is also referred to as the non-coding or long control region.

These sectors are segregated with the use of two sites composed of multiple adenosine monophosphates designated as early pA and late pA that is dependent on when they get expressed during the life cycle (Burd, 2016).

The Early Region covers around 50% of the virus genome from its 5′ half and codes for six Open Reading Frames (ORFs) (E1, E2, E4, E5, E6 and E7) that are involved in the translation of individual proteins. The Late Region, which constitutes nearly 40% of the virus genome, lies downstream of the Early Region and codes for L1 and L2 ORFs for translation of a major (L1) and a minor (L2) capsid protein that encloses the viral Deoxyribonucleic acid (DNA). They have no known role in carcinogenesis but are believed to play a vital role in final stages of virion assembly, mediating viral entry into future host cells and being of importance to the immune response to Human papillomavirus infection (Faraji et al., 2017). The Long Control Region (LCR), covers about 10% of the genome and contains the Origin of Replication together with multiple transcription factor binding sites that are key in the regulation of Ribonucleic acid (RNA) polymerase II-initiated transcription from viral early as well as late promoters (Perri, 2020).

Malignant transformation starts by integration of high-risk Human papillomavirus Deoxyribonucleic acid (HPV DNA) into the host cellular genome that disrupts the expression of the gene encoding the E2 protein. This results in overexpression of the viral E6 and E7 oncogenes in basal layer leading to a disruptive viral infection and incomplete viral life cycle and causes abrogation of cell cycle checkpoints (Aldalwg & Brestovac, 2017). E2 protein differentially regulates E6/E7 expression through control of their transcription (Faraji et al., 2017).

The three genes E7, E6 and E5 do not exist in all Human papillomavirus types. For instance, it has been noted that E5 does not seem to be present in beta Human

papillomavirus. It has been previously postulated that E5 plays a role in tumorigenic capability. However, further examination has shown that the proteins that are produced from this gene appear to be dissimilar in different Human papillomavirus types (Burd, 2016).

In high-risk Human papillomavirus types, the E5 proteins have been shown to interact with a variety of cellular targets, with consecutive effects that may lead to development of cancer which may include interference of apoptosis, dodging exposure by the immune system, over production of transmembrane protein receptors of epidermal growth factor. It is believed that E5 collaborates with E7 and E6 in the early stages and is later gotten rid of since it no longer plays a role in the subsequent steps and development (Wang et al., 2018) (Eid, 2019).

High-risk Human papillomavirus types encode virulent alleles of two viral proteins, E6 and E7, that endow viral oncogenic potential by targeting molecular pathways that underlie neoplastic transformation (Faraji et al., 2017).

In normal cells, uncontrolled cell growth usually leads to apoptosis, frequently because of the stabilization and activation of the tumor suppressor p53. The normal function of p53 is to stop cell division and repair damaged Deoxyribonucleic acid so that damaged cells do not reproduce. P53 is the protein product of the TP53 gene, a regulator of the Deoxyribonucleic acid-damage response, G2/M cell cycle transition and the most commonly mutated tumor suppressor gene in cancer (Faraji et al., 2017). High-risk Human papillomavirus E6 protein inactivates p53 and activates telomerase activity which synthesizes telomere repeat sequences and maintains a repeated cell cycle that continues to produce infected cells. It binds to p53 and induces ubiquitin-dependent proteolysis via the cellular ubiquitin ligase E6-AP (UBE3A) pathway (Riaz, 2014). The degeneration of p53 affects the stability of the cellular genome

through deregulation of both G1/S and G2/M cell cycle checkpoints (Dok & Nuyts, 2016).

This leads to: increased Deoxyribonucleic acid (DNA) damage, chromosomal instability, increased cell proliferation and eventually tumorigenesis due to loss of p21 function which is a cyclic dependent kinase (CDK) inhibitor and subsequent loss of p53 mediated apoptosis (Mwapagha, 2017). Only E6 alleles from high-risk Human papillomavirus types bind p53. The strength of the binding affinity correlates with that Human papillomavirus type's oncogenic potential. For example, Human papillomavirus (HPV) 16, which is responsible encodes for an E6 allele that binds p53 with twice the affinity of the Human papillomavirus (HPV) 18 E6 allele (Faraji et al., 2017).

In normal cells, retinoblastoma (Rb) proteins impede transcription factor E2F from upregulating a number of genes, particularly, E2F-responsive genes (ERGs), that are important for cell proliferation. Retinoblastoma enacts its primary inhibitory effect on the cell cycle by regulating the nuclear accumulation of E2F and thereby controls the G1 to S-phase cell cycle transition. The proteins that are coded for by many ERGs are concerned with nucleotide synthesis, Deoxyribonucleic acid replication and cell cycle progression (Perri, 2020).

High-risk Human papillomavirus E7 oncoprotein exerts its oncogenic effect by facilitating the proteolytic destruction of retinoblastoma (Rb)-family tumor suppressors. High-risk Human papillomavirus E7 plays a critical role in initiating Deoxyribonucleic acid synthesis by binding and inactivating the retinoblastoma and its related pocket proteins p107 and p130 which are tumor suppressor genes by targeting them for degradation. High-risk Human papillomavirus E7 induces enhanced degradation of retinoblastoma protein via a ubiquitin-proteasome pathway.

The binding of high-risk Human papillomavirus E7 protein with retinoblastoma mimics phosphorylation (pRb) resulting in the release of the transcription factor E2F from the pRb-E2F protein complex (Hübbers & Akgül, 2015).

Degradation of retinoblastoma releases E2F family mitogenic transcription factors into the nucleus, promoting entry into S-phase and activating proliferative transcriptional programs. On the other hand, low-risk Human papillomavirus E7 binds retinoblastoma at an affinity that is insufficient to induce levels of retinoblastoma degradation necessary to promote neoplastic transformation (Faraji et al., 2017).

Interruption of the normal cell cycle results in the rise of uncontrolled cell growth with the resulting formation of a lesion. Depending on one's immune system, these may decrease in size with time. It is possible to be able to isolate Human papillomavirus from such growths within 1 to 2 years. In a minority of cases ranging from 10 to 20%, the growths do advance to precancers and cancers (Burd, 2016).

CHAPTER THREE: METHODOLOGY

3.1 Study Design

This was a retrospective study with laboratory analysis. Medical records at Alexandria Cancer Centre and Palliative Care Hospital (ACCPCH) Eldoret between January 2017 and December 2018 were reviewed. For patients diagnosed with head and neck cancer data on demographics (age, gender, area of residence, occupation) and histological information (primary site and morphology of tumor) was collected. Stored tissues were then retrieved and processed [repeat microscopy, p16 Immunohistochemistry (p16 IHC) and Human papillomavirus Polymerase Chain Reaction (HPV PCR)] at the Pathologists Lancet Laboratories.

3.2 Study Site

Alexandria Cancer Centre and Palliative Care Hospital is located along Lumumba Avenue, off Uganda/Nandi Road, opposite Moi University School of Dentistry, School of Nursing and School of Public Health in Eldoret. Eldoret Town serves as the capital of Uasin Gishu County and is 310 Kilometers Northwest of Nairobi. It is the sixth most populated urban area in the country with 475,716 inhabitants according to 2019 national census.

The majority of patients that attend this facility come from about 22 counties that are part of the Western Kenya Region. There are also a small number that come from other parts of the country and sometimes even further in Eastern Uganda and Southern Sudan. The main headquarters of Lancet Laboratories in Kenya are situated in Upper Hill in Nairobi, across from Traffic Headquarters along 5th Ngong Avenue Office Suites. These group of laboratories began in South Africa and is now found in

many countries. They provide many testing services both directly to individuals or to hospitals. They offer screening, monitoring and diagnostic services.

3.3 Population

3.3.1 Target Population

The target population was all patients diagnosed with cancer among patients attending Alexandria Cancer Centre and Palliative Care Hospital between January 2017 and December 2018 and their associated records meeting.

3.3.2 Study Population

The study population was all patients diagnosed with head and neck cancer among patients attending Alexandria Cancer Centre and Palliative Care Hospital between January 2017 and December 2018 and their associated records meeting the eligibility criteria.

3.3.2.1 Inclusion Criteria

All consenting patients diagnosed with head and neck cancer and guardians attending Alexandria Cancer Centre and Palliative Care Hospital during the study period.

3.3.2.2 Exclusion Criteria

- Records with incomplete entries.
- Head and neck cancer sample records with missing sample blocks.
- Sample blocks with missing tissue.
- Samples with inconclusive repeat microscopy results.

3.4 Data Collection

Medical records of all patients diagnosed with cancer attending Alexandria Cancer Centre and Palliative Care Hospital between January 2017 and December 2018 were reviewed. A predesigned data abstraction form was used for the purposes of data collection. For patients diagnosed with head and neck cancer, data on demographics (age, gender, area of residence, occupation) and histological information (primary site and morphology of tumor) was collected. Stored tissues were then retrieved and processed at the Pathologists Lancet Laboratories. Results of the laboratory assays: repeat microscopy, p16 Immunohistochemistry and Human Papillomavirus Polymerase Chain Reaction were also recorded in the abstraction form. Collected data was then entered into a Microsoft Excel Spreadsheet, (Microsoft 2016).

3.5 Laboratory Assays

3.5.1 Sectioning and Microscopy

Formalin fixed, paraffin-embedded (FFPE) blocks of malignant head and neck cancers were retrieved. 4µm thick sections were cut using, a rotary microtome, Leica RM2125RT (Leica Biosytems, Germany). The slides were then stained with Haematoxylin and Eosin (H&E) using an Automated Slide Stainer, Thermo Scientific Gemini AS (Thermo Shandon Diagnostics Limited, United Kingdom) prior to being microscopically examined. Briefly: Sections were cut at 4µm thick on a microtome (Leica RM2125RT) and picked up on microscope glass slides. They were then placed upright on a wooden rack to drain off excess water, after which they were placed on a hot plate to dry completely. Slides were then placed in a staining rack and stained using the Thermo Scientific Gemini AS Automated Slide Stainer with Hematoxylin and Eosin as follows: Dewax in Xylene – Changes (Xylene 1– 5 minutes, Xylene 2 – 10 dips), Hydrate in alcohol – 100%, 90%, 80% (Decreasing concentrations), Rinse in

running tap water, Stain in 3 changes of Hematoxylin for 4-5 minutes, Rinse in running tap water, Place in Scotts tap water – 1 minute, Rinse in running tap water, Stain in 2 changes of Eosin for 2-3 minutes each, Rinse quickly in running tap water, Dehydrate in 3 changes of alcohol (Increasing concentrations), Clear in 3 changes of Xylene, Place it against a drop of on DPX (A combination of distyrene (a polystyrene), a plasticiser (tricresyl phosphate) and (xylene)) on the surface of slide, then use a cover slip. The results were read based on microscopic distribution with the nuclei staining blue and the cytoplasm staining varying shades of pink.

3.5.2 p16 Immunohistochemistry (IHC)

Immunohistochemistry (IHC) was performed on 4 µm thick sections cut from formalin-fixed, paraffin-embedded tissue (FFPE) blocks. The sections were then mounted on TOMO Adhesive microscope slides, (Matsunami, Japan). CINtec® p16 Histology Kit (705-4713, Ventana Medical Systems, Roche, Tucson, Arizona, United States) was used for qualitative detection. The kit is composed of one constituent, an antibody that is made against the $p16^{INK4a}$ protein, anti-$p16^{INK4a}$ (E6H4). Immunostaining was carried out on a Ventana Benchmark Ultra automated slide stainer (Ventana Medical Systems, Roche), following manufacturer protocol. Detection involved Ventana's optiView diaminobenzidine tetrahydrochloride (DAB) Detection Kit (Ventana Medical Systems, Roche) that utilizes a cocktail of enzyme-labeled secondary antibodies that locate the bound primary antibody. To be able to see the compound that is formed, a colored dye 3, 30-diaminobenzidine tetrahydrochloride (DAB) is mixed with hydrogen peroxide substrate. The tests were compared to known positive and negative controls. Continuous, diffuse staining pattern was considered positive. Focal staining represented by non-continuous staining of isolated cells or small cell clusters represented a negative staining result.

Briefly: Sections were cut at 4µm thick on a microtome (Leica RM2125RT) and picked up on TOMO Adhesive charged microscope glass slides. Positive was included at the top and the sample was included at the bottom on the same slide. The slide was then placed on a hot plate to melt the wax. Staining Protocol for CINtec p16 Histology with Opti View Universal diaminobenzidine tetrahydrochloride (DAB) IHC Detection Kit on Benchmark Ultra Instrument was carried out as follows.

Procedure Type	**Method**
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, 48 minutes
Pre Primary Peroxidase Inhibitor	Selected
Antibody (Primary)	12minutes, 36^0C
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

Standard Positive and negative controls were compared against the slides that were stained with CINtec Histology kit to ensure that staining is adequate and within the required parameters usually set that in most cases is ≥ 70%. The patient specimen is subsequently interpreted as either positive (continuous, diffuse p16 staining pattern) or negative (focal p16 staining pattern, represented by non-continuous staining of isolated cells or small cell clusters or no p16 staining).

3.5.3 Human papillomavirus (HPV) Genotyping

3.5.3.1 Deoxyribonucleic acid (DNA) Extraction and Purification

Deoxyribonucleic acid extraction and purification from the formalin fixed paraffin embedded tissue blocks was done using Maxwell® 16 Formalin-Fixed, Paraffin-Embedded (FFPE) Plus Low Elution Volume (LEV) Deoxyribonucleic acid Purification Kit (AS1135, Promega, Madison, Wisconsin, United States) that was used together with Maxwell® 16 MDx Instrument (AS3000, Promega, Madison, Wisconsin, United States). Briefly: A stock of 20mg/ml of Proteinase K solution was prepared. 1–10 × 5µm section (s) were scraped from the Formalin Fixed Paraffin (FFPE) tissue sample into a single micro tube. The samples were then centrifuged at maximum speed and the sample was then gathered at the bottom of the tube. Then 180µl of Incubation Buffer and 20µl of Proteinase K solution were added. The sample tube was the closed and incubated at 70°C through the night. The sample was then vortexed and lysis was then added at a ratio of 2:1 for instance if 100µl of the mixture of Proteinase K/ Incubation Buffer was added then put 200µl of Lysis Buffer. The lid of the microtube was the closed and saved until ready for automated DNA extraction. The cartridges were placed in the Maxwell® 16 low elution volume cartridge rack. A low elution volume was then placed in the well closest to the elution tube. Then 50µl of nuclease-free water was added to the tubes. Maxwell® 16 MDx Instrument was then turned on and the on-screen instructions followed.

3.5.3.2 Deoxyribonucleic acid (DNA) Amplification and Detection

Human papillomavirus genotyping was achieved with the Linear Array Human papillomavirus Genotyping Test Kit (Roche Diagnostics, Mannheim, Germany). It is a qualitative, *in vitro* test for the detection of Human papillomavirus in clinical specimens based on L1 consensus Polymerase Chain Reaction (PCR) amplification of

target Deoxyribonucleic acid with biotinylated PGMY primers. The next step is then identification of the thirty-seven Human papillomavirus genotypes that this kit can distinguish by hybridization of the amplification product of nucleic acids. HPV: [89 (CP108), IS39, 84 (MM8), 83 (MM7), 82 (MM4), 81, 73 (MM9),72, 71, 70, 69, 68, 67, 66, 64, 62, 61, 59, 58, 56, 55, 54, 53, 52 (XR), 51, 45, 42, 40, 39, 35, 33, 31, 26,18, 16, 11, 6]. Amplification was performed on GeneAmp PCR System 9700 (Applied Biosystems, Foster City, California, United States). Detection was done by hybridization of the genotyping strips on the the GT-Blot 48 (Bruker Hain Diagnostics, Billerica, Massachusetts, United States). Detection of probe-bound amplified products was by colorimetric determination. The ß-globin gene was amplified and detected concurrently as an internal control to assess cellular adequacy, extraction and amplification for each sample. The manufacturer's specifications were followed for each of the tests that were carried out. Briefly: Record on the worksheet sample IDs and Lot numbers from all reagents. Put on the GeneAmp 9700, choose "Linear Array Assay". Pick from the genotyping kit and put the same number of tubes of Magnesium solution (HPV Mg2+) and Linear Array HPV Master Mix onto the rack. Label 0.2 ml PCR tubes for the reactions. Combine the entire content of each HPV Master Mix with 125 µl HPV Mg2+ for each. Mix 12 times and then move the mixture of into a clean tube using a pipette then add 50 µl of working Master Mix to each of the tubes. Transfer 5 µl DNA template to the prepared PCR tubes/wells and add 45 µl nuclease free water so that the total volume will be 100 µl. Place into thermocycler and start program (approximately 3h and 15 min). Retrieve the tray after the final HOLD step and put 100 µl of denaturation solution to each tube. Warm all detection reagents to room temperature. Label HPV genotyping strips and separate into single strips. Choose assay Press "Start" then add hybridization buffer, after

which “Add amplicon” will be shown. Add 75 µl of denatured amplicon to the rounded opening at the front of the slots into the hybridization buffer. Press “Start”. Run Cleaning cycle A and B when prompted. Empty general waste containers into the drain after each run. The strips were left to dry through the night then they were placed on the blotting paper and enclosed in plastic, together they were then attached onto the Linear Array worksheet.

3.6 Data Storage

The cancer abstract forms that were used for data collection were placed in cabinets that are locked and used for reference when data analysis was done. The electronic copies were kept in a computer that has a password for confidentiality. There was also an extra copy that was made as a backup and kept in an external hard drive.

3.7 Data Analysis

Data analysis was done using Microsoft Excel (Microsoft 2016). Descriptive statistics were used to summarize the findings on demographics (age, gender, area of residence, occupation); subsites of head and neck cancer, tumor morphology, p16 status and Human Papillomavirus Polymerase Chain Reaction. Proportions were also calculated in order to determine the prevalence of head and neck cancer and high risk-Human papillomavirus head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital. The information was presented in charts and tables.

3.8 Ethical Considerations

Ethical approval for the study was obtained from Moi University/ Moi Teaching and Referral Hospital Institutional Research and Ethics Committee (MTRH-IREC). Institutional Consent was also obtained from the Management of Alexandria Cancer Centre and Palliative Care Hospital, Eldoret. Patients’ files were used within the

confines of the medical records department under the supervision of the Health Records Officer. Verbal informed consent (via telephone call) was obtained from the subjects whose samples were used. Permission to carry out the laboratory assays was obtained from the management at Pathologist Lancet Laboratories. All laboratory procedures were carried out by a trained laboratory technician. A second pathologist was tasked with the reconfirmation of diagnosis. The pathologist also reported on p16 immunohistochemistry.

3.9 Limitations of the Study

Non-availability of fresh frozen tissues because use of Formalin Fixed Paraffin Embedded (FFPE) blocks poses a challenge during Deoxyribonucleic acid extraction due to cross-linking of proteins and Deoxyribonucleic acid.

3.10 Dissemination of Findings

- The research findings were presented to staff and Management at Alexandria Cancer Centre and Palliative Care Hospital, Eldoret.
- Preparation and submission of abstracts to relevant scientific conferences for oral and poster presentations.
- Preparation of manuscripts for submission to relevant scientific journals for publication.
- A bound thesis for the library.

CHAPTER FOUR: FINDINGS OF THE STUDY

4.1 Prevalence of head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital

The total number of patients diagnosed with cancers between January 2017 and December 2018 was 1016. Of these, the number of patients who were diagnosed with head and neck cancer during this period of the study was 89. This number represented 8.8% (89/1016) of the total malignancies.

Table 2: Prevalence of head and neck cancer at Alexandria Cancer Centre and Palliative care Hospital

	Frequency
Patients diagnosed with Head and Neck Cancer	89 (8.8%)
Patients diagnosed with other malignancies	927 991.2%
Total	1016

4.2 Demographic Characteristics of patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital

4.2.1 Gender distribution of patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital

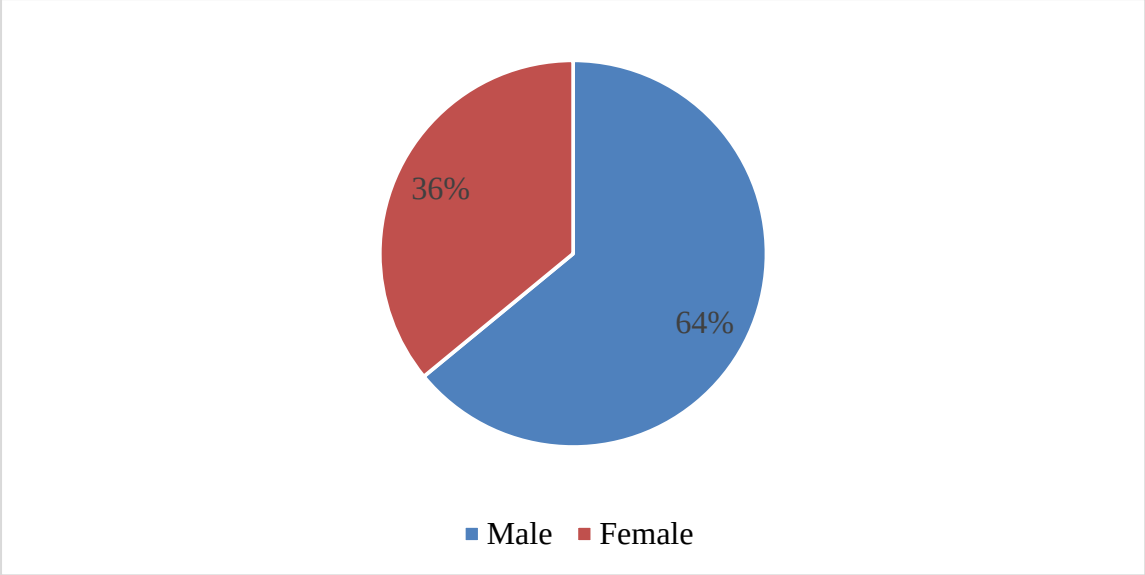

Figure 4: Gender distribution of patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital

During the period of the study, 89 patients attending Alexandria Cancer Centre and Palliative Care Hospital were diagnosed with head and neck cancer, of these, 57 (64%) were males and 32 (36%) were females.

4.2.2 Age distribution of patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital

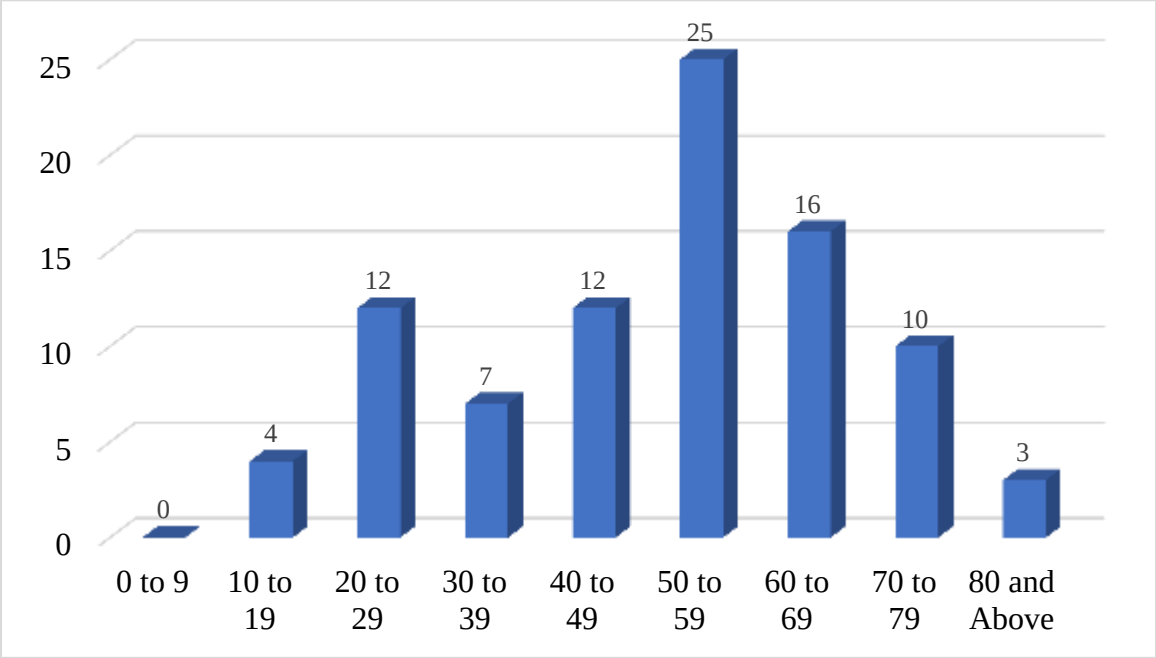

Figure 5: Age distribution of patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital

Among patients that were diagnosed with head and neck cancer: there were no patients within the 0 to 9 age group. 4 (4.5%) were within the age group 10 to 19, 12 (13.5%) were within the age group 20 to 29, 7 (7.9%) were within the age group 30 to 39, 12 (13.5%) were within the age group 40 to 49, 25 (28.1%) were within the age group 50 to 59, 16 (18.0%) were within the age group 60 to 69, 10 (11.2%) were within the age group 70-79 and 3 (3.4%) were within the age group 80 and above. The mean age was 50.9 years (Standard Deviation ± 17.6), the median age was 54.5 years old and the modal age being 57 years old, with the most affected age group being 50-59.

4.2.3 Area of residence among patients diagnosed with head and neck cancer at Alexandria Cancer and Palliative Care Hospital

Table 3: Area of residence among patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital

	County	Count		County	Count
1	Nandi	11 (12.4%)	14	Siaya	3 (3.4%)
2	Uasin Gishu	11 (12.4%)	15	Homabay	2 (2.2%)
3	Kericho	8 (9.0%)	16	Migori	2 (2.2%)
4	Elgeyo Marakwet	6 (6.7%)	17	Vihiga	2 (2.2%)
5	Kisii	5 (5.6%)	18	Busia	1 (1.1%)
6	Baringo	4 (4.5%)	19	Laikipia	1 (1.1%)
7	Bomet	4 (4.5%)	20	Lodwar	1 (1.1%)
8	Bungoma	4 (4.5%)	21	Machakos	1 (1.1%)
9	Kakamega	4 (4.5%)	22	Nairobi	1 (1.1%)
10	Nyamira	4 (4.5%)	23	Narok	1 (1.1%)
11	Trans Nzoia	4 (4.5%)	24	Nyandarua	1 (1.1%)
12	Kisumu	3 (3.4%)	25	West Pokot	1 (1.1%)
13	Nakuru	3 (3.4%)	26	Unknown	1 (1.1%)

Among patients that were diagnosed with head and neck cancer: 11 (12.4%) came from Nandi and Uasin Gishu Counties respectively, 8 (9.0%) Kericho, 6 (6.7%) Elgeyo Marakwet, 5 (5.6%) Kisii, 4 (4.5%) Baringo, Bomet, Bungoma, Kakamega, Nyamira and Trans Nzoia respectively, 3 (3.4%) Kisumu, Nakuru and Siaya respectively, 2 (2.2%) Homabay, Migori and Vihiga respectively, 1(1.1%) Busia, Laikipia, Lodwar, Machakos, Nairobi, Narok, Nyandarua, West Pokot respectively, one patient failed to indicate their area of residence.

4.2.4 Occupation of patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital

Table 4: Occupation of patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital

	Occupation	Count		Occupation	Count
1	Farmer	22 (24.7%)	10	Clerk	1 (1.1%)
2	Student	10 (11.2%)	11	Fisherman	1 (1.1%)
3	Housewife	6 (6.7%)	12	Hotelier	1 (1.1%)
4	Teacher	4 (4.5%)	13	Librarian	1 (1.1%)
5	Business	2 (2.2%)	14	Mason	1 (1.1%)
6	Casual Laborer	2 (2.2%)	15	Police Officer	1 (1.1%)
7	Driver	2 (2.2%)	16	Security Officer	1 (1.1%)
8	Priest	2 (2.2%)	17	Soldier	1 (1.1%)
9	Civil Servant	1 (1.1%)	18	Unknown	30 (33.7%)

Among patients that were diagnosed with head and neck cancer: 22 (24.7%) reported their occupation as farmer, 10 (11.2%) student, 6 (6.7%) housewife, 4 (4.5%) teacher, 2 (2.2%) business, casual laborer, driver and priest respectively. 1 (1.1%) civil servant, clerk, fisherman, hotelier, librarian, mason, police officer, security officer, soldier respectively. However, 30 (33.7%) did not indicate their occupation.

4.3 Sub-sites of head and neck cancer diagnosed at Alexandria Cancer Centre and Palliative Care Hospital

Table 5: Sub-sites of head and neck cancer diagnosed at Alexandria Cancer Centre and Palliative Care Hospital

	Subsites of HNCs	**Combined**
1	Pharyngeal Cancer	33 (37.1%)
2	Cancer of the Oral Cavity	14 (15.7%)
3	Laryngeal Cancer	12 (13.5%)
4	Esophageal Cancer	7 (7.9%)
5	Maxillary Cancer	6 (6.7%)
6	Salivary Gland Cancer	6 (6.7%)
7	Sinus and Nasal Cavity Cancer	6 (6.7%)
8	Mandibular Cancer	3 (3.4%)
9	Plexiform Ameloblastoma	1 (1.1%)
10	Temporal Zygomatic Cancer	1 (1.1%)
	TOTAL	89

33 (37.1%) were diagnosed with Pharyngeal Cancers comprising of: Nasopharyngeal (25), Hypo-pharyngeal (4), Oropharyngeal (2) and Pharynx unspecified (2); 14 (15.7%) Cancer of the Oral Cavity comprising of: Tongue (10), Oral cavity (3) and Lip (1); 12 (13.5%) Laryngeal Cancers, 7 (7.9%) Esophageal Cancer (upper third), 6 (6.7%) Maxillary, Salivary Gland and Sinus and Nasal Cavity Cancer each respectively, 3 (3.4%) Mandibular Cancer and 1 (1.1%) Plexiform Ameloblastoma and Temporal Zygomatic Cancer each respectively.

4.4 Laboratory Findings of samples obtained from patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital

4.4.1 Introduction

Out of the 89 patients that were diagnosed with head and neck cancer, 29 were excluded because their files did not have pathology reports. Among the 60 that had pathology reports, the following 29, were further excluded from the study: 7 patients that did not have contact details recorded in their files, 9 patients that could not be reached (5 of the telephone contacts in the files were out of service and 4 did not pick their calls even after repeated attempts), 6 patients declined to take part in the study, 7 samples could not be retrieved from the archives (2 could not be located, 5 had been reclaimed by the patients for a second opinion). Therefore, the results presented here after are for 31 patients.

4.4.2 Microscopy of samples obtained from patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital

31 samples underwent repeat microscopy. Of these, 29 (93.5%) were Squamous Cell Carcinomas (SCC). Among the SCC, 15 (48.3%) were well to moderately differentiated, 12 (38.7%) were poorly or undifferentiated and 2 (6.5%) were not otherwise specified. 2 did not meet criteria for further testing.

Table 6: Microscopy of samples obtained from patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital

Microscopy	**Count**
Well to Moderately differentiated Squamous Cell Carcinoma	15 (48.3%)
Poorly or Undifferentiated Squamous Cell Carcinoma	12 (38.7%)
Squamous Cell Carcinoma not otherwise specified (SCC nos)	2 (6.5%)
Others	2 (6.5%)
TOTAL	31

4.4.3 p16 Immunohistochemistry of samples obtained from patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital

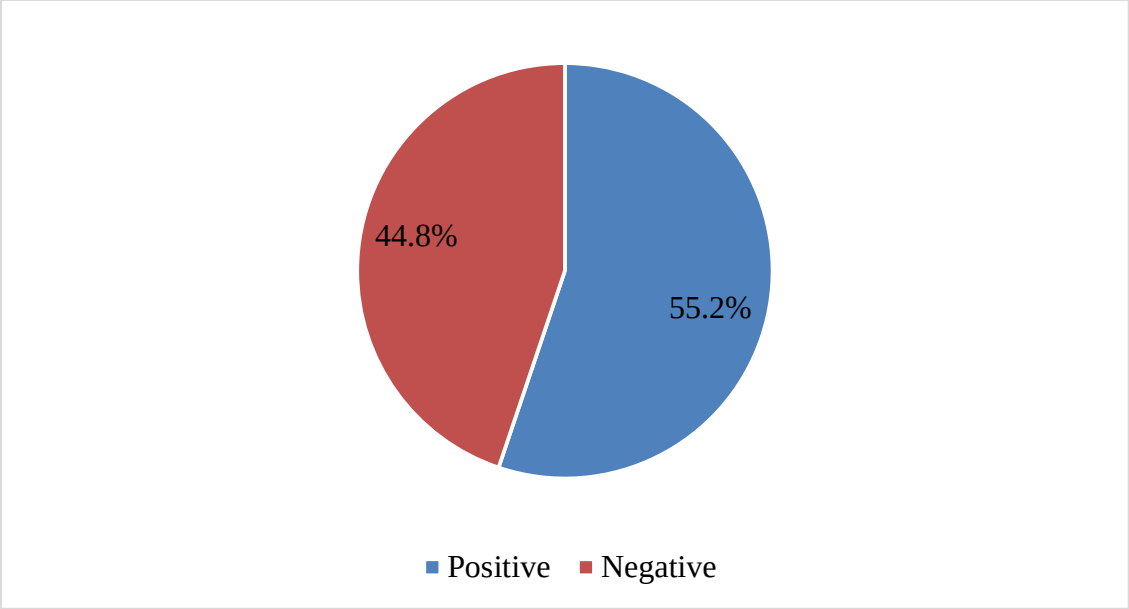

Figure 6: p16 Immunohistochemistry of samples obtained from patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital

Of the 29 samples tested, 16 (55.2%) were p16 Positive while 13 (44.8%) were p16 Negative. Among those that tested positive; 62.5% (10/16) were male and 37.5% (6/16) were female. By sub-site: 3 (18.75%) Esophagus (upper third), 3 (18.75%) Nasopharyx, 3 (18.75%) Tongue, 2 (12.5%) Hypopharyx, 2 (12.5%) Larynx, 1 (6.25%) Maxilla, 1 (6.25%) Pharynx unspecified, 1 (6.25%) Post Nasal Space.

4.4.4 Human papillomavirus Polymerase Chain Reaction (HPV PCR) of samples obtained from patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital

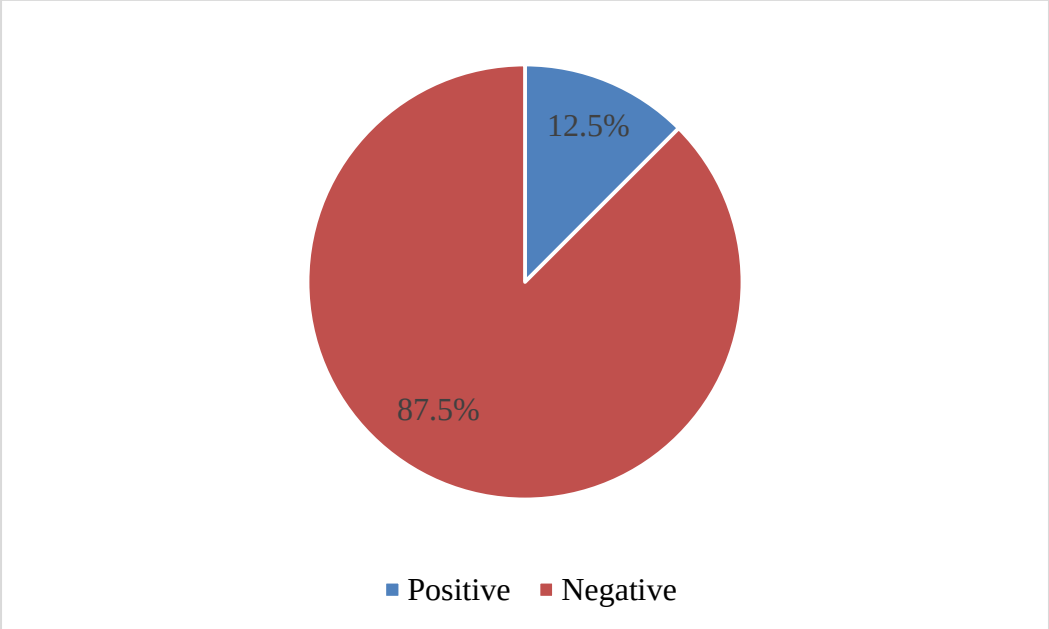

Figure 7: Human papillomavirus Polymerase Chain Reaction (HPV PCR) of samples obtained from patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital

Of the 16 samples that tested positive for p16 (p16 positive), 2 (12.5%) tested positive with Human papillomavirus Polymerase Chain Reaction (HPV PCR). One of the samples was obtained from an 87-year-old female that was diagnosed with Tongue Cancer. After analysis with Human papillomavirus Polymerase Chain Reaction (HPV PCR) revealed the genotype to be HPV 52. The other sample was obtained from a 49-year-old male who was diagnosed with Cancer of the Post Nasal Space. After analysis with Human papillomavirus Polymerase Chain Reaction (HPV PCR) it was determined that this sample had co-infection with HPV: 35, 52 and 59 genotypes.

4.5 Summary of p16 positive samples on sub-site, age, sex, morphology, Human papillomavirus Polymerase Chain Reaction and Occupation

Table 7: Summary of p16 positive samples on sub-site, age, sex, morphology, Human papillomavirus Polymerase Chain Reaction and Occupation

Sub-site	Age	Sex	Morphology	HPV-PCR	Occupation
Nasopharynx	15	M	Poorly differentiated	Negative	Student
Nasopharynx	26	M	Poorly differentiated	Negative	Farmer
Pharynx Unspecified	58	M	Moderately differentiated	Negative	Teacher
Hypopharynx	63	M	Poorly differentiated	Negative	Farmer
Esophagus	48	M	Poorly differentiated	Negative	Security Officer
Tongue	70	M	Well differentiated	Negative	Farmer
Larynx	56	M	Well differentiated	Negative	Librarian
Larynx	69	M	Well differentiated	Negative	Navy Officer
Maxilla	51	M	Moderately differentiated	Negative	Priest
Post Nasal Space	49	M	Poorly differentiated	35, 52, 59	Unknown
Nasopharynx	29	F	Poorly differentiated	Negative	Unknown
Hypopharynx	49	F	Well differentiated	Negative	Police Officer
Esophagus	61	F	Moderately differentiated	Negative	Farmer
Esophagus	65	F	Moderately differentiated	Negative	Unknown
Tongue	59	F	Moderately differentiated	Negative	Housewife
Tongue	87	F	Poorly differentiated	52	Unknown

CHAPTER FIVE: DISCUSSION OF FINDINGS

5.1 Prevalence of head and neck cancer

89 patients were diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital during the period of this study which represented 8.8% (89/1016) of the total malignancies. This figure is comparable to similar studies carried out elsewhere 8.2 % as reported by Korir et al., 2015 in a study on Cancer Incidence in Nairobi using figures from Nairobi Cancer Registry; 8.8% in Gabon Globocan, 2018 and in 9.5% in a study carried out on the clinical and pathological profile and the challenges presented in the handling of patients diagnosed with head and neck cancer in Tanzania by Gilyoma et al., 2015. However, this figure was higher than 3% in the United States as indicated in Cancer Statistics 2019 by Siegel et al., 2019 and the figure of 4% that was reported from a large population based study that was carried out in Europe in early 2000s in a study by Gatta et al., 2015. On the other hand, this figure was lower than 17-20% in Egypt in a study by Attar et al., 2010 on head and neck cancer in a developing country. Bhattacharjee et al., 2006 reported 54.48% in a study titled population-based perspective across 8 years in India on prevalence of head and neck cancers in the North East - An Institutional Study.

The figure of 8.8% obtained in this study was higher than the global prevalence that was 4.9% according to 2018 global cancer statistics (Globocan, 2018). It was also higher than the national figure of 5.7% as stipulated in the Kenya national treatment protocols by Kalebi A et al., 2019. This may point to a higher prevalence of head and neck cancer in the region because preliminary findings from the Eldoret Cancer Registry indicate the prevalence of head and neck cancer in 2017 as being 6.5% that's both higher than the national and global figure. Further investigations need to be carried out before this conclusion is made.

5.2 Demographics of patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital

5.2.1 Gender

During the period of this study, there were 89 patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital, of these, 57 (64%) were males and 32 (36%) were females, with a ratio of 1.8:1. These findings are comparable to other studies. In a study carried on four years trend of head and neck cancer at Nairobi Cancer Registry, Gathere et al., 2011, reported a male to female ratio of 2:1. da Lilly-Tariah et al., 2009 in a meta-analysis review on the burden of head and neck cancer in Nigeria reported a male to female ratio ranging from 1:1 to 2.3:1. Claire E Faggons et al., 2017 in a study carried on human papillomavirus on head and neck squamous cell carcinoma in Malawi reported more males (65%) than females. On the other hand, other studies have shown a higher male to female gender ratio. For instance Li et al., 2018, in a study involving data on a large sample of patients extracted from the National Cancer Database (NCDB) in the United States, reported 74.0% male and 26.0% female (2.8:1). Other studies from Brazil (Ruback et al., 2012), Korea (Kim et al., 2003) and India (Alam et al., 2017) showed a much higher male to female gender ration of 3.9:1, 5:1 and 16:1 respectively, when compared to findings in our study. However, the findings of this study differ from the slight female predominance observed by Ologe et al., 2005 in Nigeria, Tsai et al., 2019 Taiwan and Argirion et al., 2019 in Khon Kaen, Thailand. These differences point out the need to have a closer look at the risk factors that are associated with Head and Neck Cancer in this region.

5.2.2 Age

The frequencies of patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital increased with increasing age (except the 30-39 age group) to a peak at the 50-59 age group and decreased gradually up to the age group 80 and above. The youngest was 15 and the eldest was 87 years old (age range 72). The mean age was 50.9 years ± 17.6 with the most affected age group being 50-59. These findings are comparable to other studies. In a study carried out by Mwansasu et al., 2015 on pattern of head and neck cancers among patients attending Muhimbili National Hospital Tanzania reported the age at the time of diagnosis was 16 years to 88 years (age range 72) with a mean age of 51 years ± 18. Gilyoma et al., 2015 in a study carried out in Tanazania on head and neck cancers: a clinico-pathological profile and management challenges in a resource-limited setting, reported the age of patients at diagnosis ranging from 3 to 82 years (age range 79) with the majority aged between 41 and 60 years. In a study carried on four years trend of head and neck cancer at Nairobi Cancer Registry, Gathere et al., 2011, reported that the most affected age group was 50-54 years' and Claire E Faggons et al., 2017 in a study carried on human papillomavirus on head and neck squamous cell carcinoma in Malawi reported a mean age of 52 years. On the other hand, Li et al., 2018 in a study involving data on a large sample of patients extracted from the National Cancer Database (NCDB) in the United States reported a mean age, 63.1 years [SD ± 11.9]. The reasons for higher percentage of occurrence in younger age group among African are not so clear but they may be connected to issues of race and genetics. Worldwide, there is an increasing incidence of head and neck cancer in younger age groups which is thought to be associated with changing sexual behavior such as increase in oral-genital contact, sexual debut at early age and a high number of lifetime sex partners.

There is also the matter of a shorter life span among Africans as compared to Caucasians.

5.2.3 Area of Residence

25 counties were represented with the top 15 accounting for 85% of patients diagnosed with head and neck cancer during the period of the study. Frequencies are closely related to proximity of residence to Alexandria Cancer Centre and Palliative Care Hospital with most patients coming from the West Kenya Region especially Uasin Gishu and Nandi Counties. Cancer diagnosis and treatment is an expensive and long process. Patients will opt to seek such services in a place that is easily accessible and hence more coming from surrounding counties. There are few hospitals in Kenya and in the Western Kenya region that offer treatment for cancers. Therefore, some of the patients were referrals from other institutions, both within Eldoret and from other counties.

5.2.4 Occupation

Combined the top 3 occupations (farmer, student, housewife) accounted for 42.6% of head and neck cancer diagnosed at Alexandria Cancer Centre and Palliative Care Hospital during the period of the study. Alexandria Cancer Centre and Palliative Care Hospital (ACCPCH) is one of the major cancer facilities in the Western Kenya Region that is National Hospital Insurance Fund (NHIF) accredited which means that people of all social classes can be able to afford treatment here. In a study carried out on the clinical and pathological profile and the challenges presented in the handling of patients diagnosed with head and neck cancer in Tanzania by Gilyoma et al., 2015 they reported that most of the patients were farmers. In a study carried on Human Papillomavirus Attributable Head and Neck Cancer in Sudan by Ahmed et al., 2012 reported that a high number HPV-positive cases were from employees and

housewives each having 10/31 (32%), next were labors 9/31 (29%) and students 2/31 (6%). In this study, information on the occupation of 30 (33.7%) could not be found in the records.

5.3 Sub-sites of head and neck cancer

Nasopharyngeal (25/89) was the leading subsite among the head and neck cancers diagnosed at Alexandria Cancer Centre and Palliative Care Hospital during the period of the study.

According to Muchiri, 2008, Korir, 2015 among others, the occurrence of modestly raised rates of nasopharyngeal cancer in Kenya has been known for many years. Nasopharyngeal cancer commonly starts in the flat cells that make up the lining of upper part of the throat that is behind the nose. It easily and quickly spreads because of the abundant blood supply to this region of the body. Some of the symptoms like mild nose bleeding, nasal blockage and pain free neck swellings are not very specific and end up getting overlooked until it's too late. The short course in 1993 by the American Society for Radiology and Oncology (ASTRO) put the spotlight on Kenya as a region with a higher frequency of occurrence Nasopharyngeal cancer. It carries a much better prognosis than the rest of head and neck cancers despite the fact that it is usually diagnosed late.

Nasopharynx was also the leading subsite according to the figures from Eldoret Cancer Registry in 2017 (46/119). A finding also reflected in global cancer statistics Kenya Globocan, 2018. The nasopharynx has also been reported to be the leading head and neck cancer subsite in other African countries Uganda, Senegal and Sudan Onyango et al., 2006; Tunisia and Algeria Muchiri, 2008 and Nigeria Oga et al., 2016. Nasopharyngeal cancer was once considered endemic in the southern part of China. During the beginning of the 20th century, Nasopharyngeal cancer was so common

parts of Southern China and was referred to as ‘Guangdong cancer’ after Guangdong Province. In the rest of the world nasopharyngeal cancer is common in China, Hong Kong and the Far East Ho, 2017.

The incidence and pattern of occurrence of head and neck cancers vary greatly by race and geographic location. In addition, specific tumor types also show an uneven geographic distribution even within the same country. The reason for this anatomical difference is not clear but may have to do with geographical location and the socio-cultural practice of the people in that region. In this study, following nasopharynx, the other subsites were: Laryngeal (12) and (Tongue 10). For Eldoret Cancer Registry, following nasopharynx, were Tongue (28), Esophagus (upper third) (10), Larynx (10). Onyango et al., 2006 in a study on pattern of occurrence of head and neck cancer presenting at Kenyatta National Hospital reported the subsites larynx (15%), mouth (13.5%) and nasopharynx (12.5%). In a study carried on four years trend of head and neck cancer at Nairobi Cancer Registry Gathere et al., 2011 reported: oral cancers at 40.6% followed by cancers of the nasopharynx at 20.8% and then larynx (14%). Ahmed et al., 2012 in a study carried out on Human papillomavirus attributable head and neck cancer in the Sudan reported: oral, larynx, pharynx, esophagus and others, constituting, 51, 19, 17, 53 and 10 respectively. Kofi et al., 2019 in a study titled detection of human papillomavirus infection in head and neck cancers in the Central African Republic reported that 60.7% of study specimens were from oral cavity or larynx. Akinshipo et al., 2019 in a histopathologic review of head and neck cases in Nigeria reported the commonly identified sub-sites: eyes, oral cavity, nasal and paranasal structures. Li et al., 2018 in a study involving data on a large sample of patients extracted from the National Cancer Database (NCDB) in the United States

reported that 40% was oral cancers, 25% was laryngeal cancer and 20.8% was Nasopharyngeal cancer.

5.4 Microscopy

In this study, of the 31 samples of patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital underwent repeat microscopy, 29 were Squamous Cell Carcinomas accounting for 93.5%. These findings are in concurrence with various studies in Africa and other parts of the world. Galbiatti et al., 2013 in a study carried out on head and neck cancer: causes, prevention and treatment in Brazil reported 90%. Aldalwg & Brestovac, 2017 in a study on Human papillomavirus associated cancers of the head reported 90%. A figure of 91% was reported from a large population based study that was carried out in Europe in early 2000s in a study by Gatta et al., 2015. Aboagye et al., 2019 in a study carried out in Ghana on Human papillomavirus detection in head and neck squamous cell carcinomas at a tertiary hospital in Sub-Saharan Africa reported 95%. However, this figure was higher than that reported from other studies. da Lilly-Tariah et al., 2009 in a meta-analysis review on the burden of head and neck cancer in Nigeria reported 66.7%. Jakobsen et al., 2018 in a nation-wide study on increasing incidence and survival of head and neck cancers reported 69%. In a study carried out by Mwansasu et al., 2015 on pattern of head and neck cancers among patients attending Muhimbili National Hospital Tanzania reported 74% and Gilyoma et al., 2015 in a study carried on in Tanazania on head and neck cancers: a clinico-pathological profile and management challenges in a resource-limited setting, reported 75.7%.

5.5 p16 Immunohistochemistry (IHC) and Human papillomavirus Polymerase Chain Reaction (HPV PCR)

In this study, prevalence of high-risk human papillomavirus (hr-HPV) associated head and neck cancer based on p16 status was 55.2% (16/29). Among these; 10 were male and 6 were female.

This figure was higher than that reported by Claire E Faggons et al., 2017 in a study carried on human papillomavirus on head and neck squamous cell carcinoma in Malawi where 17% (7/42) were p16 positive with 4 male and 3 female. Ahmed et al., 2012 in a study carried out on Human papillomavirus attributable head and neck cancer in the Sudan reported 20.7% (31/150) were p16 positive with 18 male and 13 females. Alsbeih et al., 2019 in a study on prevalence of Human papillomavirus in Saudi patients with head and neck cancer, a subset of 50 representative specimens were processed for p16, comprising 10 Human papillomavirus-positive tumors along with 40 Human papillomavirus-negative cases. Out of these, 21 (42%) were found to be p16 positive. Among these, 10 were those that were initially confirmed to be positive for HPV, thus making them double-positive with overexpression of p16 and Human papillomavirus. The other 11 were those that were initially found to be negative for Human papillomavirus, thus making them to be single positive for overexpression of p16. The remaining 29 were found to be double negative for both Human papillomavirus and p16 overexpression. Among those that tested positive for p16, 12.5% (2/16) tested positive with Human papillomavirus Polymerase Chain Reaction. Molecular analysis revealed that one sample had HPV 52 and the other one had multiple co-infection with HPV: 35, 52 and 59. These findings were similar to those by Aboagye et al., 2019 in a study carried out in Ghana on human papillomavirus detection in head and neck squamous cell carcinomas at a tertiary

hospital in Sub-Saharan Africa which reported that 18 out of the 100 head and neck squamous cell carcinomas samples were positive for Human papillomavirus Deoxyribonucleic acid (HPV-DNA) with 15 (83.3%) males and 3 (16.7%) females.

However, the findings of this study were higher than those reported in other studies. In a study on Human papillomavirus related head and neck cancer in Nigeria, Oga et al., 2016 reported that none of the samples tested were positive for Human papillomavirus Deoxyribonucleic acid (HPV-DNA) (0/149). Kofi et al., 2019 in a study titled detection of human papillomavirus infection in head and neck cancers in the Central African Republic reported 0.74% with only one (1/135) oropharyngeal squamous cell carcinoma biopsy sample testing positive for HPV-16. Alsbeih et al., 2019 in a study on prevalence of Human papillomavirus in Saudi patients with head and neck cancer reported 3.5% (10/285) being Human papillomavirus positive of there, 7 were isolated from males and three from females.

These findings were lower than figures reported in other studies. Shaikh et al., 2017 in a study on Prevalence and types of high-risk human papillomaviruses in head and neck cancers from Bangladesh had 21% (36/174) of blocks being positive for HPV DNA with 30 males and 6 females.

Kumar et al., 2015 reported 31.13% (33/106) with 23 male and in a study on high-risk Human papillomavirus infection in head and neck cancer patients in North-East Region of India. Auguste et al., 2017 reported 36% (36/100) with 32 males and 4 females in a study involving Human papillomavirus infection in people of African descent in the French West Indies. Faust et al., 2016 reported, 65.2% (92/141) in oropharyngeal carcinomas and 11.1% (15/134) in non-oropharyngeal carcinomas. This was from a study on HPV 16 particularly prevalence, physical status and viral

load in head and neck squamous cell carcinoma in the South Health Care Region of Sweden.

This disparity maybe due to race and genetics compared to whites. In meta-analysis study on prevalence of Human papillomavirus infection in racial ethnic subgroups of head and neck cancer, (Ragin et al., 2017) reported that amongst Blacks and Asians, Human papillomavirus negative/p16 positive (HPV−/p16+) disease comprises the majority of disease, in distinction to Whites, where Human papillomavirus positive and p16 positive (HPV+/p16+) disease is the predominant disease. There are several reasons that give rise to such conflicting results for instance, number of cases, difference in patient samples, different geographical areas from which the sample is selected, alcohol and tobacco use of the patients and the criteria used as a measure of $p16^{INK4a}$ as positive. These results that are not in agreement with what is expected in regions known to have high incidence of cervical cancer which has been known to have be highly associated with cervical cancer. These differences may be an indication in the differences of sexual practices among these populations with oral sex being strongly associated with Human papillomavirus associated head and neck squamous cancer.

The Human papillomavirus genotypes identified in this study (HPV 35, 52 and 59) have also been isolated in other studies though rarely with HPV 16 being the most isolated in most studies. HPV 52 was reported in a study by Faust et al in a study on prevalence of human papillomavirus types, viral load and physical status of HPV16 in head and neck squamous cell carcinoma from the South Health Care Region in Sweden. In a study carried out in French West Indies carried out by Auguste et al., 2017 managed to isolated HPV52 and HPV 59 among other genotypes in a study that involved people of African descent.

Both samples that tested positive for Human papillomavirus were poorly differentiated Squamous Cell Carcinomas. These findings agree with those by Boscolo-Rizzo et al., 2013 in a study on New insights into human papillomavirus-associated head and neck squamous cell carcinoma who also found out that poorly differentiated tumors had significant higher incidence of Human papillomavirus-positivity compared to well differentiated tumors. However Aboagye et al., 2019 in a study carried out in Ghana on human papillomavirus detection in head and neck squamous cell carcinomas at a tertiary hospital in Sub-Saharan Africa reported that many, 50% (9/18) of the tumors that tested positive for Human papillomavirus were those with a morphology of moderately differentiated with 27.8% (5/18) being well differentiated. Shaikh et al., 2017 in a study carried out in Bangladesh on prevalence and types of high-risk human papillomaviruses in head and neck cancers reported on their morphology. The highest number (20/36) reported in this study were moderately differentiated, next was (12/36) poorly differentiated and the least was (4/36) well differentiated. The difference in genetic makeup, different environments and the duration of being exposure to carcinogens may be the reason why such results come about. Akinshipo et al., 2019, pointed out that structures in the head and neck are many and they are situated very close to one another. On top of that, these organs are made up of tissue types that are almost alike, this makes things challenging for the pathologist when selecting which area to focus on considering that the final diagnosis is dependent on histology. What makes even more dire is that since these are crucial organs, the tissue samples taken are usually small, often deformed and burdensome to prepare for paraffin embedding. This further makes the subsequent steps of evaluation and diagnosis difficult.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

- The prevalence of head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital between January 2017 and December 2018 was 8.8%.
- The prevalence of high-risk head and neck cancer based on p16 was 55.2% (16/29) at Alexandria Cancer Centre and Palliative Care Hospital during the period of the study.
- There were three Human papillomavirus genotypes that were isolated in this study: HPV 35, 52 and 59. HPV DNA was isolated from two samples. One of the samples was obtained from an 87-year-old female that was diagnosed with Tongue Cancer. After analysis with Human papillomavirus Polymerase Chain Reaction (HPV PCR) revealed the genotype to be HPV 52. The other sample was obtained from a 49-year-old male who was diagnosed with Cancer of the Post Nasal Space. After analysis with Human papillomavirus Polymerase Chain Reaction (HPV PCR) it was determined that there was co-infection with HPV: 35, 52 and 59 genotypes.
- In this study there were 4 parameters that were looked at in demographics:
  - There more males (64%) than females (36%) diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital.
  - The frequencies of patients diagnosed with head and neck cancer at Alexandria Cancer Centre and Palliative Care Hospital increased with increasing age (except the 30-39 age group) to a peak at the 50-59 age group and decreased gradually up to the age group 80 and above.
  - Looking at the area of residence, 25 counties were represented with the top 15 accounting for 85% of patients diagnosed with head and neck cancer.

Frequencies were closely related to proximity to at Alexandria Cancer Centre and Palliative Care Hospital with most patients coming from the West Kenya Region especially Uasin Gishu and Nandi Counties.

- Combined the top three occupations (farmer, student, housewife) accounted for 42.6% of head and neck cancer diagnosed at Alexandria Cancer Centre and Palliative Care Hospital, however, information on the occupation of 30 (33.7%) could not be found in the records.

6.2 Recommendations

- Further prospective studies with a larger sample size in order to try and gain a better understanding of the prevalence of Human papillomavirus and high-risk genotypes associated with head and neck cancer in Kenya. Additional data will allow to close the gap of knowledge between Western countries and the sub-Saharan African region, especially in Kenya which has a high burden for head and neck cancer. This could play a big role in assisting health authorities in implementing public health strategies like vaccinations and will aid in allocating necessary resources.
- p16 immunohistochemistry is advocated as a surrogate marker of Human papillomavirus.

  This technique can be used in most laboratories since the same laboratory technician can section and stain the slides and the same pathologist can read the slides as well. This means that this test can be easily integrated into routine testing with no additional manpower. This test can be used to separate Human papillomavirus associated and non-Human papillomavirus associated Head and Neck Cancer. In some countries it is compulsory for the tumor boards to know the Human papillomavirus before planning and proceeding with treatment of Head and Neck Cancer.

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APPENDICES

APPENDIX I: ETHICAL APPROVAL FROM INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

MU/MTRH-INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
COLLEGE OF HEALTH SCIENCES
P.O. BOX 4606
ELDORET

Reference: IREC/2018/214
Approval Number: 0003176

6th December, 2018

Eva Mokeira Ombiro,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

06 DEC 2018
APPROVED

Dear Ms. Ombiro,

RE: FORMAL APPROVAL

The MU/MTRH- Institutional Research and Ethics Committee has reviewed your research proposal titled: -

"Genotypes and Prevalence of High –Risk Human Papilloma Virus among Patients Diagnosed with Head and Neck Cancers at Alexandria Cancer Centre".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 3176*** on 6th December, 2018. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; hence will expire on 5th December, 2019. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date. You will be required to submit progress report(s) on application for continuation, at the end of the study and any other times as may be recommended by the Committee.

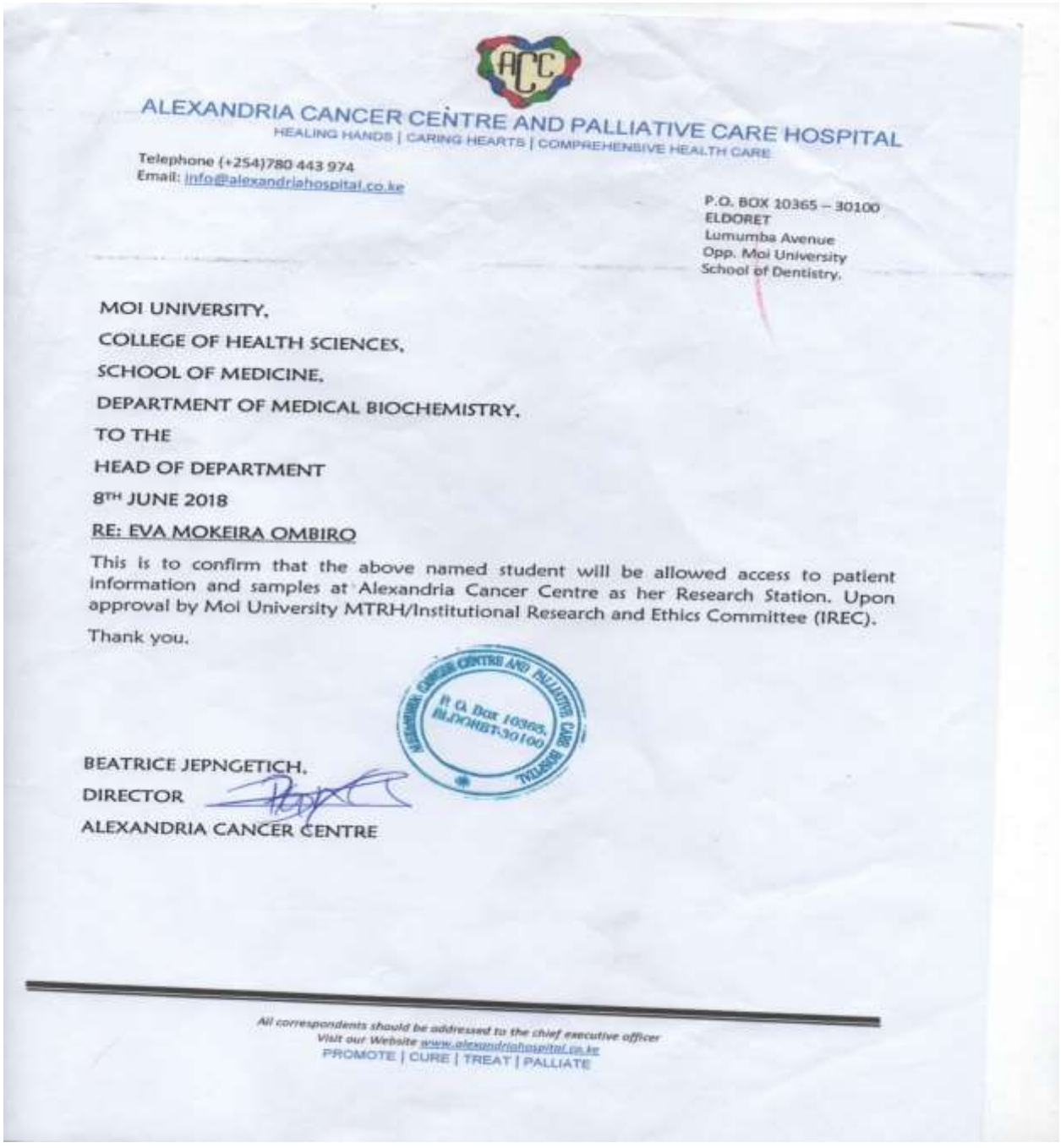
Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. You will also be required to seek further clearance from any other regulatory body/authority that may be appropriate and applicable to the conduct of this study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc CEO - MTRH Dean - SOP Dean - SOM
Principal - CHS Dean - SON Dean - SOD

APPENDIX II: APPROVAL TO USE ALEXANDRIA CANCER CENTRE AND PALLIATIVE CARE HOSPITAL AND RESEARCH STATION



ALEXANDRIA CANCER CENTRE AND PALLIATIVE CARE HOSPITAL
HEALING HANDS | CARING HEARTS | COMPREHENSIVE HEALTH CARE

Telephone (+254)780 443 974
Email: info@alexandriahospital.co.ke

P.O. BOX 10365 – 30100
ELDORET
Lumumba Avenue
Opp. Moi University
School of Dentistry.

MOI UNIVERSITY,
COLLEGE OF HEALTH SCIENCES,
SCHOOL OF MEDICINE,
DEPARTMENT OF MEDICAL BIOCHEMISTRY,
TO THE
HEAD OF DEPARTMENT
8TH JUNE 2018

RE: EVA MOKEIRA OMBIRO

This is to confirm that the above named student will be allowed access to patient information and samples at Alexandria Cancer Centre as her Research Station. Upon approval by Moi University MTRH/Institutional Research and Ethics Committee (IREC).

Thank you.

BEATRICE JEPNGETICH,
DIRECTOR
ALEXANDRIA CANCER CENTRE

All correspondents should be addressed to the chief executive officer
Visit our Website www.alexandriahospital.co.ke
PROMOTE | CURE | TREAT | PALLIATE

APPENDIX III: CANCER ABSTRACT FORM

A. PATIENT INFORMATION

1. Unique Identifier

   ……...……

2. Age

   ……

   ……

3. Gender

   ……

   …...

4. Place of Residence

   ……

5. Occupation

   ……

B. TUMOR INFORMATION

6. Primary site

   ……..**.....**

7. Morphology

   a) Well and Moderately differentiated Squamous Cell Carcinoma

   b) Poorly or Undifferentiated Squamous Cell Carcinoma

   c) Squamous Cell Carcinoma not otherwise specified (SCC nos)

   d) Others

8. P16 Immunohistochemistry

   a) Positive

   b) Negative

Note:

A sample is considered positive with continuous, diffuse p16 staining pattern. A sample is considered negative if there is focal p16 staining pattern that is represented by non-continuous staining of isolated cells or small cell clusters or no p16 staining

C. Human Papillomavirus Polymerase Chain Reaction (HPV PCR) (Tick as Appropriate)

Human Papillomavirus Polymerase Chain Reaction (HPV PCR)			
HPV Genotypes	Positive	HPV Genotypes	Positive
HPV 6		HPV 59	
HPV 11		HPV 61	
HPV 16		HPV 62	
HPV 18		HPV 64	
HPV 26		HPV 66	
HPV 31		HPV 67	
HPV 33		HPV 68	
HPV 35		HPV 69	
HPV 39		HPV 70	
HPV 40		HPV 71	
HPV 42		HPV 72	
HPV 45		HPV 73	
HPV 51		HPV 81	
HPV 52		HPV 82	
HPV 53		HPV 83	
HPV 54		HPV 84	
HPV 55		IS39	
HPV 56		CP6108	
HPV 58			

APPENDIX IV: PARTICIPANT CONSENT FORM

Research Topic: Genotypes and prevalence of High-Risk Human Papillomavirus among patients diagnosed with Head and Neck Cancer cases at Alexandria Cancer Centre and Palliative Care Hospital (ACCPCH).

Investigator: Eva Mokeira Ombiro

Designation: Masters Student in the Department of Biochemistry and Clinical Chemistry, Moi University

Mobile: 0726-517384

This study is seeking to determine Human papillomavirus (HPV) genotypes and their prevalence rates in histologically confirmed Head and Neck Cancer cases at Alexandria Cancer Centre and Palliative Care Hospital (ACCPCH). This study exposes you to minimal risks as it will utilize tissue samples initially collected for purposes of diagnosis and currently archived. Apart from giving this verbal consent, no additional samples or procedures whatsoever will be required of you. There is no monetary inducement that will be provided. Your participation is completely voluntary. In case you wish to withdraw at any point in time, there will be no intimidation for you to remain in the study. The information collected during this study will be strictly confidential.

Investigator	Health Records Officer
Name: …….	Name:
……...	
Date: ……	Date:
…….	

APPENDIX V: LABORATORY SERVICE AGREEMENT WITH LANCET LABORATORIES

LABORATORY SERVICE LEVEL AGREEMENT FOR RESEARCH STUDY

BETWEEN, on the one hand Dr.Eva Ombiro having a principal place of business in Eldoret, hereinafter referred as to (CLIENT)

AND, on the other hand,

Pathologists Lancet Kenya Limited, a company duly incorporated under the Laws ofKenya having a principal place of business at Fifth Avenue Building, 5th Ngong Avenue, Upper Hill, Nairobi, Kenya, hereinafter referred to as "LANCET"

WITNESSETH:

Whereas, the Clientis planning to conduct a Project and has requested LANCET to perform central laboratory services in this research study or project program on **Genotypes and Prevalence of High-Risk HPV among patients diagnosed with head and neck Cancers at Alexandria Cancer Centre, Eldoret** and LANCET desires to perform such activities, all in accordance with the terms and conditions of this Agreement;

NOW, THEREFORE, the parties agree as follows:

1. Purpose of the Agreement

The CLIENT selected LANCET as the central laboratory to perform different taskssuch as,analysis, result reporting, and facilitation of transportation of samples related to evaluatingthe said samples in a cohort.

LANCET will perform the analysis of the biological specimens from The Project as requested from time to time by the CLIENTat its respective investigator sites. The specific details of the analysis and the services requested from LANCET as accepted between parties, are defined in the Substantive Provision on Recipricol Obligations by the parties, attached and incorporated herein as Appendix II