PATTERN OF PRESENTATION AND SOME RISK FACTORS OF ORAL SQUAMOUS CELL CARCINOMA AT KENYATTA NATIONAL HOSPITAL

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Pattern of presentation and some Risk factors of Oral Squamous Cell Carcinoma at Kenyatta National Hospital

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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DEDICATION

This thesis is dedicated to all the persons affected by oral cancer across the world.

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ABBREVIATIONS AND ACRONYMS

AJCC	American Joint Commission on Cancer
CI	Confidence interval
Cm	Centimetre
CRP	C-reactive protein
CDKs	Cyclin dependent kinases
DNA	Deoxy-rinoneucleic acid
EGFR	Endothelial growth factor receptor
ELISA	Enzyme linked immune-sorbent assay
HNSCC	Head and neck squamous cell carcinoma
GSI	Gamma secretase inhibitors
HPV	Human Papilloma Virus
IL6	Interleukin 6
KNH	Kenyatta National Hospital
MAML	Mastermind like
MDSCC	Moderately Differentiated Squamous Cell Carcinoma
Ml	Millilitre
NGS	Next Generation Sequencing
NICD	Notch1 intracellular domain

OSCC	Oral Squamous Cell Carcinoma
PDSCC	Poorly Differentiated Squamous Cell Carcinoma
OR	Odds ratio
TNM	Tumour Nodes Metastasis
WDSCC	Well Differentiated Squamous Cell Carcinoma
WHO	World Health Organization

ABSTRACT

It is estimated that the annual incidence and mortality of OSCC are 450,000 and 194,000 respectively with a 5 year survival of about 51%. The main objective of the study was to describe the pattern of presentation of OSCC and to determine the association between some correlates and the disease at the Kenyatta National Hospital. The cases were persons who presented with OSCC while controls were age and gender matched persons from the same hospital. Data including sociodemographic characteristics, body weights and behavioral factors were obtained from cases and controls. In addition, clinical features of the disease were recorded from the cases. Swabs were obtained from the lesions on cases and equivalent mucosal sites on controls and analysed for the 28 HPV viral subtypes. Biopsy specimen from lesions of cases and swabs from equivalent mucosal sites of controls were obtained and analysed for P53 and Notch1 gene mutations. Blood was obtained from cases and controls and analysed for CRP levels by using a biochemistry machine. The data was analyzed using descriptive statistics, Mantel Haenszel and unconditional logistic regressions and presented using narratives, tables and figures. The mean age of the study subjects was 58 years (SD 13.2) with more males (61.8%) presenting at the hospital with the disease. The tongue was the most affected site (38%) and majority of the cases (93.4%) presented with pain and stage 4 disease. There were Significant associations between farming (OR=2.16), weight loss (OR=2.6), tobacco (OR=16.96), inflammation (OR=2.66), P53 (OR=75), Notch1 (OR=140) and OSCC. From this study, it is recommended that health care providers should be sensitized about the signs and symptoms of OSCC and the need for early referral to a tertiary facility. Furthermore, nutritional support and pain control should be instituted at an early stage to reduce the morbidity of the disease. Regarding clinical care, CRP assays should be done for all cases of OSCC in order to control inflammation in the course of the disease. Finally, more studies should be done on the gene mutations and their role in choice of treatment and prognosis of OSCC.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Cancer is one of the leading causes of morbidity and mortality today, with reports indicating that there are more than 10 million new cases and more than 9.6 million deaths each year worldwide. Of great concern is that approximately 70% of deaths from cancer occur in low- and middle-income countries. It estimated that more than 20 million persons around the world live with cancer with more than half of these cases living in developing countries where late-stage presentation and inaccessible diagnosis and treatment are common. In more than 90% of high-income countries treatment services are available compared to less than 30% of low-income countries(WHO, 2018). According to a Kenyan study by the World Bank the high cost of treatment of cancer and the loss of income can impoverish families, considering that the majority of the population are not under medical insurance covers and the few that have the benefit ultimately exhaust the prescribed limits(Lehmann J, 2020).

Up to 30% of cancer deaths are linked to risk factors which include tobacco use, alcohol consumption, unhealthy diets, inactive lifestyles and some infections including the high risk human papilloma virus [HPV](Collaborators, 2016). The incidence of OSCC, which is the 8th most common cancer worldwide, shows regional variations with reports indicating higher incidences in developing than in developed countries(Bray et al., 2018). Not surprisingly, as with other cancers, it has been observed that there is a sharp increase in incidence rates of oral/pharyngeal cancer in several countries and regions. Sadly, these increases are consistently shown to be prominent among younger individuals aged 30-50 years(Davis & Severson, 1987; Macfarlane, Boyle, & Scully, 1992). It is estimated the current and future estimated burden of OSCC is shifting to the less developed countries which are poorly equipped to handle the increasing burden (Gupta, Johnson, & Kumar, 2016). The survival trends for OSCC has not significantly improved over the years and according to a study by Chen(2018), the average 5 year survival for all oral sites was

51.3% with females having a slightly better outlook than males(S. W. Chen et al., 2018).

There is a wide geographical variation in the incidence OSCC with regions having higher tobacco consumption recording higher rates(Warnakulasuriya, 2009). Data from Africa are scanty and restricted to hospital registries. A recent study from South Africa, a multi- racial/multi-cultural society, based on pathology records reported ethnic and gender differences in proportions of oral and oropharyngeal squamous cell carcinoma(Khammissa, Meer, Lemmer, & Feller, 2014). The data is, however, inadequate in planning control strategies. Regarding the aetiology and risk factors for OSCC, tobacco use, alcohol consumption, local and systemic inflammation; and the oncogenic HPV have been associated. In Kenya tobacco consumption has not been high and might actually be on the decline as a result of the new anti-tobacco legislation. In 2019 a report from the WHO estimated that tobacco was consumed by 12.1% of the Kenyan population with 21.4% males and 2.9% of females engaged in the habit(WHO, 2019). Sadly, this has not translated into a reduction in the anecdotally observed incidence of OSCC raising the possibility that other risk factors are responsible for the increasing rates.

Mutations involving the P53 tumour suppressor gene have been noted in up to half of OSCC and are thought to worsen the prognosis of the tumours (Agrawal et al., 2011; Stransky et al., 2011). More recently Notch1 gene mutations have been observed in 14-15% of cases and, therefore, becomes the second most commonly mutated gene(Agrawal et al., 2011; Stransky et al., 2011). Interventional strategies targeting inflammation and gene mutations are in various stages of development. With the paucity of reports from Kenya this study, therefore, provides baseline data for future investigations.

1.2 Statement of the Research Problem

Anecdotal evidence shows an increase of OSCC involving non-tobacco users, particularly in countries where tobacco consumption is on the decline including Kenya. These cases have been noted to be more aggressive and demonstrate higher recurrence rates. Evidence shows that previously, these carcinomas occurred among

an older age group, a scenario that is hardly true at present. Despite these findings, there is the paucity of data on OSCC in general and in particular those involving non-tobacco users, especially in Kenya. In addition, there are hardly any studies on the association between tobacco use and OSCC in Kenya, making it difficult to plan any control strategies. Such information would go a long way in guiding policy on tobacco control.

Studies that have been done across the world have given varying data regarding the presentation of OSCC in general. These variations have been attributed to racial, as well as geographical difference among populations. Although tobacco use, betel quid and areca nut chewing have been strongly associated with the etiology of OSCC In studies from other countries, other possible etiological risk factors including inflammation, dietary habits and HPV infection could play a role. Several studies that have focused mainly on tobacco users have reported a direct link between inflammation and cancer but with no consensus on its role in carcinogenesis. These studies have involved, to a large extent IL-6, a pro-inflammatory cytokine that is produced at a site of disease; and acute phase proteins including C-reactive protein (CRP) both of which have shown increased levels in OSCC. However, these biomarkers have been noted to show wide geographical and racial variations, possibly due to genetic and environmental factors. There are hardly any studies linking inflammation and non-tobacco cases of OSCC and in this study an attempt will be made to compare cases that used tobacco and those who did not. In addition, some studies have suggested that increased levels of CRP in apparently healthy individuals might predict the development of cancer in future. This needs to be investigated further due to its far-reaching implications with the possibility of including the biomarker as a screening tool. Regarding the role of high risk HPV in OSCC studies give very wide prevalence figures, attributed mainly to the detection technique and the site and type of sampling used. With newer highly sensitive molecular methods it will be interesting to know the types of HPV and the proportion of OSCC associated with the virus in Kenya where such a study has not been carried out before.

Turning to the molecular biology of OSCC, common mutations involving the TP53 gene and more recently the NOTCH1 Gene have been associated with OSCC. They also show a wide geographical variation due to genetic and environmental factors. It has been demonstrated that cases with TP53 mutations are resistant to radiation and chemotherapy. This is attributed to cellular resistance to undergo apoptosis, a process that is driven by normal or wild type TP53. Interventional strategies including the introduction of wild type TP53 using adenoviral vectors have shown promising results. In addition, identification of the mutated gene protein has led to development of monoclonal antibodies which can target cells carrying the mutated gene. The Notch1 mutation has recently been described, and is reportedly involved in up to 15% of head and neck squamous cell carcinoma (HNSCC) cases in some studies. Interventional studies are currently on-going, and with time it may be possible to incorporate the findings in the treatment of OSCC.

1.3 Justification

Part of this study set out to describe the clinical presentation of OSCC at kenyatta National Hospital. By improving the understanding of the presentation of the disease we might be able to anticipate the challenges which patients face and offer solutions including early interventions at the primary point of care and early referral to a tertiary facility .The other part of the study would determine modifiable aetiological correlates and therefore recommend control measures at the population level. The interventions would go a long way in reducing the incidences of OSCC that are attributable to the risk factors. The laboratory aspects of the study will set a foundation for diagnosing HPV infections in the oral mucosa and to understand its role in oral carcinogenesis. Additionally, the molecular component will lay a framework for conducting mutation analysis in oral cancer and also for participating in the developments of targeted therapies of the disease. Finally, this study at will provide baseline data to enrich current situational knowledge for future research, policy strengthening and development in Kenya and other developing countries.

1.4 Objectives

1.4.1 Broad Objective

To describe the pattern of presentation and determine the risk factors of Oral squamous cell Carcinoma at Kenyatta National Hospital .

1.4.2 Specific Objectives

1. To describe clinical and pathological characteristics of Oral squamous cell Carcinoma at Kenyatta National Hospital.
2. To determine the association between some behavioural factors and Oral squamous cell Carcinoma at Kenyatta National Hospital.
3. To determine the association between inflammation and Oral squamous cell Carcinoma at Kenyatta National Hospital.
4. To determine the association between HPV and Oral squamous cell Carcinoma at Kenyatta National Hospital
5. To determine the association between some gene mutations and Oral squamous cell Carcinoma at Kenyatta National Hospital.

1.5 Study Hypotheses

1.5.1 Null Hypothesis.

The risk factors of Oral Squamous Cell Carcinoma are not significantly associated with the disease at the Kenyatta National Hospital.

1.5.2 Alternative Hypothesis

The risk factors of Oral Squamous Cell Carcinoma are significantly associated with the disease at the Kenyatta National Hospital.

1.6 Research Questions

1. What are the clinical and pathological features of Oral Squamous Cell Carcinoma at the Kenyatta National Hospital?

2. Which behavioural factors are significantly associated with Oral Squamous Cell Carcinoma at the Kenyatta National Hospital?
3. Is inflammation significantly associated with Oral Squamous Cell Carcinoma at the Kenyatta National Hospital?
4. Is HPV significantly associated with Oral Squamous Cell Carcinoma at the Kenyatta National Hospital?
5. Are gene mutations significantly associated with Oral Squamous Cell Carcinoma at the Kenyatta National Hospital?

1.7 Variables

Variable	Measurement
Sociodemographic Variables	
• Age	• Number of years since birth
• Gender	• Whether male of female
• Residence	• Where the person lives
• Occupation	• Type of work done
Anthropometric variables	
• Height	• In meters while standing
• Weight	• In Kg
• BMI	• In kg per Meter squared (kg/M^2)
Independent variables	
	• Type (whether chewed or smoked)
• Tobacco consumption	• Used or Not
• Alcohol consumption	• Used or not
• Khat consumption	• In Mg/l of blood
• CRP	• Type
• HPV	• Pathogenic Type
• P53	• Pathogenic Type
• Notch1	
Dependent variables	
• Pathologic grade	• Well, moderately or poorly differentiated
• Site	• Anatomical location
• Size	• Widest dimension in cm
Cervical lymph nodes;	
• Level	
• Size	• 1,2,3,4,5 or 6
• Number of nodes	• Widest dimension in cm
• Chest metastasis	• Single or multiple
	• Present or absent Metastasis.

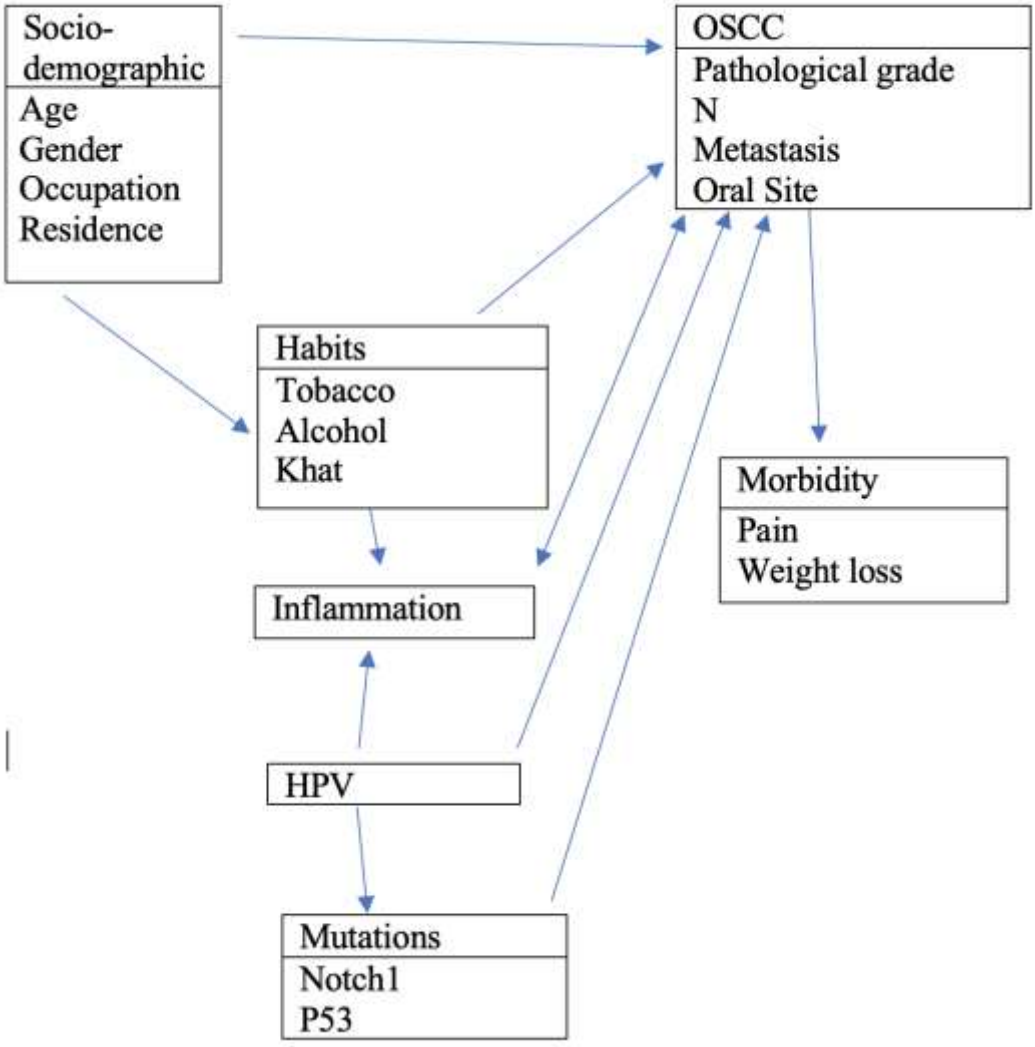

Figure 1.1: Conceptual framework of the study

CHAPTER TWO

LITERATURE REVIEW

2.1 Distribution and Clinical Features of OSCC

The worldwide incidence rates of OSCC are inaccurate largely because the databases from which the estimates are based are far from ideal. Many parts of the world produce no data at all while in others the data may be obtained from biased sources including hospital cancer registries. As can be expected in many developing countries cases may not come to attention at all either because of fear or inability of the poor to access the often scarce hospital facilities. In these areas death rates attributed to cancer may be even more unreliable because follow-up is often impossible. In addition the causes of death may be inaccurate because of lack of standardization in the categories of causes of death (Shah JP, 2003). An earlier review by Johnson [2003](Shah JP, 2003) showed that there was a wide geographical variation in the incidence of OSCC with Melanasia showing the highest incidence despite its small population and not surprisingly, followed by countries in southern Asia where tobacco consumption is high. The same comprehensive review found that females were less likely to have OSCC owing to their lower exposure to traditional risk factor of alcohol and tobacco use. Statistics from western countries indicate that men are affected two to three times more than women. Regarding age distribution, the incidence of OSCC is significantly higher among those above the age of 45 years and has continued to increase in developed countries where the life expectancy has been rising(Shah JP, 2003).

A review of global trends by Warnakulasuriya[2009] (Warnakulasuriya, 2009) shows an unchanged picture in terms of incidences of oral and oropharyngeal cancer. As a group , Oral and pharyngeal cancer is the sixth most common cancer in the world(Warnakulasuriya, 2009).With two-thirds of these cases occurring in developing countries ,the annual estimated incidence is around 275,000 for oral and 130,300 for pharyngeal cancers excluding the nasopharynx. There is a wide geographical variation of up to twenty times in the incidence of this cancer. The areas characterized by high incidence rates for oral cancer are found in the South and

Southeast Asia including Sri Lanka, India, Pakistan and Taiwan), parts of Western Europe such as France and Eastern Europe including Hungary, Slovakia and Slovenia, parts of Latin America and the Caribbean including Brazil, Uruguay and Puerto Rico and in Pacific regions such as Papua New Guinea and Melanesia (Fig. 2.1). In high-risk countries including Sri Lanka, India, Pakistan and Bangladesh, OSCC is the most common cancer in men, and could contribute up to 25% of all new cases. Considering that data from Africa are limited to few hospital cancer registries, it is difficult extrapolate the true incidence in these countries. However, the reported rates do not show evidence that oral cancer is a serious problem in the African continent, a situation that might be misleading. Studies from the Sudan suggest that the use of "toombak" , a traditional tobacco product containing sodium bicarbonate is linked to high rates of OSCC in males (Warnakulasuriya, 2009). While OSCC of the lip has been associated with occupations including agricultural work probably due to prolonged exposure to the sun the evidence has not been strong for the mouth (Kachuri et al., 2017). However, a study among farmers in Iran reported increased risk of HNSCC (OR=3.26, 95%CI 1.13-9.43) and noted that pesticide use was independently associated with an increase in the total HNSCC cases (OR=7.45)(Amizadeh, Safari-Kamalabadi, Askari-Saryazdi, Amizadeh, & Reihani-Kermani, 2017). Furthermore, It has been reported that agricultural pesticides including the organophosphate and thiocarbamate groups increase the risk of cancer (OR=1.58, 95% CI 1.1-2.28)(Weichenthal, Moase, & Chan, 2010).

Regarding site involvement, the tongue remains the most common area for intraoral cancer among European and the US populations, accounting for 40–50% of the oral cancers. On the hand, buccal cancer is more common among Asian populations due to betel quid/tobacco chewing habits. This has been noted In Sri Lanka where 40% of oral cavity cancers are found on the buccal mucosa(Warnakulasuriya, 2009). Other sites of OSCC include the floor of the mouth, lip, palate, retromolar trigone and gingiva of the upper and lower alveolar ridges(JG, 2003). Turning to the symptoms of OSCC, they are variable and depend on the location and extent of the primary tumour, ranging from non-healing ulcers with varying degrees of pain, an exophytic lesion with several contiguous loose teeth, a non-healing extraction socket to excessive salivation with reduced mobility of the tongue and severe pain. With

further progression and delay of treatment, tumour growth can result in impairment of chewing and swallowing leading to significant loss of weight. In addition, halitosis secondary to a fungating necrotic tumour is present in some patients with massive lesions. Progressive trismus is a manifestation of local progression of tumour in the masticator space and medial pterygoid muscles (Carew JF, 2003). Among all human cancers, oral cancer has the highest prevalence of pain, ahead of gastrointestinal and gynaecolgical malignancies(van den Beuken-van Everdingen et al., 2007). Pain in OSCC is caused by the secretion of mediators into the cancer microenvironment including Endothelin-1(ET-1), proteases and nerve growth factor (NGF). In addition, there are neurological pathways which are particularly prominent in head and neck, that produce and maintain opioid tolerance thus presenting challenges in the clinical management of oral cancer pain(Viet & Schmidt, 2012). Significantly, Patients with OSCC have high levels of ET-1 in the cancer microenvironment. Proteases activate cell surface receptors on afferent nerves while NGF is secreted to promote local growth and survival of norciceptive nerves(Viet & Schmidt, 2012). Furthermore, NGF has also been shown to regulate body weight through lipid and glucose metabolism as well as feeding behaviour and may induce cachexia(Ye et al., 2011). In order to further the understanding of cancer related weight loss a panel of experts developed an international consensus that defined Cancer cachexia as a multifactorial syndrome defined by an on-going loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment. Its pathophysiology is characterised by a negative protein and energy balance driven by a variable combination of reduced food intake and abnormal metabolism(Fearon et al., 2011). It has been observed that up to 60% of patients having HNSCC present with weight loss at the time of diagnosis as a result of the tumour burden, obstruction of food intake or cachexia and anorexia from cancer and recommendations have been made for early nutritional interventions(Alshadwi et al., 2013). In addition, the risk of developing critical weight loss, defined as unintentional weight loss of $\geq$ 5% at one month or $\geq$10% at six months from the start of treatment has been reported to increase with advancing stage of the disease(Iftikhar et al., 2018).

With regard to prognosis, the single most important factor impacting on the outcome of patients with squamous cell carcinoma of the upper aerodigestive tract is the stage of the disease at the time of initial diagnosis and treatment. Patients who present with tumours localized at the primary site without dissemination to regional lymph nodes enjoy an excellent prognosis. On the other hand, once dissemination to regional lymph nodes takes place, the probability of a 5- year survivor-ship, regardless of the treatment, reduced to nearly one half of that seen in early staged patients. Several factors pertaining to the characteristics of the regional lymph node metastasis directly influence prognosis. These include the presence or absence of clinically palpable cervical lymph node metastasis, the size of the metastatic lymph node, the number of lymph nodes involved, and the location of lymph nodes involved by metastatic cancer. In addition to this, the presence of extranodal spread of metastatic disease by capsular rupture of the lymph node with invasion of the soft tissues clearly impacts on prognosis. Perivascular and perineural infiltration by tumour as well as the presence of tumour emboli in regional lymphatics also have an adverse impact on prognosis. Involvement of regional lymphatic structures by primary squamous cell carcinomas of the upper aerodigestive tract is dependent on various factors related to the primary tumour, including the site, size, T stage and location of the primary tumour. In addition, histo-morphologic features of the primary tumour also influence the risk of nodal metastasis. The risk of nodal metastasis increases in relation to the location of the primary tumour as one progresses from the anterior to the posterior aspect of the upper aero-digestive tract. In general, the T (tumour) stage usually reflects tumour burden and, therefore, the risk of nodal metastasis increases with increasing T stage of the primary tumour at any site.

Certain histo-morphological features of the primary tumour also increase the risk of nodal metastasis. Thus, endophytic tumours are more inclined to metastasize than exophytic tumours. It has been well documented that for tongue and floor of mouth cancers, tumour thickness is related to the risk of nodal metastases. Poorly differentiated carcinomas have a higher risk of nodal metastases compared to well-differentiated lesions. Within the oral cavity certain primary sites have significantly increased risk of nodal metastasis compared to the other sites, for example, the floor

of mouth versus hard palate(Carew J. F, 2003). The anatomical arrangement of the cervical lymphatics is illustrated in Fig. 2.2.

Figure 2.1: Countries with high incidence and mortality from oral cancer

Adopted from Warnakulasuriya, S (2009).

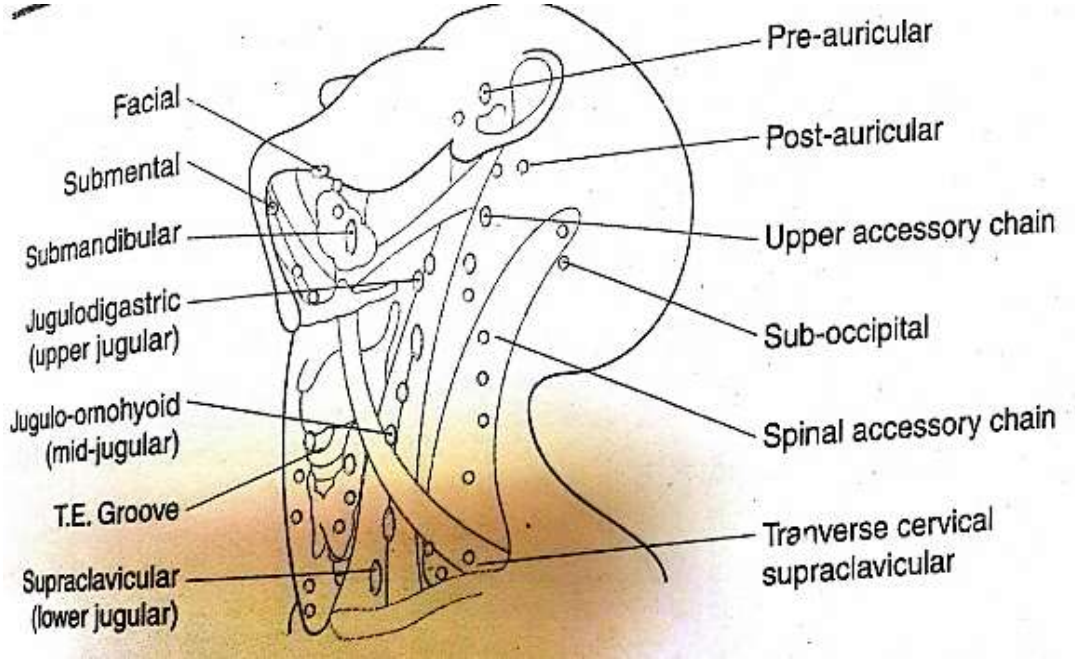

Figure 2.2: Anatomical arrangement of regional cervical lymph nodes.

2.2 Habits and OSCC

Tobacco in all its forms is by far the main risk factor which when combined with poor oral hygiene and alcohol intake is responsible for more than 90% of OSCC(Johnson, 2001). Among adults in Kenya the WHO estimated that there were up to 12.1% tobacco consumers with 21.4% males and 2.9% of females (WHO, 2019). Whether smoked, chewed or sniffed tobacco use produces carcinogenic chemicals such as tobacco specific nitrosamines(TSNA), N'nitrosonornicotine, 4-(methylnitrosoamino-)1-(3-pyridil)-1butanone) and also free radicals which result in alterations in the antioxidant enzymes glutathione –s-transferase(GST), glutathione reductase, superaoxide dismutase,catalase and glutathione peroxidase, as well as lipid peroxidation and total thiols (Brunnemann, Prokopczyk, Djordjevic, & Hoffmann, 1996; Hoffmann & Hoffmann, 1997).Tobacco is either smoked or used in smokeless forms including chewing, snuff and as an ingredient in Pan (betel) and although all forms carry a risk of oral cancer some authors have suggested a lesser danger from the chewed variety(Johnson, 2001; Warnakulasuriya, 2009).

A study from India reported that there is about a twenty times risk of OSCC with heavy tobacco smokers and a strong dose dependent relationship has been observed.

The risk of disease appears to increase with the duration of smoking and number of cigarettes smoked per day. In the same study it was observed that smokeless tobacco is also a risk factor and a dose-response relationship for the frequency and duration of tobacco chewing (including pan) and bidi smoking was noted (Muwonge et al., 2008). Tobacco use is widespread all over the world but changing lifestyles and anti-tobacco legislations have brought about a decline in many countries. There is a wide geographical variation in the use of tobacco and tobacco related products with the countries in the south east Asia such as Sri Lanka having relatively high occurrence of smoking with reports indicating that up to 54.8% and 0.8% of men and adult women, respectively, are engaged in the habit. This high prevalence of tobacco consumption has been associated with the highest prevalence of OSCC among the south-eastern Asian countries(Warnakulasuriya, 2009). Similar findings have been reported by Lin Wen-Jiun et al.(2011) in a prospective cohort hospital based study which demonstrated a 40-fold increased risk of OSCC among those who smoked(Lin, Jiang, Wu, Chen, & Liu, 2011).

Regarding alcohol consumption most heavy drinkers also use tobacco and, therefore, from epidemiological data it is difficult to separate the effects. Earlier studies described several ways, both systemic and local in which alcohol is thought to contribute to head and neck cancer. There is clear evidence that ethanol causes an increase in mucosal permeability to water itself and to other water-soluble molecules probably including carcinogens. These include tobacco products and in particular nitrosornicotine, which has been demonstrated in in vitro experiments (T. C. Hsu, Furlong, & Spitz, 1991; Lesch, Squier, Cruchley, Williams, & Speight, 1989). When alcohol is consumed, the immediate metabolite is acetaldehyde and a considerable amount has been found in saliva after moderate alcohol drinking owing to the action of bacterial alcohol dehydrogenase (Homann et al., 2000). In an experiment, production of the metabolite is significantly reduced after 3 days use of chlorhexidine, an antiseptic mouthwash, possibly explaining why poor oral hygiene appears to be an independent risk factor in OSCC in some studies(Homann et al., 2001; Moreno-Lopez et al., 2000). Alcoholic liver disease is also common among heavy drinkers and this probably reduces the detoxification of active carcinogens(Kato & Nomura, 1994). In addition, hand heavy alcohol drinkers have

been known to suffer from nutritional deficiencies due to poor eating habits and such deficiencies may contribute significantly to a lowered resistance to malignancies (Kato & Nomura, 1994).

Turning to khat, there is paucity of literature regarding its consumption and the occurrence of OSCC. “Khat” (*Catha edulis*) , a shrub whose leaves are chewed as a stimulant mostly in parts of East Africa and middle east but also in many parts of the world, has been associated with development of white mucosal lesions(Yarom et al., 2010). A case series from Yemen reported that close to 60% of patients with OSCC consumed “Khat” although a clear association was not established(Sawair et al., 2007). By using the micronucleus test to determine genetic damage, Khat extracts exhibited genotoxic effects on mucosal cells in vitro , raising the possibility of carcinogenic properties(Kassie, Darroudi, Kundi, Schulte-Hermann, & Knasmuller, 2001).

A report from Ethiopia indicated that khat farmers spray the crop with several pesticides including profenofos, dimethoate, and chlorpyrifos which could be detected after the leaves were harvested. These residues are consumed by the khat chewers and could cause malignancies (Atnafie, Muluneh, Getahun, Tsegaw Woredekal, & Kahaliw, 2021). Another toxicology study from Saudi Arabia on fresh khat leaves indicated the presence of Penconazole, Triademinol, Tebuconazole, Bifenethrin ,Lambda-Cyhalothrin,Carbaryl, Keroxim-methyl and Trifloxystrobin . However, There were no invitro studies to show any carcinogenic potential of the chemicals (Hassan AA, 2016).

2.3 Human Papilloma Virus and OSCC

Human papillomaviruses are small double-stranded DNA viruses that comprise a heterogeneous family consisting of more than 130 different subtypes. The viruses are classified as high-risk (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82) or low-risk (HPV-26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85), based on their association with cervical cancer (Bouvard et al., 2009; zur Hausen, 2002). Accounting for approximately 600,000 new cases of cancers worldwide annually, it is known that high-risk HPV types contribute significantly to virally associated

neoplasms (Moore & Chang, 2010). Is has been reported that (HPV-16) accounts for approximately half of cervical carcinomas and is associated with more than 90% of HPV (+) carcinomas of the oropharynx and the other ano-genital sites(Moore & Chang, 2010). The possibility that HPV might be involved in oral and laryngeal SCCs was noted from the findings that the morphologic features of genital and oral HPV associated lesions are similar (K. Syrjanen, Syrjanen, & Pyrhonen, 1982; K. Syrjanen et al., 1983). At present, only HPV-16 is classified as carcinogenic in the head and neck (Bouvard et al., 2009; Stransky et al., 2011). However, the presence of HPV in OSCC and normal mucosa show a wide geographical variation with some studies indicating positivity of between 34 and 74% in OSCC and 5.5 and 55% in normal mucosa(Giovannelli et al., 2002; Sugiyama et al., 2003; Zhang, Sdek, Cao, & Chen, 2004). These variations appear to depend on the laboratory technique used and the site and methods of sample collection.

The mechanisms through which high-risk HPV causes OSCC has been comprehensively discussed by Sugarman and Shillitoe (1997) and illustrated in Fig.2.3, (Sugerman & Shillitoe, 1997). Although it is suggested that HPV is associated with OSCC, this is not equivalent to causation. It has been observed that the immortalizing ability of the virus in the living tissues is not necessarily present in vitro. In order to explain the disease causation by viruses, Murphy (1996) presented ten criteria in the 9th edition of fields' virology (Table 2.1)(Murphy, 1996). Regarding HPV, very few criteria have been met in studies involving OSCC and more studies are clearly needed.

Table 2.1: Criteria for disease causation by viruses

1.	Prevalence of the disease is significantly higher in subjects exposed to the putative virus than in those not so exposed.
2.	Incidence of the disease is significantly higher in subjects exposed to the putative virus than in those not so exposed (prospective studies). Evidence of exposure to the putative virus is present more commonly in subjects with the disease than in those without the disease.
3.	Temporally, the onset of the disease follows exposure to the putative virus and an incubation period that follows a normal pattern.
4.	A spectrum of signs and symptoms follows exposure to the putative virus, presenting a pattern of response, from mild to severe
5.	A measureable host response, such as an antibody response and/or a cell-mediated immune response, follows exposure to the putative virus. In individuals lacking prior experience, the response appears regularly, and in those individuals with prior experience, the response is anamnestic.
6.	Experimental reproduction of the disease follows deliberate exposure of animals or humans to the putative virus, but non-exposed control subjects remain disease free. Deliberate exposure may be in the laboratory or in the field, as with sentinel animals.
7.	Elimination of the putative virus and/or its vector decreases the incidence of the disease.
8.	Prevention or modification of infection, via immunization or drugs, decreases the incidence of the disease. ‘The whole thing should make biologic and epidemiologic sense

Adapted from Murphy, FA(1996)

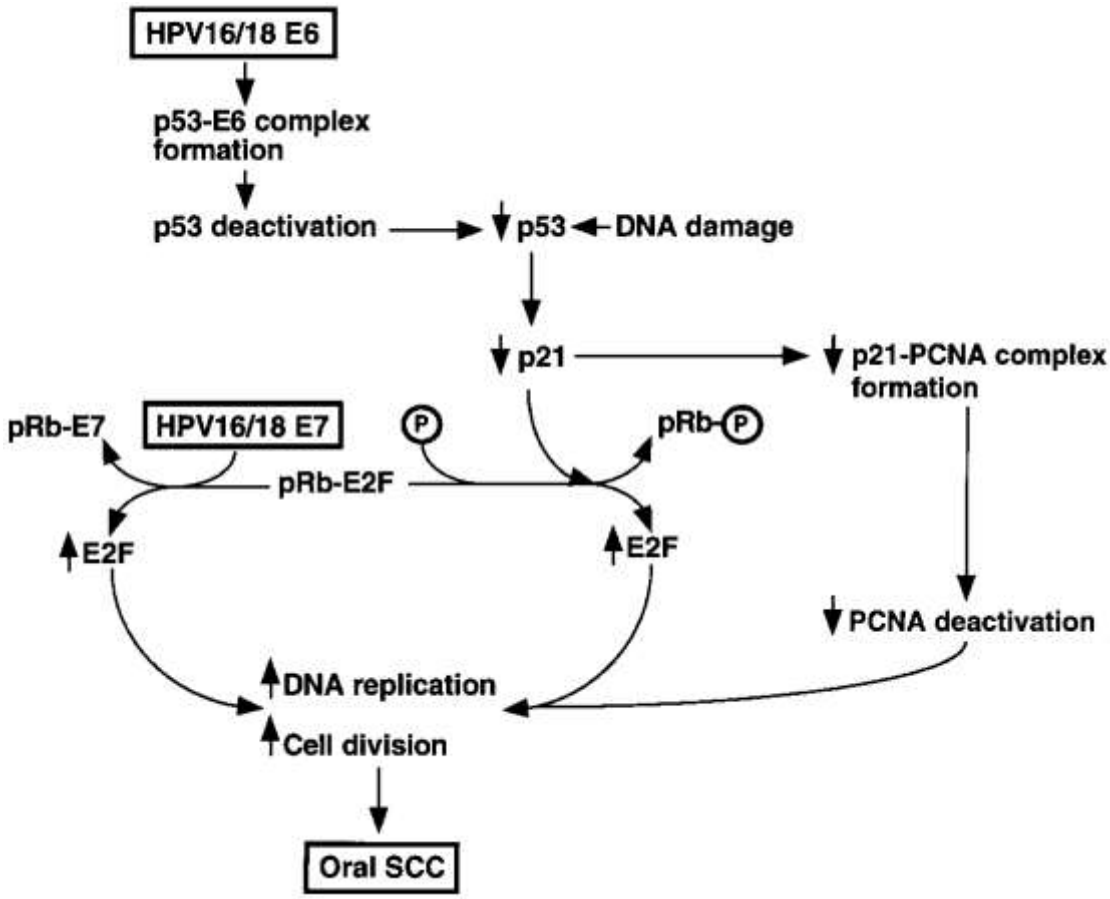

Figure 2.3: Hypothesis linking high risk HPVs(16/18) and oral squamous cell carcinoma.

Adapted from Sugarman and Shillitoe (1997)

2.4 Inflammation and OSCC

Inflammatory agents including viruses, bacteria and chemicals will in some cases elicit a prolonged low-grade immune response that does not result In the clearance of the pathogens, but to a state of continuous low-grade inflammation(Colotta, Allavena, Sica, Garlanda, & Mantovani, 2009). This state of chronic inflammation may predispose to a diversity of diseases, including cancer (Grivennikov, Greten, & Karin, 2010). According to the WHO, infections cause up to 20% of cancer deaths in developing countries and 9% of cancer deaths in high-income countries, and chronic inflammation constitutes an important component of these infections (http://www.who.int/mediacentre/factsheets/ fs297/en/) (WHO).

Regarding markers of inflammation interleukin 6(IL-6) , a pro-inflammatory cytokine, is consistently found to be elevated in patients with OSCC, although at present its clinico-pathological role is not clear (Chang et al., 2011). This cytokine is produced at inflammatory sites and secreted into the blood, thereby initiating systemic inflammatory reaction and production by hepatocytes of acute phase proteins including CRP, fibrinogen and a1-antitrypsin(Casas, Shah, Hingorani, Danesh, & Pepys, 2008). In a case control study done using an ELISA method to measure IL-6 levels in patients with OSCC, Chang et al (2003) found statistically higher serum levels of IL-6 in patients with OSCC tumours compared with those of healthy controls and among those with the oral premalignant lesions. They also found that the higher IL-6 serum levels were associated with higher Tumor size, overall stage, and deeper tumour depth and bone invasion(Chang et al., 2013).

The other marker of inflammation is CRP , a classical acute-phase protein which displays a rapid and pronounced rise of its plasma concentration in response to acute inflammation, infection, and tissue damage (Casas et al., 2008). It is known that genetic and environmental factors both have an influence on an individual's basal CRP concentration, with normal levels varying from 0.1 to 10 mg/l (Brull et al., 2003; Greenfield et al., 2004). Underscoring its non-specific nature, increased CRP concentrations have been reported in many diseases, including cardiovascular diseases, type 2 diabetes, arthritis and many types of cancers(Brull et al., 2003; Wieland et al., 2003). However, as a prognostic biomarker CRP has been found to predict recurrence, tumour destruction including bone invasion, lymph node metastasis as well as overall survival(Y. P. Hsu et al., 2015; S. F. Huang et al., 2012). When CRP is measured by high-sensitivity assays it has been described as not just a marker of existing cancer but also a predictor of increased risk of cancer in apparently healthy individuals (Chaturvedi et al., 2010; Heikkila, Ebrahim, & Lawlor, 2007; Heikkila et al., 2011).

There are several possible mechanisms that could explain the association between circulating levels of CRP and malignant neoplasms. The first one is causality, where elevated levels of CRP could play a causal role in the development of cancer. The second mechanism is reverse causality where existing cancer causes elevated levels

of CRP and thirdly a confounder causes both elevated CRP and the risk of cancer(Coussens & Werb, 2002). The association between chronic inflammation and cancer appears to be in two directions: inflammation can precede and promote tumour development and progression, but tumour development and progression can also induce inflammation through elaboration of various cytokines. This reinforces the fact that therapies, including non-steroidal anti-inflammatory drugs (NSAIDS) may thus have a role to play in reducing progression of tumours and the associated symptoms (Coussens & Werb, 2002).

2.5 Molecular Biology of OSCC Carcinoma

2.5.1 Oncogenes and tumour suppressor genes

Almost all cancer genes including oncogenes and tumour suppressor genes are involved in the complex pathways of signal generation, receipt and response that regulate cell growth and differentiation. To ensure normal development and cellular homeostasis there are important multiple layers of control. Escape from this tight control requires several mutations to be accumulated within the cell (Batsakis, 2003).

Tumour suppressor genes encode proteins that may counteract the effects of proto-oncogenes. In a normal cell proto-oncogenes and tumour suppressor genes modulate growth promoting signals, transcription, DNA repair and replication through their protein products, which act at crucial points in the cell cycle to maintain homeostasis. Deletional or mutational loss of function of oncogenes and tumour suppressor genes could, therefore, contribute to tumour formation (Batsakis, 2003).

2.5.2 Molecular biology of the cell cycle

Cells engaged in proliferation pass through four phases, namely G1, S, G2 and M, which together constitute the cell cycle. In G1 preparation is made to enter DNA synthesis while in S, DNA synthesis occurs. In G2 the cell assembles the machinery for distributing the newly replicated chromosomes to the daughter cells, which are generated in M, the mitosis phase. Although it lasts between 30 minutes and one hour, mitosis involves more changes in the cell structure than all the rest of the cell

cycle (Alberts B, 2002). The cell division cycle may be viewed as a series of checkpoints or transitions at which certain criteria have to be met before the cell proceeds to the next phase. When DNA is damaged, a checkpoint is activated to prevent cell cycle progression. This is presumed to prevent the cell from replicating the damaged DNA (S phase) or erroneous chromosomal segregation during mitosis. The molecules that drive cells through the cell cycle are called cyclin dependent kinases (CDKS). The kinases remain inactive until bound by its corresponding cyclins to form a kinase –cyclin complex. These complexes are key regulators of cell progression catalysing transition both into the S phase and into mitosis (Gao & Zelenka, 1997).

OSCC results from the accumulation of multiple genetic and epigenetic changes in a variety of cellular pathways. The processes of genetic alteration and selection result in the clonal expansion of those cells with the most favourable genetic aberrations, resulting in tumour development and eventual progression to invasive carcinoma (Califano et al., 2000). The study of the molecular pathogenesis of OSCC is complicated by the biological complexity of the disease with the current understanding that OSCC is known to be heterogeneous at both the histopathology and molecular levels (Agrawal et al., 2011; Leemans, Braakhuis, & Brakenhoff, 2011). Genetic alterations in cancer may occur in the form of small intra-genic mutations such as point mutations and insertions/deletions or large alterations including genomic deletions, amplifications and chromosomal rearrangements. Whole cancer exomes/genomes can be evaluated for genetic aberrations using either next-generation sequencing (NGS) or comparative genomic hybridization (CGH) technologies(Tan, Myers, & Agrawal, 2013) . In 2011, a group of researchers performed whole exome sequencing using NGS and confirmed mutations in several genes, including in TP53 , CDKN2A , FAT1 , PTEN , HRAS , PIK3CA, and EGFR that had been previously implicated in HNSCC. They also identified mutations in NOTCH1, which had never previously been associated with HNSCC(Agrawal et al., 2011; Stransky et al., 2011). Interestingly, NOTCH1 mutations were the second most common mutation in HNSCC of which OSCC is a subset.

2.5.3 TP53 gene mutations

It is estimated that half of HNSCC tumours harbour mutations of the tumour suppressor gene TP53, often called the guardian of the genome, which is located on chromosome 17p13.1 making this the most commonly mutated gene in these tumour types (Agrawal et al., 2011; Stransky et al., 2011).

When DNA damage is detected, the TP53 is activated and through its protein, a process is set in motion where transcription of genes responsible for cell cycle arrest is achieved. These processes provide the cell with the necessary pause to attempt DNA repair. If the damaged DNA cannot be repaired, TP53, through its factors will direct the cell to undergo apoptosis or senescence. Thus, damaged cells are prevented from propagating and accumulating more cellular damage. In cells in which loss of function of TP53 has occurred restorative processes, apoptosis, and/or senescence are unable to take place and this may lead to malignancy (Skinner et al., 2012).

Mutation of TP53 has been identified early in OSCC, present even in so-called premalignant lesions. Oral premalignant dysplastic diseases have been shown to contain TP53 mutations in 15% to 27% of cases(Boyle et al., 1993; Forastiere, Koch, Trotti, & Sidransky, 2001). The presence of TP53 mutations has been associated with increased risk of malignant transformation and indeed the rates of mutations appear to increase with histologic progression from mild dysplasia to invasive carcinoma(Boyle et al., 1993). Exposure to tobacco and alcohol has been associated with increased TP53 mutation rates in patients with OSCC and studies have shown that the rate of mutation is almost double in exposed patients compared with non-smokers (Brennan et al., 1995; Soussi, Kato, Levy, & Ishioka, 2005).

The presence of TP53 mutations in HNSCC is associated with poor clinical outcomes and disease progression. A large multicentre trial analysing 420 patients using a hybridization approach with a TP53 chip that carries all known TP53 mutations showed a decrease in survival by more than 1.5 times in patients with HNSCC with disruptive TP53 mutations(Poeta et al., 2007). Poor tumour response to chemotherapeutic agents including Cisplatin and Fluorouracil has been associated with the presence of TP53 mutations. In addition, patients with HNSC have shown

resistance to radiotherapy(Cabelguenne et al., 2000; Temam et al., 2000). Furthermore, it has been found that TP53 mutations are strongly associated with loco-regional recurrence following primary radiotherapy (Ganly, Soutar, Brown, & Kaye, 2000).

Therapeutic strategies aimed at restoration of wild-type TP53 using tumour injections of viral vectors continue to show promising results in patients with HNSCC. Phase 1 clinical trials done as early as the year 2000 have established the safety profile of the use of intralesional injections. Subsequently, multiple phase II trials have shown encouraging responses to therapy (Khuri et al., 2000; Nemunaitis et al., 2000; Nemunaitis et al., 2001). In 2009, a phase III trial comparing adenovirus TP53 gene therapy Advexin (Introgen Therapeutics Inc., Austin, TX), versus methotrexate for recurrent advanced HNSCC showed that wild-type TP53 patients had better response to Advexin, whereas patients with mutant TP53 responded better to methotrexate(Nemunaitis et al., 2009). Therapies targeting the TP53 pathway will continue to be explored in clinical trials and are expected to soon translate into approved therapeutic options. The drug ONYX-015 (Onyx Pharmaceuticals Inc., San Francisco, CA), a TP53 adenoviral-based treatment for patients with HNSCC has recently been approved for use in China with promising results (Nemunaitis & Nemunaitis, 2011). More recently, a review by Li et al[2019] (H. Li et al., 2019) describes novel therapeutic interventions targeting mutant P53 with the aim of depleting, restoring to wild type, inducing synthetic lethality and disrupting its tumour promoting signalling. Molecules including 17AAG and Ganetespib are currently being tested for lung cancer with promising results(H. Li et al., 2019).

2.5.4 Notch1 Mutations

NOTCH1 is the second most commonly mutated gene in HNSCC, with a mutation rate of 14 to 15% and it is important in regulating normal cell differentiation, lineage commitment and embryonic development(Agrawal et al., 2011; Bolos, Grego-Bessa, & de la Pompa, 2007; Stransky et al., 2011). It appears to function as a tumour suppressor gene in HNSCC based on the position and characteristics of the mutations and the inactivation of both alleles (Agrawal et al., 2011). The NOTCH1 protein is a

transmembrane ligand receptor with intracellular and extracellular domains. Upon ligand binding, the NOTCH1 intracellular domain (NICD) is cleaved and translocated to the nucleus. In the nucleus, the NICD activates transcription by binding to CBF1 in the presence of co-activators from the Mastermind-like family (MAML). Proteasomal degradation and down-regulation are mediated through the PEST intracellular domain. Downstream target genes of NOTCH1 signalling are crucial for cell differentiation and normal embryonic development (Bolos et al., 2007). Activating and loss-of-function mutations preferentially occur in different regions of the NOTCH1 gene. Deletions and mutations of the PEST regulatory domain may prevent proteasomal degradation and prolong downstream activation. Likewise, mutations of the extracellular heterodimer domain may allow constitutive NOTCH1 signalling in the absence of ligand binding. These previously reported mutations help to explain the oncogenic role of NOTCH1 in some cancers. In contrast, the majority of NOTCH1 mutations in HNSCC affect either the EGF-like ligand-binding domain or the NICD domain, suggesting loss of function(Agrawal et al., 2011).

As expected, therapeutically targeting NOTCH1 presents a dilemma, considering that the pathway has both oncogenic and tumour suppressor activity. A variety of γ - secretase inhibitors (GSI) are available and these can target the constitutively active NOTCH1 pathway by preventing NICD cleavage and nuclear translocation(Fortini, 2002).GSIs have shown promise in animal and in vitro studies of melanoma and Kaposi sarcoma (Curry et al., 2005). In a recent review of GSIs that have been developed for NOTCH1 signalling inhibition De Kloe and De Strooper (2014) paint a hopeful future in spite of the current challenges of non-selectivity of the molecules and gastro-intestinal toxicity. Some of these molecules have been entered in clinical trials including for metastatic solid tumours in combination with chemotherapy and glucocorticoids with promising results(De Kloe & De Strooper, 2014).

In summary the current study aimed at determining the aetiological correlates among cases and controls and to establish associations between the habitual, viral and genetic risk factors and OSCC. Figure 2.3 depicts a conceptual framework which shows the relationships between the independent and the dependent variable

CHAPTER THREE

RESEARCH METHODOLOGY

3.1 Study Site

This study was conducted at the Kenyatta National Hospital. KNH is situated along Ngong road in Nairobi, the capital city of Kenya with a population of about 3 million people. It is managed as a state corporation under the ministry of health. The referral hospital has in-patient and outpatient facilities in the departments of Internal Medicine, Surgery, Ear, Nose and Throat Surgery [ENT], Ophthalmology, Dentistry, Obstetrics and Gynaecology and Paediatrics. The departments of laboratory medicine and diagnostic radiology provide investigations for the clinical departments. As a government of Kenya referral hospital it receives patients requiring specialized care from the whole country and not infrequently from the neighbouring countries. The Oral and Maxillofacial surgery unit has outpatient as well as inpatient facilities within the main hospital. Patients with a wide range of oral and maxillofacial conditions including tumours, infections, congenital and developmental disorders, trauma and infections are managed by staff comprising specialists, trainee registrars and interns in a hierarchical manner, depending on the complexity of the conditions. Patients who present with tumours are fully clerked by the interns to establish a differential diagnosis before they are discussed with a specialist. In the specialist clinic the clinical presentation is reviewed and investigations including incisional biopsies,haematological tests and imaging studies are ordered. The incisional biopsies are done under the guidance of a specialist or a trainee registrar under local anaesthetic in the clinic. The specimen obtained is fixed in 10% formalin and taken to the pathology laboratory. The results of histopathological examination are usually received in the clinic after about 2 weeks. Upon receipt of the results confirming a squamous cell carcinoma a TNM staging is carried out followed by a treatment plan which may include surgery alone, with adjuvant chemo–radiotherapy or palliative care. The last modality often includes radiotherapy, counselling, nutritional support and symptom control. On average, the hospital receives about 100 OSCC patients every year as referrals from county facilities.

The pathology laboratory is situated within the hospital and is responsible for tissue diagnosis from all departments that obtain tissue for histopathological examination. Various cadres and specialists including oral and maxillofacial pathologists run the laboratory. The diagnosis of squamous cell carcinoma is made based on the light microscopic features.

3.2 Research Design

This was a combination of a case series and a case control study. The case series part was a descriptive prospective study which only involved persons who presented with OSCC. The analytical part was the case control study which involved 2 groups; the persons with OSCC and an equal number of controls who were age and gender matched subjects without the disease.

3.3 Study Population

Kenyatta National Hospital is a referral facility which receives patients from across the country .The cases were patients with a confirmed diagnosis of OSCC while age and gender matched controls were recruited among non–OSCC patients seeking outpatient services and particularly at the blood collection points where routine laboratory samples are obtained, to minimize as much as possible the discomfort and inconvenience.

3.3.1 Definition of cases.

Cases were all male and female patients presenting to the outpatient clinic with diagnosis of OSCC.

Inclusion criteria for cases.

1. Persons who gave informed consent
2. Persons who had a diagnosis of OSCC

Exclusion criteria for cases.

1. Persons who had previously been treated for OSCC

2. Persons who were too ill to participate

3.3.2 Definition of controls

Controls were patients who didn't have OSCC and were seeking outpatient services within the hospital. They were matched with cases for age and gender only.

Inclusion criteria for controls

1. Persons who gave informed consent
2. Persons who did not have OSCC

Exclusion criteria for controls

1. Persons who were too sick to participate

3.4 Sample Size Determination

The sample size for cases and controls was calculated using the following formula for differences in proportions(Schlesselman, 1974; woodward, 1992)

$$n = \frac{(r+1)}{r} \frac{(p)(1-p)(Z\beta + Za/2)^2}{(\bar{p}1 - \bar{p}2)^2}$$

Where;

1. r = 1; ratio of cases to controls
2. Z=1.96 , the level of confidence measure at (at 95% confidence interval)
3. $\bar{p}$ = Measure of variability (average proportion of exposure in cases and controls)
4. Z_{β} = Desired power (.84 for 80% power)
5. $Z\boldsymbol{a}_{/2}$=The desired level of statistical significance (1.96)
6. P_1= proportion of exposure among cases
7. P_2= proportion of exposure among controls

To calculate the proportion of cases exposed and to detect an odds ratio of 2 and above the following formula was used.

$$\text{Pcasesexp} = \frac{\text{ORp controlsexp}}{\text{Pcontrolsexp}(\text{OR}-1)+1}$$

According to a 2004 WHO report 26.2% of males and 1.9% of females in Kenya consumed tobacco (10). The proportion exposure among males was used to calculate the sample size in this study.

P2= .26

$$P1 = \frac{2x\ .26}{.26x(2-1)+1}$$

P1= 0.41

$$\bar{p} = \frac{P1+P2}{2}$$

$\bar{p}$= .36

$$n = \frac{1+1}{1}x\ \frac{0.36[1-.36][0.84+1.96]^2}{[0.41-.26]^2}$$

n= 157 pairs

Therefore,157 cases and a similar number of controls were recruited during the study.

3.5 Sampling Procedure

Sequential sampling method was used to select the study participants. Cases who met the inclusion criteria were enrolled at the department of Oral and Maxillofacial Surgery until the sample size of 157 was achieved.This method was chosen because of the relatively small number of persons with OSCC. Thereafter, an equal number of controls, who were matched for age and gender were recruited at the blood samples collection clinic using the inclusion criteria.

3.6 Research Tools

The study instruments included data abstraction forms, clinical, radiological and laboratory records; weighing scales and measuring tapes; dental mirrors, sterile gloves, measuring rulers, tissue brushes, swab sticks, specimen bottles and biopsy instrument sets.

3.7 Data Collection Procedure for Cases

At enrolment patients with lesions consistent with clinical diagnosis of OSCC were first subjected to a questionnaire (Appendix 4), which was used to record socio-demographic characteristics and information regarding tobacco use in all its forms and alcohol consumption, including type and duration. Thereafter, anthropometric measurements (height, weight) and examination were carried out and recorded on a clinical examination form (appendix 1). Height was determined using a tape and recorded in meters while the patient's weight (in kg) was measured using a regularly calibrated weighing scale (Salter,UK). The examination of the participants was done in artificial light from a power source (dental chair light) while seated on a dental chair. Regarding the lesion, the site, size in cm (measured in widest diameter using a ruler), presence or absence of induration and the effect on the adjacent structures including teeth was determined by inspection and palpation. In addition, the status of the cervical lymph nodes was determined clinically using digital examination. For the laboratory part of the study three types of samples were obtained from each patient including tissue scrapings, biopsy and 6ml of blood. The first samples were tissue scrapings collected from the lesions using special brushes and transported

within 2 hours in special media to be stored frozen at -70^{o} centigrade. After explaining the nature of the surgical procedure including benefits, risks and complications; and after obtaining consent , incisional biopsies were obtained from the lesions under a local anaesthetic in the standard way, divided into 2 portions and fixed in 10% formalin, with one portion being frozen at -70^{o} centigrade and the other submitted for histopathological examination. All the patients who had biopsies taken were confirmed to have OSCC and were subjected to further standard blood investigations including CRP, baseline full haemogram and Urea and electrolytes during their subsequent visits for management purposes. Apart from the collection of blood samples, which were done by laboratory staff, the principal investigator carried out all the clinical procedures. The blood samples were obtained from visible veins of the upper limb using sterile needles and syringes after cleaning the sites with surgical spirit. The blood was divided into equal portions of 3ml each, with one portion being used for full haemogram while the other for urea and electrolytes and also to estimate CRP levels. Finally, the patients were sent to the radiology department for chest X-ray to rule out chest metastasis. During the study period, 30 cases underwent surgery including neck dissection and the lymph nodes were submitted to the histopathology laboratory for examination.

3.8 Data Collection Procedure for Controls

At enrolment control subjects were subjected to a questionnaire (appendix 1), which was used to record socio-demographic characteristics and information regarding tobacco use in all its forms and alcohol consumption, including the type and duration. Thereafter anthropometric measurements (height and weight) and a careful oral examination were carried out to exclude the presence of any suspicious lesion. Heights were determined using a tape and recorded in meters while the patient's weights (in kg) were determined using a regularly calibrated weighing scale (Salter, UK). For the laboratory part of the study 3 samples, namely mucosal scrapings and 6ml of blood will be obtained from each participant. The first samples were mucosal scrapings obtained using brushes from equivalent anatomical sites as the lesions in the matching cases and kept in 2 types of bottles; one set was dry and the other had antibacterial media. The third samples was blood, which were obtained by the

laboratory staff for routine haematological investigations. Out of these, 3ml was placed in separate containers and used for CRP determination. Apart from blood samples which were collected by the laboratory staff, the other clinical procedures were carried out by the principal investigator.

3.8.1 Histopathology procedures

Histopathological examination of the biopsies and node dissection specimen was carried out at the KNH laboratory in the the standard manner by one pathologist and the reports entered in the data collection form, with the main copy being filed with the patient's records.

3.8.2 C-Reactive protein profiles for cases and controls.

The CRP levels of the serum samples were determined using an automatic biochemistry machine, BiOLiS 50i superior^{R} (Tokyo Boeki Medisys Inc) and the results reported in mg/l. The machine is regularly calibrated and operated by trained laboratory Technologists.

3.8.3 P53 and Notch1 gene mutation studies for cases and controls

For this part of the study a section of the fixed block of biopsy tissue and the mucosal scrapings were analysed. The design of the primers was done based on the identified pathological variants of the P53 and Notch1 genes. These included the rs28934575 ,rs121913343 ,rs28934577,rs28934576 and rs28934574 for P53 and rs587777735, rs587781259, rs864622061, rs587777734 and rs587777736 for Notch1 genes. The formalin fixed tissues and swabs which had been frozen at -70^{0}C were equilibrated at 19^{0}C-25^{0}C taken through the following process. First, Preparation of the formalin fixed tissue was done using the *Quick*DNA^{TM} FFPE miniprep kit (ZYMO Research Corp, California, USA). Thereafter, DNA extraction from the tissues and swabs was carried out using the *Quick*-DNA^{TM} 96 Plus Kit (ZYMO RESEARCH CORP, California, USA). This was followed by DNA amplification using the designed primers and SNP detection using the Agena massARRAY platform.

The Agena Bioscience MassARRAY platform was applied for performing high throughput DNA methylation analysis, quantitative gene expression and CNV analysis, SNP genotyping, quantitative mutation detection in heterogeneous samples and allele frequency determination on pooled samples of DNA. The system has the ability to use a single extension primer to interrogate all alleles at a SNP site for optimal data quality, accuracy and reproducibility. The system analyses up to 192 separate reactions in a single unattended run with no robotic feeder required since two 96-well chips can be assayed at the same time. The system provides allele-specific analysis products through robust and accurate primer extension reaction that extends through the polymorphic site and generates completely unambiguous results. In addition, the assay provides discrimination by both signal and size separation. It provides an accuracy genotype call rate of more than 99%. The system uses ultra-low amplification reaction volume of 5 *u*l. The assay has the ability to detect small insertions and deletions in addition to SNPs with the standardized assay. It detects the third alleles (tri-allelic SNPs), even if unexpected. The data was entered into an excel sheet and analyzed against the SNPs in the database.

3.8.4 Human Papilloma Virus identification

For this part of the study the scrapings samples from cases and controls were used to identify and quantify the HPV subtypes. The scrapings were collected using Copan diagnostics nylon flocked dry swabs (FLOQSwabs®, Copan Diagnostics, California USA), transported in capped plastic tubes containing antibacterial media and stored in -70^{0}C. Just before processing, the containers were removed from the freezer and equilibrated at 19^{0}C-25^{0}C. After vortexing to obtain a good mix , 50*u*l of the liquid mixture using a sterile pipette. The following steps were used in the detection of HPV.

First, DNA extraction was done using the *Quick*-DNA^{TM} 96 Plus Kit (Zymo Research Corp, California, USA). This was followed by DNA amplification and detection using the Anyplex^{TM}11 PCR system (Inqaba Biotech, Pretoria, SA). A comprehensive assay for the detection, differentiation and quantification of 28 HPV distinct genotypes (Anyplex^{TM}11 PCR system,Inqaba Biotech, Pretoria, SA) was

applied on the samples. These included 19 high-risk HPVs (16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 69, 73 and 82) and 9 low-risk HPVs (6, 11, 40, 42, 43, 44, 54, 61, 70). The data was entered into an excel sheet against the record numbers of the participants.

3.8.5 Minimizing errors and biases

Patients and controls were interviewed in Kiswahili or English and where a language barrier was encountered attempts were made to get a translator who was a native speaker of the patient's language. To ensure accuracy of the data all the personnel were trained and calibrated on the use of the data capture tools . Thereafter, the instruments were pretested and validated. In addition to the principal investigator, 2 trainee registrars collected all the data after appropriate training and calibration.

Only patients with confirmed diagnosis of OSCC were recruited and where the diagnosis was equivocal immunohistochemistry was used as a confirmatory investigation. Careful symptoms enquiry and examinations were carried out on controls to ensure that they did not have OSCC. The samples were obtained in a sterile manner and every care undertaken to ensure that no contamination occurred at any stage. In addition, all the equipment in the laboratory was periodically calibrated as per their standard operating procedure to ensure the integrity of the results. To avoid disintegration while awaiting analysis unfixed tissue was stored at below -70^{o} centigrade. Tissues for histopathology were fixed in 10% formalin. The concentration of the chemical was verified periodically by a trained laboratory technologist. Quality control and assurance was maintained at all times through various ways including training of research assistants on the study protocols and data collection as well as pretesting of data collection tool. All the processes were subjected to strict protocols to ensure validity and reproducibility of the results.

3.9 Ethical Considerations

This proposal was presented to the ethics and research committee for evaluation. The study only commenced after approval from the committee and permission had been obtained from the heads of clinical services in KNH. Confidentiality was maintained

at all times and apart from the assigned numbers no other participant identifiers were analysed. In addition, all paper records were kept in a lockable cabinet and electronic data password protected. The purpose of the study, the expected benefits and risks were explained to the participants clearly in a language they understood. In addition, the participants were informed about the voluntary nature of the study and that they could opt out at any stage without jeopardizing their treatment. Any questions/queries regarding the study were answered appropriately. Before enrolling in the study, a written informed consent was obtained from each participant (Appendix 2). Each participant meeting the inclusion criteria had an equal chance of being included in the study. The participants were at liberty to terminate participation at any time without victimization. Emergency treatment was given to any participant needing it and referrals were given to those requiring them.

The patients were managed as per KNH standard of care protocols and no new treatments or interventions were introduced. Patients who presented with lesions consistent with OSCC were informed about the need for a biopsy that would provide tissue for a histological diagnosis. Additionally, they were provided with a consent form for the surgical procedure. Apart from the sample required for routine haematological investigations no extra blood was obtained from the participants. In this study, no patient samples were exported out of the country.

3.10 Data Management

Data was keyed-in into MS Excel and later transferred to a password protected statistical package for social sciences (SPSS version 25, IBM) software.. The database was developed from the data collection tool (appendix 3). Range and consistency checks were carried out in real time using a built-in facility in SPSS. After data collection was complete a final data cleaning exercise was carried out for ease of analysis.

3.10.1 Data Analysis

Continuous variables were analysed for means and standard deviations while the student t test was used to compare the means. Categorical variables were analysed

using chi-square tests for associations. Mantel Haenzel test was used to calculate Odds ratios and to test the association between some independent variables (exposure) and OSCC. In addition, logistical regression was applied on all the associated factors to isolate the actual risk factor. Although the cases were matched for age and gender this was considered loose matching and the matched pairs analysis was not necessary (Kuo, Duan, & Grady, 2018; Pearce, 2016). Consequently, unconditional logistic regression was done to isolate the actual risk factors while the Mantel-Haenszel method was applied on the significant factors in order to get adjusted odds ratios. Statistical significance was set at P values of equal or less than 0.05 and the confidence intervals were calculated at 95% certainty.

The results have been presented in the standard way using narratives, figures and tables.

3.10.2 Dissemination of findings

The findings of the study have been bound and displayed in libraries of the Jomo Kenyatta University of Agriculture and technology and Kenya Medical Research Institute. In addition, two abridged versions have been published in a peer-reviewed journal.

CHAPTER FOUR

RESULTS

4.1 Sociodemographic Characteristics

Out of the 157 cases there were 97 (61.8%) males and 60(38.2%) females with age range of 28 to 96 years (mean age = 58, SD 13.2 years). There was no significant deference between the mean ages of the males and females.

Majority of the patients (n=78, 48.4%) were farmers while 47% (n=74) were in the informal sector in towns. The rest were employed in the formal sector. A higher percentage of females (68%) than males (36%) were engaged in farming of vegetables. Among the farmers majority (65.2%, n=45) used chemical pesticide on their crops while 31% (n=22) used chemical fertilizer. On the other hand, 31%(n=49) of the controls were farmers while 67% (n=106) and 1.9% (n=3) were in the informal and formal sectors respectively. There was an association between farming and OSCC (OR=2.16, 95% CI 1.37-3.39). Among the cases majority (67%, n= 105) came from outside of Nairobi County while 33% (n= 52) resided in different parts of Nairobi city.

Among the controls majority (65% , n=102) came from counties outside Nairobi while 35%(n=55) resided in different parts of the city. Table 4.1 shows the sociodemographic characteristics of the participants.

Table 4.1: Comparison of Sociodemographic features between cases and controls

Variable	Characteristics	Cases (%)	Controls (%)	p	OR	95% CI
Mean age (years)	Male 56					
	Female 60			>0.05		
Gender	Male	61.8	61.8			
	Female	38.2	38.2			
Occupation	Farmer	48.4	31		2.16	1.37-3.39
	Informal	47	67	-		
	Employed	4.6	1.9	-		
Residence	Nairobi	67	65	-		
	Outside Nairobi	33	35	-		

4.1.1 Body Mass Index

Among the cases, the mean and median BMI was 23.03kg/m^2 (SD= 6.4) and 21.7kg/m^2 respectively (range 13.6-45.4kg/m^2) with a significant differences between the mean BMI for males (21.55kg/m^2) and females (25.33kg/m^2) [p<0.001]. On the other hand, controls had mean and median BMI of 24.7kg/m^2 (SD=5.48) and 23.4kg/m^2 respectively (range 14.1-43kg/m^2) with no difference between the values for males and females. There was a significant difference between the mean BMI for the cases and the controls (critical ratio=2.39, p<0.05). Among the cases 56% reported recent weight loss with 28.2%(n=44) having a BMI below 18.5kg/m^2 compared to 5.7%(n=9)of the controls (OR=6.44 95% CI 2.98-13.5). When the low BMI was adjusted for age those above 60 years of age were significantly underweight (OR=5.28 95% CI 1.89-16.8.). Furthermore, males were likely to be under weight than females (OR=7.65,95% CI 2.71-26.33; vs OR= 4.85, 95% CI 1.38-21.3). The distribution of BMI values among the study subjects is depicted in Table 4.2.

Table 4.2: Comparison of BMI levels among participants in kg/m^2

	<18.5	**19-25**	**25.5-30**	**30.5-40**	**>40.5**
Cases (%)	28.2	42.5	15	12.1%	2.2
Controls(%)	5.7	45.9	20.2	18.1	1
OR	6.44	0.83	0.71	0.62	2.23
95% CI	2.98-13.5	0.48-1.46	0.34-1.46	0.28-1.34	0.22-22.19

4.2 Clinical and Pathological Presentation of OSCC

4.2.1 Site predilection of OSCC

Among the cases 38.2%(n=60) had the tongue involved, followed by buccal mucosa 41(26.1%), mandibular gingiva 21(13.4%) and maxilla 10(6.4%). While the tongue was the most common site among males at 42.3%, OSCC showed a higher predilection

for the buccal mucosa among females at 36.7%. These findings were, however, not statistically significant. Fig. 4.3 shows the distribution of cases according to site of the lesions.

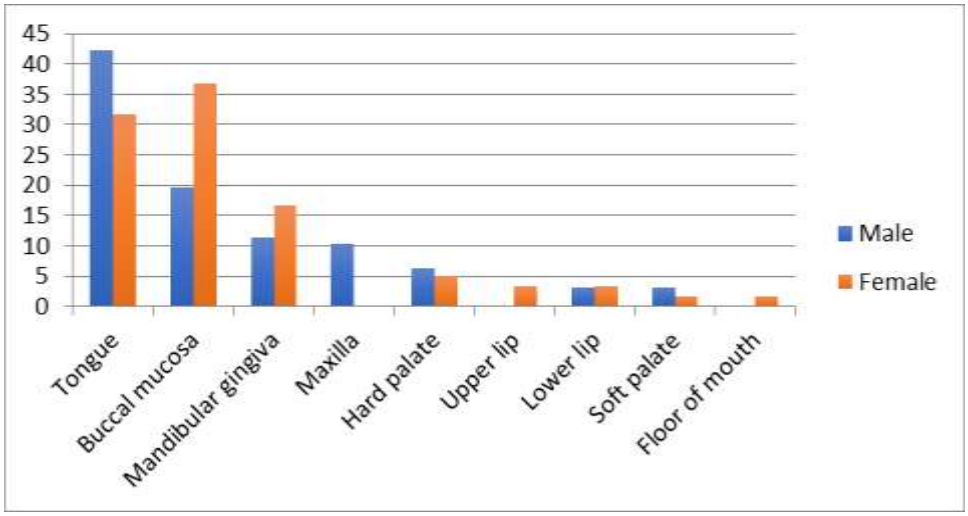

Figure 4.1: Distribution of cases according to the site of the lesions

4.2.2 Sizes of the lesions

The ulcerated lesions had a mean diameter of 4.6cm and a median of 5cm. There were no significant differences in ulcer sizes between males and females. Fig.4.4 shows the distribution of the cases according to the size of the ulcers.

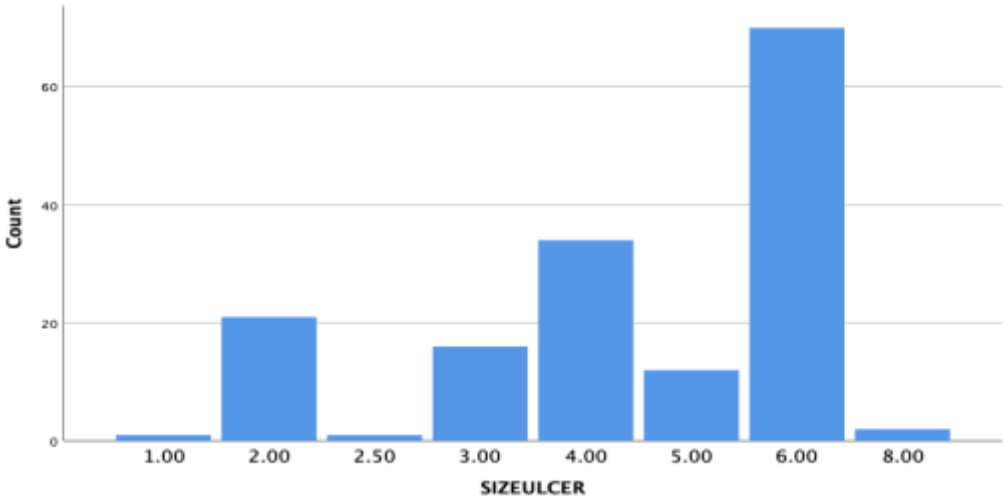

Figure 4.2: Distribution of the cases according the sizes of the ulcers in cm

4.2.3 Symptoms of OSCC

Among the cases 98% complained of a non-healing wound in the mouth of durations ranging from 1 month to 30 months. Most of the cases (93.4%, n= 147) presented with symptoms of pain with severities ranging from 1 to 10 on the NRS (mean=5), while 29.3%(n=46) and 56.1%(n=88) complained of odynophagia and weight loss respectively. Fig.4.5. shows the distribution of cases according to severity of pain.

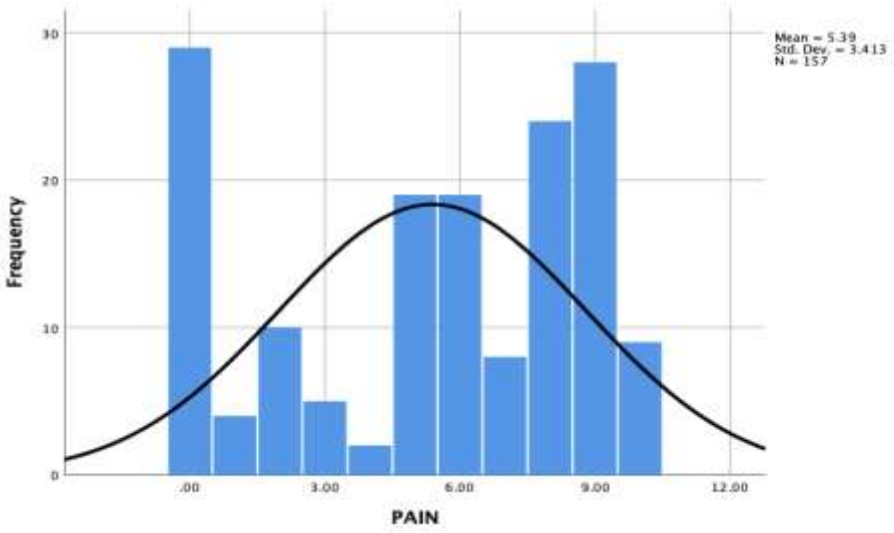

Figure 4.3: Distribution of the cases according to severity of pain measured on NRS

4.2.4 Cervical lymphnodes involvement

Among the study participants 55.5% had clinical involvement of cervical lymph nodes. Out of this level 1 node were more clinically palpable (67.9%) with sizes of about 2cm.

This was followed by level 2 nodes (23.86%), with most of the nodes not exceeding 3cm and level 3 nodes (8.1%). Following evaluation 30 patients underwent surgery including neck dissection and out of these 50% of the lymph nodes were found to have been positive for OSCC on histopathology. There was no significant relationship between the sites and sizes of the primary lesions with the lymph node status.

4.2.5 Chest metastasis

Chest X-rays were done as part of the investigations and 4.5% of the patients had evidence of chest metastasis.

4.2.6 Pathological grading of OSCC

The majority of participants (73.2%) had well differentiated squamous cell carcinoma(WDSCC) while 22.9% had moderately differentiated squamous cell carcinoma(MDSCC). The rest (4.9%) had poorly differentiated squamous cell carcinoma(PDSCC).

The distribution of patients according to pathological grading is demonstrated in Fig.4.4.

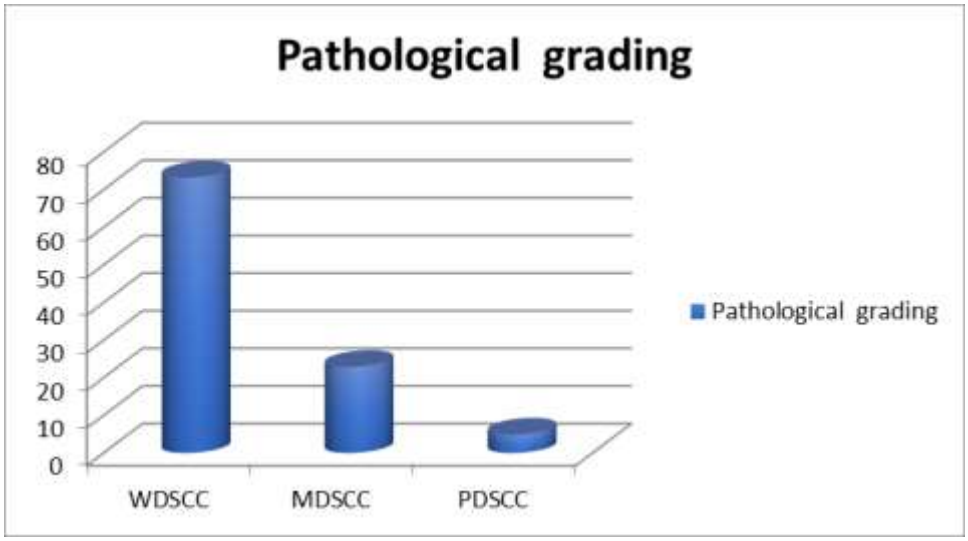

Figure 4.4: Distribution of the cases according to pathological staging

The clinical and pathological presentation of the OSCC lesions is depicted on plates 4-16 in appendix 1.

4.3 Tobacco Use

Among the cases 47.8% (n=75) consumed tobacco out of whom, 71% (n=53) smoked cigarettes while 29%(n=22) chewed it. Tobacco consumption was significantly higher among males (58.7%, n=57) than females (30%, n=18) [X^2=17.335, p<0.0001). On the other hand, tobacco consumption among controls was lower (5%, n=8). This study showed that tobacco consumption may be positively associated with OSCC (OR=16.96, 95% CI 8.33-35.87). When the Tobacco use was adjusted for age, those between 40 and 50 years and above 60 years

were significantly associated with the habit (OR=31.85, CI 3.89-1392 and OR=30.18, CI 8.45-159 respectively) [Table 4.3].

4.4 Alcohol Consumption

Among the cases 28.8%(n=42) were consumers of alcohol, out of whom 92.8%(n=39) and 7.2%(n=2) were males and females respectively. On the other hand, alcohol consumption among controls was very low (6.4%, n=10). Therefore, there was

an association between alcohol consumption and OSCC (OR=4.62, 95% CI 2.25-10.06). The comparison of alcohol consumption between cases and controls is shown on Table 4.3.

4.4.1 Tobacco use and alcohol consumption

Among the cases 21.7% (n=34) consumed both tobacco and alcohol while 26.1% (n=41) and 5.5% (n=8), respectively, engaged in tobacco use and alcohol consumption independently. On the other, among the controls 3.8% (n=6)subjects used both alcohol and tobacco while 6.3%(n=4) and 1.3%(n=2) respectively ,used alcohol and tobacco independently .

In order to identify the true effects of alcohol, adjustments for tobacco was done and alcohol was not found to be significantly associated with OSCC. Table 4.3 shows the comparison of Tobacco and alcohol use between cases and controls.

Table 4.3: Comparison of tobacco and alcohol use between cases and controls

Exposure	Cases (%)	Controls (%)	OR	95%-CI
Tobacco	47.5	5	16.96	8.33-35.87
Alcohol	28	6.4	4.62	2.25-10.06
Alcohol with tobacco	21.7	3.8	0.37	0.57-1.94
Alcohol without tobacco	5.5	1.3	3.15	0.95-11.16

Logistic regression analysis of the relationship between tobacco use, alcohol consumption and OSCC showed that only tobacco was significantly associated with OSCC (OR 20.8, 95% CI 6.56-65.88). Mantel Haenszel adjustment of tobacco use for age showed that 40-50 years groups and those above 60 years were significantly engaged in the habit. (Table 4.4)

Table 4.4: Logistic regression analysis of the relationship between tobacco, alcohol use and OSCC; and Mantel Haenzel adjustment of tobacco for age

Variable	OR	Std. err.	z	P>z	95%	CI
Tobacco	20.80	12.23	5.16	0.000	6.56	65.88
Alcohol	1.41	0.95	0.51	0.607	0.37	5.32
Tobacco adjustment for age using Mantel-Haenszel method						
Age group	**OR**	**95% CI**				
<40	.					
40-50	31.83	3.88 1382				
51-60	3.63	0.99-14.86				
>60	30.18	8.45-159				
Crude	16.98	7.88-40.2				

4.5 Khat Chewing

Chewing of "Khat" was not reported among controls but only 10% (n=16) of cases used the stimulant. Of note was that all the "khat" consumers also used tobacco.

After applying the Haldane-Anscombe correction(Lawson, 2004), there was an association between "khat" consumption and OSCC (Odds ratio= 36.86, 95%CI 2.19-614)[Table 4.5].However , its true effects were masked by tobacco.

4.6 C-Reactive Protein Levels

In this study CRP measurements were done as an estimate of inflammation. The mean and median CRP measurements for the cases were 10.55mg/l and 5.8mg/l respectively (range 1.0-81mg/l). On the other hand, the controls had a mean of 20.92mg/l and a median of 8.2 mg/l (range 1.7-121mg/l). There was a difference between the mean CRP values of cases and controls (critical ratio 3.91, $p<0.01$). Considering that the parameters were continuous, cut-off points of 3mg/l, 5mg/l and

10mg/l were used in order to compare cases against those of controls, with odds ratios of 8.65, 2.53 and 0.3 having been determined. (Table 4.5).

Table 4.5: Comparison of CRP levels between cases and controls

CRP levels(mg/l)	Cases(n)	Controls(n)	OR	95% -CI
>3mg/l	149	124	4.96	2.2-11.2
>5mg/l	114	89	2.66	1.66-4.1
>10mg/l	28	69	0.3	0.18-0.50

Considering that tobacco has been associated with elevated CRP levels, the true association between CRP levels and OSCC independent of usage was established by adjusting for tobacco usage and an independent association was established. Table Table 4.6 shows logistical regression analysis of the relationship between CRP levels and OSCC ; and adjustment for tobacco use.

Table 4.6: Logistic regression analysis of the relationship between CRP levels and OSCC; and Mantel Hanszel adjustment of CRP levels according to tobacco

Variable	OR	Std. err.	z	P>z	95% CI
CRP>3	1.09	0.29	0.33	0.738	0.64 1.84
CRP>5	19.98	11.21	5.33	0.000	6.65 60.05
CRP>10	0.022	0.016	-5.08	0.00	0.005 0.096

Adjustment for tobacco			
	OR	**95%**	**CI**
Tobacco	1.01	0.15	5.2
No Tobacco	4.24	2.08	9.08
Crude	2.51	1.51	4.18

4.6.1 C-Reactive Protein Levels According to Clinicopathological Presentation of Cases

There was no significant relationship between the CRP levels and cervical lymph node status (X2=0.634,p=0.426), site (X^2=6.05,p=0.735) as well as histopathological types [well differentiated (X^2=0.56,p=0.75), moderately differentiated (X^2=0.22, p=0.64) and poorly differentiated (X^2= 0.215,p=0.64)]. However, pain score above 5 on the NRS was significantly associated with CRP levels above 5mg/l [Table 4.4]. This study demonstrated that at cut-off levels of

3mg/l and 5mg/l CRP may be associated with OSCC (OR 4.96, 95%CI 2.22-11.2 and OR 2.66, 95% CI 1.66-4.1) [Table 4.7].

Table 4.7: Relationship between Clinicopathologic features and CRP levels

Variable	CRP>5 mg/l	CRP<5mg/l	X^2	P
Pain score >5 (%)	49.7	17.2	4.5	0.03
Lymph Nodes(%)	25.4	5.7	0.63	0.426
WDSCC (%)	56.7	17.2	0.56	0.755
MDSCC (%)	18.5	4.5	0.22	0.64
PDSCC (%)	1.9	1.2	0.22	0.64

4.7 Human Papilloma Virus Infection and OSCC

High risk HPV infection was not demonstrated in the oral mucosa of all study subjects. Among cases 7.3% had low risk HPV infection while controls had a prevalence of 5.5% of the virus with subtypes 43 and 69 being demonstrated.However , this finding was not significant (OR= 1.3, 95% CI 0.51-3.2).

The presentation of the raw laboratory data for HPV is depicted on plate 1 in appendix 1.

4.8 P53 Mutations

During the laboratory analysis 21 samples from cases had poor DNA yield and could not be taken through the PCR. Out of the 135 samples that were successfully analysed 21.4% (n=29) had mutations of the P53 gene that involved variants rs28934576, rs28934575, rs121913343 and rs28934577. The rs28934575 variant was the most commonly mutated P53 in 14.3% of the cases. This was followed by variant rs121913343 (5.4%), rs28934577 (3.5%), rs28934576 (1.7%) and rs28934574 (1.7%). Some of the cases had more than one variant involved in the mutations. On the other hand, the matched controls did not have mutations of the P53 gene. After using the Haldane-Anscombe (Lawson, 2004) correction, a significant association between P53 mutations and OSCC was determined(OR= 75, 95% CI 4.53-1232) . Fig.4.5 shows the distribution of the P53 variants among the cases.

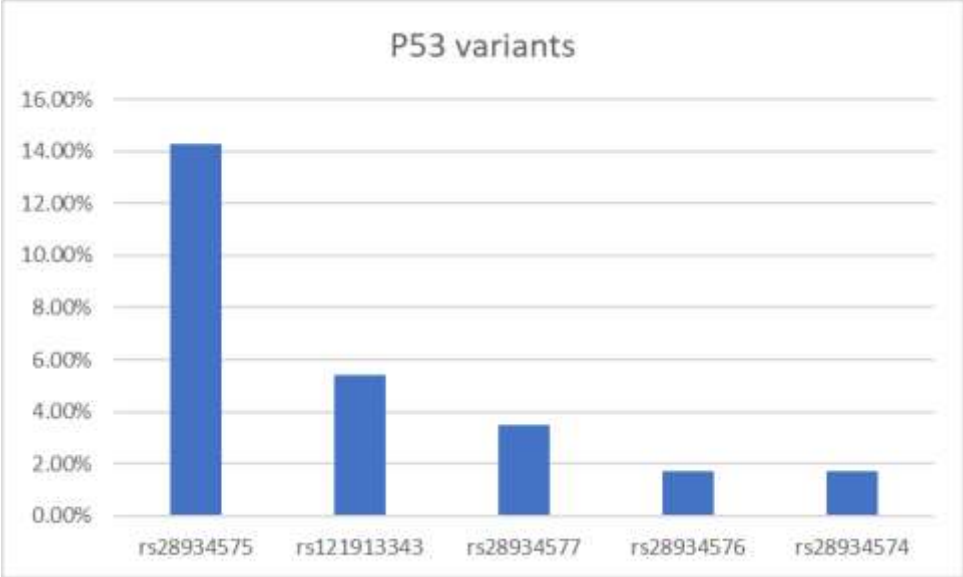

Figure 4.5: Distribution of the P53 variants

4.8 Notch1 Gene Mutations

Among the 135 samples that were fully analyzed, 26.6%(n=36) of cases had mutations of the Notch1 gene, involving the rs587777735, rs587781259, rs864622061, rs587777734 and rs587777736 variants. Among controls, Notch1 mutations was 32% (n=43) and involved only the rs587781259. However, this finding was not significant (OR 0.78, 95% CI 0.44-1.36). The Haldane-Anscombe correction (Lawson, 2004) was applied to compare the other variants between cases and controls and Notch1 was found to be significantly associated with OSCC(OR 140 , 95% CI 8.58-2321) . Fig.4.6. shows the distribution of the Notch1 variants

among the cases.

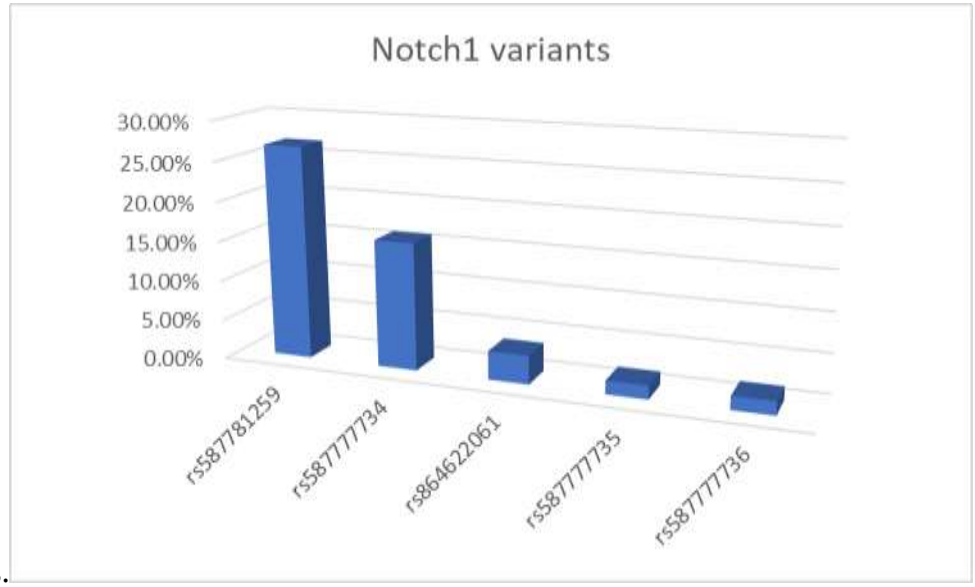

Figure 4.6: Distribution of the Notch1 variants among the case

The presentation of the raw SNP data for P53 and Notch1 mutations are depicted on plates 2 and 3 in appendix 1.

A summary of the comparison of the associated factors between cases and controls is presented in table 4.8.

Table 4.8: Comparison of all associated factors between cases and controls

Variable	cases(n)	controls(n)	OR	95%-CI
Farming	78	49	2.16	1.37-3.39
Low BMI	42	18	2.6	1.41-4.75
Tobacco	75	8	16.96	8.33-35.87
Alcohol	42	10	5	3.45-7.23
Alcohol with tobacco	34	6	0.276	0.026-1.69
Alcohol without tobacco	8	4	1.5	0.49-4.42
Khat	16	0	36.89	2.19-614
Inflammation				
>3mg/l	149	124	4.96	2.2-11.2
>5mg/l	114	89	2.66	1.66-4.1
>10mg/l	28	69	0.3	0.18-0.50
HPV	11	9	1.3	0.51-3.2
P53	29	0	75.06	4.53-1232
Notch1 rs587781259	36	43	0.78	0.46-1.33
Other Notch1	46	0	140.8	8.58-2321

Logistic regression analysis was carried out to determine the relationships between OSCC and all the associated factors and only tobacco use and CRP levels above 5mg/l were significantly associated with OSCC (Table 4.9.).

Table 4.9: Logistic regression analysis of the relationship between all the associated factors and OSCC

Exposure	**Odds Ratio**	**Std. err.**	**z**	**P>z**	**95%**	**CI**
Low BMI	2.59	1.73	1.43	0.154	0.700	9.63
Tobacco	20.80	12.23	5.16	0.000	6.56	65.88
Alcohol	1.41	0.95	0.51	0.607	0.37	5.32
CRP>3mg/l	1.09	0.29	0.33	0.738	0.64	1.84
CRP>5mg/l	19.98	11.2	5.33	0.000	6.65	60.05
CRP>10mg/l	0.022	0.016	-5.08	0.00	0.005	0.096
rs587781259	0.65	0.33	-0.85	0.398	0.24	1.75
Other NOTCH1	1 (omitted) controls had zero numbers					
P53	1 (omitted) controls had zero numbers					

CHAPTER FIVE

DISCUSSION

5.1 Overview of the Study Methods

The main purpose of this study was to describe the pattern of presentation and to determine the association between the correlates of OSCC and the disease at Kenyatta National Hospital in Kenya. This was done by determining sociodemographic features of all participants, clinico-pathological presentation of cases, tobacco use, alcohol consumption, ”khat” consumption, CRP levels, HPV infection and mutations of P53 and Notch1 genes among cases with OSCC and selected persons who did not have the disease. To answer the research questions a combination of case series and case control study design was considered suitable in describing the disease and determining the strength of association of the correlates and the disease. The case series was necessary in describing clinical characteristics of cases. These can be used to enrich guidelines of managing the disease and in generating new areas of research (Chidambaram & Josephson, 2019). A case-control study design is an efficient research method for investigating risk factors of a rare disease which takes unpredictable time to develop. When appropriately designed, case-control studies can provide the same information as a cohort study in a more rapid and efficient manner(van Stralen, Dekker, Zoccali, & Jager, 2010).

In this study, the sample size was calculated by taking into consideration the prevalence of tobacco consumption in the Kenyan population. This was used because the prevalence of the other factors in the Kenyan population is not known. Determining a minimum sample size is important to avoid wastage of resources when the sample is too large, or inaccurate results when the sample is too small (Noordzij et al., 2010). Sample size calculations are usually influenced by the study design, purpose of the study, degree of variability, level of confidence and level of precision. In a case control study sample size calculation takes into consideration expected OR between exposed and non-exposed groups, the probability of exposure in cases and controls, Statistical power; Alpha: usually 0.05 and the number of

controls per subject in the cases group (1 in case of equal groups) and either the OR or the probability of exposures in the cases (Fahim, Negida, & Fahim, 2019).

This study used convenience sampling method to select the participants. While this method is not random and likely to be biased, it is appropriate in clinical situations where the condition under study is not common(Stratton, 2021). In addition, precisely accurate statistics may not be necessary in observational studies where relationships between factors are being determined(Tyrer & Heyman, 2016) .

Oral brushes were used to exfoliate mucosal cells from asymptomatic persons for purposes of mutation studies. An audit of the efficacy of the oral brush technique in collecting cytological material found that the method was convenient and carries less morbidity while providing adequate tissue for diagnosis(Poate et al., 2004). For the HPV studies, flocked swabs were used to collect samples from both cases and controls. This type of swabs have been found to yield better cellular retrieval than the traditional cotton swabs(Viviano et al., 2018).

During part of the data analysis, the Haldane Anscombe correction was used during the calculation of the odds ratios where controls did not have any numbers(Lawson, 2004). In the cases control part of the study, matched pairs were selected based on age and gender. This was to make cases and controls to be as similar as possible except for the exposure factors. This type of matching eliminates some confounders while improving on the efficiency of the study and cutting the anticipated costs of recruiting more controls(Mansournia, Jewell, & Greenland, 2018; Setia, 2016). The analysis of the matched pairs was done using unconditional logistic regression since it been found to be easier to use and to give similar results as the conditional analysis in situations where pairs are loosely matched for age and gender (Kuo et al., 2018; Pearce, 2016).

5.2 Discussion of the Study Findings

5.2.1 Sociodemographic characteristics.

In the current study, there were more males than females, in concurrence with other studies. Global literature generally reports almost twice as much OSCC in males than in females (Zini, Czerninski, & Sgan-Cohen, 2010). This observation is possibly due to the higher exposure of males to the suspected risk factors including tobacco use and alcohol consumption (Zini et al., 2010). However, studies from Thailand and Malaysia, neighboring countries which share some cultural habits showed a slightly higher female to male ratio(Dhanuthai, Rojanawatsirivej, Subarnbhesaj, Thosaporn, & Kintarak, 2016; Kruaysawat, Aekplakorn, & Chapman, 2010). The current study found the mean age of the cases to have been 58 years with no difference in the mean age of females and males, concurring with a large multicenter study which placed the peak age of presentation at 50-59 years but with a significantly lower age among the Asian patients than European (Dhanuthai et al., 2018).

Among the cases, slightly more were farmers while the rest were in the informal sector and an association between farming and OSCC was established. This finding seemed to suggest that farming might have some occupational risk factors, which were not investigated in the current study. In the global literature there was paucity of studies that implicated farming and the disease. One study from the Nordic countries only associated lip cancer to prolonged solar exposure among farmers(Pukkala et al., 2009). In addition, the current study found that among the farmers there were more females than males who engaged in vegetable farming and that the majority used chemical pesticides while the rest used fertilizer. Although chemical pesticides were not investigated as risk factors in this study it is possible that some of the chemicals might have carcinogenic properties. A study from Iran reported an increased risk of HNSCC among farmers and a strong association was established between the use of pesticides and the occurrence of the cancers(Amizadeh et al., 2017). An in vitro toxicological study on Cypermethrin, a common type-II pyrethroid pesticide demonstrated cytotoxicity of macrophages (F. Huang et al., 2016).In addition , the organophosphate and thiocarbamate family of

pesticides have been associated with all cancers(Weichenthal et al., 2010). Arising from these findings more studies should be carried out in order to answer the question as to whether different chemical pesticides that are used in Kenya could be carcinogenic.

5.2.2 Symptoms of Oral Squamous Cell Carcinoma

Depending on site and stage of the disease, the symptoms of OSCC are varied including non-healing ulcers and varying degrees of pain(Carew JF, 2003). In the current study majority of the cases complained of a pain with a mean severity of 5 on the numerical rating scale (NRS), associated with a non-healing ulcer in the mouth. Several subjective methods have been used to measure pain including the visual analogue scale (VAS), numerical rating scale (NRS) and verbal rating scale (VRS)(Haefeli & Elfering, 2006).In the current study the NRS was chosen due to its simplicity and the fact that it is easy to administer. This is supported by Hjermstad et al.(2011) who reported that compared to VAS and VRS, NRS is widely preferred due to its ease of use and good applicability (Hjermstad et al., 2011).

Pain has been reported in all stages of OSCC, from precancer to advanced disease with no correlation between the size of the tumour and intensity of the pain(Yang, Zhang, & Li, 2017).An extensive systematic review found that up to 50.7% of cancer patients have pain ranging from moderate to severe in all stages of the disease(van den Beuken-van Everdingen, Hochstenbach, Joosten, Tjan-Heijnen, & Janssen, 2016). The fact that over 90% of the cases in the current study had pain is possibly a reflection of the advanced stage of the disease at presentation, which on average was T4.

In the current study slightly more than half of the cases complained of recent weight loss, close to previously reported prevalence of up to 46% in HNSCC (Richey et al., 2007) . When compared with controls the cases had significantly lower mean BMI. The current study established that 21.6% of the cases were underweight with BMI below 18.5 kg/m2. Additionally, being underweight was significantly associated with OSCC. These findings might be related to the advanced stage of the disease which could have resulted in poor feeding among our cases. Alternatively, it could be due

to the general wasting that is observed in malignant diseases. In a large Chinese study 15.4% of patients with OSCC had BMI below 18.5kg/m2 , a finding that was also significantly associated with worse prognosis(Bao et al., 2020). This was in concurrence with other reports which have indicated that pretreatment weight loss is also associated with reduced survival(Gannavarapu et al., 2018). In order to further the understanding of cancer related weight loss a panel of experts developed an international consensus that defined cancer cachexia as a multifactorial syndrome defined by an on-going loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment. Its pathophysiology is characterised by a negative protein and energy balance driven by a variable combination of reduced food intake and abnormal metabolism(Fearon et al., 2011).

5.2.3 Clinical and pathological presentation of OSCC

In the current study, the tongue was the site that was most commonly affected by OSCC, in agreement with other reports(Anis & Gaballah, 2013; Ariyoshi et al., 2008; Sargeran, Murtomaa, Safavi, Vehkalahti, & Teronen, 2006). There is no clear explanation for the propensity of OSCC to involve the tongue more commonly but one can only speculate that pooling of toxins in the floor of the mouth and trauma from the teeth might be responsible. However, other studies have found the mandibular gingiva to have been the most common site putting to question the role of dental friction(Chidzonga, 2006; Dhanuthai et al., 2016).

Most of the patients in the current study presented to the hospital late with advanced disease. Many factors including age, socio-economic conditions and gender of the patients have been identified as contributing towards delay in seeking treatment (Guneri & Epstein, 2014). In addition, many patients are not aware of cancer while others thought that it was a minor condition that would resolve on its own(Guneri & Epstein, 2014; Rogers, Vedpathak, & Lowe, 2011).

OSCC mainly metastasizes through lymphatic channels and involvement of cervical lymph nodes can occur early in the disease (Y. Li et al., 2019). In the

current study slightly more than half of the cases had clinically positive cervical lymph nodes detected in Levels 1-3. These are the most commonly involved lymphatic sites in OSCC (Dogan, Cetinayak, Sarioglu, Erdag, & Ikiz, 2014; Farmer et al., 2015). Actual metastasis to the lymph nodes is confirmed through histopathological examination following neck dissection and the positive rate varies widely depending on the stage of the disease (Abu-Ghanem S 2016; Y. Li et al., 2019; Suzuki et al., 2007). Out of all the cases in the current study 30 patients underwent surgery including neck dissection and 50% had positive cervical lymph nodes at histopathology. There was no relationship between the site of OSCC and lymph node metastasis. This finding demonstrates the need to consider each site as having the potential for lymph node spread.

Compared to other sites the incidence of distant metastasis in HNSCC is relatively small, with pulmonary spread accounting for up to 66 % of the metastasis(Ferlito, Shaha, Silver, Rinaldo, & Mondin, 2001). Although there was no attempt to look for all distant metastasis the current study established that a few of the cases had chest radiographic features of metastasis. This was close to the findings of Hazegawa et al (Hasegawa et al., 2015) in a case series which established that 80% of the 6.7% of the patients with distant metastasis had pulmonary involvement. In the same study, there was a relationship between the T and N stage of the disease as well as the histological grading of the tumour.

In the current study majority of the cases had well-differentiated tumors, compared to moderately-differentiated and poorly-differentiated. These findings contrasted with studies that have reported higher prevalence of moderately and poorly differentiated and tumors(Liu S A, 2019; Rodrigues R M, 2019), possibly due to geographical differences and other factors that haven’t been identified. These studies established relationships between the pathological grade of the tumour , recurrence and cancer specific survival. Significantly, poorly differentiated and moderately differentiated tumors had worse prognosis than well differentiated counterparts. The

differences in data could be due to population dynamics including the aetiologic factors and genetics.

5.2.4 Tobacco and OSCC

The current study established a strong association between tobacco consumption and OSCC, with the majority having smoked while the rest having used smokeless varieties. In addition, it was determined that almost twice as many males consumed tobacco when compared to the females, which may explain why more males were afflicted by the malignancy .These findings are at variance with studies from Asia, which show a more widespread use of smokeless tobacco among the consumers with women having a higher burden of OSCC than males, possibly due to their higher rate of tobacco chewing (Gupta S, 2018; Khan, Tonnies, & Muller, 2014). A paper by Khan et al(2017) established a stronger relationship between smokeless tobacco and OSCC when compared with smoked varieties [OR 21.2 vs 2.2] (Khan et al., 2017). In South America , a large case control study in Brazil(Ferreira Antunes et al., 2013) established a significant risk of OSCC from combined alcohol consumption and smoking. This finding was also highlighted by Radoi et al.(2015) who described a 10-fold risk of OSCC when alcohol consumption was combined with tobacco use(Radoi et al., 2015). Interestingly, the same study found no risk attributed to alcohol consumption on its own. It thus seems that tobacco use is a risk factor for OSCC even in our case.

Several theories have been advanced about the effects of tobacco use including epigenetic alterations of oral epithelial cells, inhibition of multiple systemic immune functions, oxidative stress alteration of DNA and possible cooperation with viruses such as the Epstein-Barr virus (EBV) and HPV infections (Jiang, Wu, Wang, & Huang, 2019). In a laboratory experiment where oral keratinocytes were exposed to tobacco extracts phenotypic changes were observed in the cells. In addition, microRNAs which have been established as key regulators of oncogenic potential in cells were dysregulated leading to altered expression of their target proteins (Bhat et al., 2018).

5.2.5 Alcohol and OSCC

Although the causal relationship between alcohol consumption and OSCC has been known since the 1950s, its independent carcinogenic effect was first described in early 1960s(Boffetta P, 2006). In the current study an association between alcohol and OSCC was observed. However, there was no independent association between of alcohol and OSCC when the effect of tobacco was considered, in concurrence with the findings of Radoi et al.(2015)(Radoi et al., 2015). However, an analysis of research from Korea found a 29.3% alcohol consumption population attributable fraction for OSCC, particularly for men (OR-1.45-1.98) (Park et al., 2014), similar to a meta-analysis from china (Li Y, 2011). Among the cases in the current study male alcohol drinkers were the majority and this underscored the fact that men suffered from OSCC more than females. Interestingly, there was a very strong link between alcohol and tobacco consumption. Studies have shown that alcohol has an additive synergistic effect with tobacco in all its forms with effects being amplified up to 10 times(Lin et al., 2011) (Petti, Masood, & Scully, 2013).This might be explained by the fact that alcohol has been shown to increase the permeability of oral mucous membrane producing an alteration in the morphology characterized by epithelial atrophy, which in turn leads to easier penetration of carcinogens into the oral mucosa (Kumar, Nanavati, Modi, & Dobariya, 2016).

5.2.6 "Khat" and OSCC

In the current study a minority of the cases and none in the control group consumed "khat". Interestingly, all the "khat" users also consumed tobacco making it difficult to isolate its role in OSCC. Furthermore, the 95% confidence interval was very wide and this can be attributed to a small sample size which failed to capture adequate numbers of khat chewers. These findings seemed to be in concurrence with a review by Thomas and Williams (2013) which could not clearly link *Khat* Consumption with OSCC(Thomas, 2013).Although there was paucity of studies that linked "khat" use to OSCC one old case series of 8 cases suggested a causal relationship based on the fact that the subjects did not consume tobacco and that the lesions occurred on the buccal pouch where they kept the bolus of *khat* (Soufi, Kameswaran, & Malatani,

1991). However, it would not be reasonable to draw conclusions based on such a small sample size and more studies are clearly needed in this area. Although not tried on laboratory animals, in vitro experiments using *khat* extracts on engineered buccal mucosa showed a significant reduction in mucosal thickness and premature differentiation of epithelial cells which in vivo, might result in carcinogenesis(Lukandu, Neppelberg, Vintermyr, Johannessen, & Costea, 2010).

5.2.7 Inflammation and OSCC

Two theories, namely the Induction and response hypothesis could be associated with increased inflammation in cancer. The induction hypothesis suggests that chronic inflammation results in excessive cell proliferation and activation of a cascade of cellular actions, leading to induction of irreversible DNA damage. On the other hand the response hypothesis states that the immune response of the host is a consequence of tumor growth itself and could be the reason for the elevation in the levels of the inflammation marker CRP (Coussens & Werb, 2002). Although the role of CRP in OSCC is controversial some case series have demonstrated the prognostic value of the cytokine in OSCC (Jablonska E, 1997; Khandavilli, Ceallaigh, Lloyd, & Whitaker, 2009; Kruse, Luebbers, & Gratz, 2010; Tai et al., 2017). After comparing the cases and hospital-based controls the current study found an association between CRP and OSCC. When compared with controls the cases had a significantly lower mean CRP level than controls , a difference that might be explained by the Berkson's bias which was described in 1949 by Joseph Berkson who pointed out that diseases which are independent in the population may become associated in hospital based case control studies and could share exposure factors(Berkson, 1946). The higher mean CRP among controls in the current study was attributed to isolated high outlier values, considering that the cytokine is elevated in several other inflammatory diseases. In order to establish whether there was a relationship between different CRP levels and OSCC, cut- off points of 3mg/l, 5mg/l and 10mg/l were used and odds ratios of 4.96 (95% CI 2.22-11.02), 2.66(95%CI 1.66-4.1) and 0.3 respectively were obtained. After comparing the cases and hospital-based controls the current study established that at CRP level above 3mg/l and 5mg/l there might be associated with OSCC. Furthermore, the possible confounding by tobacco, which has been

known to contribute to increase CRP levels (Aldaham, Foote, Chow, & Hakim, 2015; McEvoy et al., 2015) was excluded in the current study. In addition, this study found a positive relationship between moderate to severe pain and CRP levels above 5mg/l, indicating that pain is an important subjective marker of inflammation. This was in concurrence with a study by Laird et al (2010) involving a cohort of 718 cancer patients which demonstrated a strong correlation between pain and systemic inflammation(Laird et al., 2011). However, this study did not demonstrate a positive relationship between clinicopathological features and elevated CRP levels. This was possibly because the majority of the cases presented with advanced disease. Arising from these findings it can be postulated that OSCC might result from inflammation that is sufficient enough to cause elevated levels of CRP. On the other hand, OSCC might result in an elevation of the inflammatory marker and that inflammation is the driver of tumour growth. Therefore, therapeutic interventions using anti-inflammatory agents including non-steroidal anti-inflammatory drugs(NSAIDS) should be used in the management of the cancer patient(Roxburgh & McMillan, 2014).

5.2.8 Human Papilloma Virus and Oral OSCC

In the current study the prevalence of HPV was very low with slightly more cases than controls having had the viral infection. The HPV subtypes which were detected comprised only the low-risk types 43 and 69. These findings were in agreement with a study from China which did no detect high risk HPV infection in OSCC tissue samples (X. J. Chen, Sun, & Jiang, 2016). Additionally, there was no association between the low-risk HPV infection and OSCC, leading to the conclusion that the virus is probably not responsible for the OSCC in the current cases. However, some low but noteworthy proportion of healthy subjects have been found to have HPV infection with types known to be carcinogenic in the oral region (Kreimer et al., 2004).The low prevalence of HPV infection in the study participants could also be an indication that orogenital sex, the main route of infection is not widely practiced in the Kenyan population. In contrast, a case control study from Wuhan found a strong association between HPV types 16 and 18 infection and OSCC (OR=11.5) with prevalence of 27.5% and 2.9% among cases and controls respectively(Gan, Zhang,

Guo, & Fan, 2014) .This seemed to concur with a systematic review by Syrjanen et al (S. Syrjanen et al., 2011) which found a significant associated between HPV and OSCC((OR = 3.98; 95% CI: 2.62-6.02) . In addition, other studies have reported a prevalence ranging from 4 to 73% with suggestions that the differences could be due to geographical variations as well as detection methods (Hubbers & Akgul, 2015; Yete, D'Souza, & Saranath, 2018).

5.2.9 P53 mutations and OSCC

While in the current study controls did not exhibit the p53 mutations, 21.4% of the cases exhibited the mutant gene involving different variants, lower than reports from other centres. In high tobacco consumption population Poeta et al.(2007) reported a prevalence of 53.3% in a study that involved all head and neck sites with OSCC contributing about 30% of the cases(Poeta et al., 2007). This was lower than data from India which reported 52% P53 mutation rate of OSCC (Singh, Patel, & Patel, 2016). In the current study, there was no relationship between P53 mutation status and tobacco consumption, findings that were in agreement with those of Zanaruddin et al(Zanaruddin et al., 2013) who found a 27.7% prevalence of P53 mutations in an Asian population and no association with any risk habit including tobacco use. The current study did not identify high risk HPV infection among the cases and the relationship with P53 mutations could, therefore, not be established. This was at variance with other studies that reported occurrence of P53 mutations ranging from 48 to 62% and also established an inverse relationship with HPV infection (Perdomo et al., 2018; Westra et al., 2008). In these studies, tobacco consumption was also very high. As a prognostic indicator the role of P53 gene is controversial. While some studies have associated the mutant gene with a decrease in overall survival and increased risk of extranodal spread (Poeta et al., 2007; Sandulache et al., 2018) a review and meta-analysis by Tandon et al (Tandon, Tudur-Smith, Riley, Boyd, & Jones, 2010) was inconclusive. Not surprisingly, the current study did not establish any associations between the P53 mutations and stage of the disease probably because most of the cases presented with advanced disease leaving no room for comparison.

5.2.9.1 Notch1 mutations and OSCC

Among the participants Notch1 mutations were identified in 33.9% of the cases and 32% of controls who harbored only the rs587781259 variant. Interestingly, this variant was also more prevalent among the cases at 26.8%, giving an odds ratio of 0.74, 95%CI 0.44-1.36. This finding suggests that the Notch1 variant was not associated with OSCC among our cases. However, other variants including rs587777734 and rs864622061 were identified in 16% and 3.6% of the cases respectively and none among the controls suggesting that the variants might be associated with OSCC. In the current study there was no relationship between tobacco consumption and Notch1 mutations, at variance with the findings from a multicenter study involving a 97% tobacco consuming population of OSCC cases Perdomo et al [2018] (Perdomo et al., 2018) which reported 31% prevalence Notch1 mutation but lower than a prevalence of 54% in Chinese patients (Izumchenko et al., 2015). Regarding prognosis Song et al (Song et al., 2014) reported a significantly shorter overall survival among participants with Notch1 mutations. Unfortunately, as previously indicated, most of the cases in the current study presented with advanced disease and in addition, follow-up was not possible and, therefore, the role of Notch1 mutations was not determined. It seems plausible that the variations in the occurrence of Notch1 mutations appears to be due to geographic as well as environmental factors including tobacco consumption.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary of the Findings

The main objective of the study was to describe the pattern of presentation of OSCC and to determine the association between some correlates and the disease at the Kenyatta National Hospital by using a combined descriptive case series and case control study design.The findings of the study showed that the mean age of the study subjects was 58 years (SD 13.2) with more males (61.8%) being affected by the disease. Regarding the clinical features, the tongue was the most affected site among males while more females presented with buccal involvement. Although all the cases presented with non-healing ulcers in the mouth, most of them (93.4%) had pain ranging from moderate to severe, in line with their advanced stage 4 disease. Regarding occupation, this study determined that there was significant association between farming and OSCC (OR=2.16). Although slightly more than half the cases presented weight loss, being underweight was significantly associated with OSCC (OR=2.6). Regarding habits, this study determined that there was a strong relationship between tobacco use and OSCC (OR=16.96). This study did not find a relationship between alcohol, 'Khat' and OSCC although strong relationships between tobacco, alcohol and "khat' were described. Regarding CRP, the inflammatory marker had a significant association with OSCC at lower levels (OR=2.66). Although HPV was found among a small number of cases, high risk types of the virus was not demonstrated among the participants. Turning to genetic study, P53(OR=75) and Notch1(OR=140) mutations were significantly associated with OSCC . When all the associated factors were subjected to logistic regression analysis, only tobacco and CRP levels above 5mg/l were significantly associated with OSCC, implying that there were confounding effects.

6.2 Conclusions

This study described the clinical and pathological presentation of OSCC and analysed the association between some risk factors and the disease. From the

findings of the study, OSCC affects the older male more, a reflection of his heavier use of tobacco. Most patients presented late to KNH, with advanced disease characterized by large ulcerations and Pain. Pain is a debilitating symptom which has been noted in all stages of the disease, underscoring its importance in the management of OSCC. Another important symptom was weight loss, which afflicts most patients with OSCC. Weight loss could be an indicator of several nutritional deficiencies and could lead to severe morbidity. Regarding occupations, Farming was strongly linked with OSCC and more females were engaged in this occupation. From the farming practices, it is possible that some farming chemicals could be carcinogenic and are unintentionally consumed by farmers through the crops. Looking at site involvement, the tongue was the most commonly involved with OSCC and confirms what has been reported in other countries. lymph node metastasis is usually a poor prognosis indicator and was common among our patients, indicating a grim outlook of the disease. Regarding habits, while tobacco use was a risk factor for OSCC, alcohol consumption and Khat chewing were not. These might be a true finding but it could also mean that the sample size, which was based on tobacco use was not adequate to get positive associations. Turning to the viral factors, high risk HPV was not isolated from the participants and is probably not involved in the aetiology of OSCC. However, detection methods are also complex raising the possibility of false negative tests .Regarding inflammatory markers, elevated CRP levels were associated with OSCC, possibly a reflection of the role of inflammation in the disease. Lastly, as regards genetic factors, P53 and Notch1 mutations were commonly seen in OSCC although their actual roles have not been established.

6.3 Recommendations

Based on the findings of this study education of the public on the need to seek care when they notice a wound in the mouth. In addition, healthcare workers at primary and secondary facilities should be educated on how to identify high risk oral lesions and refer the patients at the earliest opportunity. Regarding symptoms, early recognition and appropriate control of pain among patients with OSCC should be done at the first point of contact to reduce the suffering that is associated with the

disease. Early interventions should be carried out to counter weight loss and cachexia in patients with OSCC in order to reduce the morbidity of the disease. In addition, clinicians at the first point of contact should understand that unplanned weight loss might be a sign of serious illnesses including cancer and carry out appropriate investigations. As regards farming, the use of farm chemicals should be monitored and the farmers educated on the possible risks that they pose. In terms of surgical management, Neck dissection should be carried out in all cases of OSCC presenting with enlarged cervical lymph nodes from the understanding that there is a 50% chance of metastasis. Turning to habits, tobacco cessation campaigns should be strengthened across the country in order to reduce the cases of OSCC that can be attributed to the habit. In addition, more studies should be done on the association between alcohol, khat and OSCC. Regarding inflammation, CRP measurements should be done on all patients presenting with OSCC in order to reduce the morbidity and progress of the disease. Further, more studies should also be designed to establish the actual role of inflammation in OSCC. These may involve the use of other inflammatory markers such as interleukin 6 at the tumor level. As far as gene mutations are concerned, more studies with follow-up components should be designed to establish the prognostic role of the P53 and Notch1 mutations and OSCC.

6.4 Limitations of the Study

The study investigated many facets of OSCC including some laboratory procedures that could only be done in few laboratories in Kenya. As a result, the cost of the tests was very high. In addition, many processes had to be repeated due to errors because the laboratories had not carried out similar tests before thus adding to the costs. OSCC is a relatively rare disease and, therefore, the time taken to recruit cases was very long. Consequently, although the tissue samples were stored in frozen state changes could have taken place in the older specimen by the time of analysis. In addition, some of the reagents that were supplied and stored at the beginning of the study could have undergone changes. Sequential sampling method was used to recruit the cases and it is possible that they might not have been representatives of the victims of OSCC across the country. The studied gene variants were selected at

the proposal stage of the study and it is possible that by the time the samples were being analyzed new variants had been identified. Furthermore, the selection of hospital controls might have introduced the Berkson's bias. However, this has been taken care of during the discussion of the results.

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APPENDICES

Appendix I: Plates

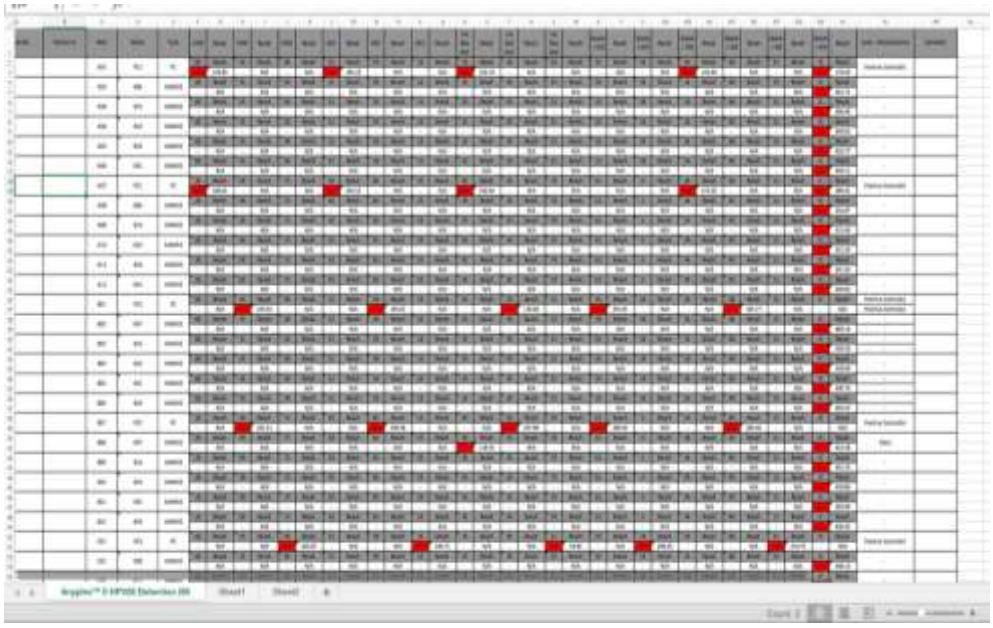


Plate 1. Sample of raw HPV data.

	A	B	C	D	E	F	G	H	I
1	AGE	SEX	rs121913343	rs28934577	rs28934575	rs28934576	rs28934574	rs11540652	rs17849781
2	74.0	M	G	A	C	C	G	C	G
3	51.0	M	G	A	C	C	G	C	G
4	65.0	F	G	A	C	C	No call	No call	G
5	50.0	F	G	A	C	C	G	C	G
6	68.0	M	G	No call	C	C	G	C	G
7	52.0	M	G	A	C	C	G	C	G
8	71.0	M	G	A	C	C	G	C	G
9	45.0	M	G	A	C	C	G	C	G
10	62.0	F	No call	No call	C	C	G	C	G
11	64.0	M	G	A	C	C	No call	No call	G
12	50.0	F	G	A	C	C	G	C	G
13	46.0	M	G	A	C	C	G	C	G
14	59.0	F	G	A	C	C	G	C	G
15	63.0	F	G	A	C	C	G	C	G
16	64.0	F	G	A	C	C	G	C	G
17	65.0	M	G	A	C	C	G	C	G
18	49.0	F	G	A	TC (pathoger	C	GA (Pathoge	C	G
19	71.0	F	G	A	TC (pathogeni	C	G	C	G
20	40.0	M	G	A	TC (pathoger	C	G	C	G
21	49.0	F	G	A	TC (pathogeni	C	G	No call	G
22	95.0	M	G	A	C	C	G	C	G
23	77.0	M	G	A	C	C	G	C	No call
24	56.0	F	G	A	C	C	G	C	G
25	67.0	F	G	A	C	C	G	C	G
26	66.0	M	G	A	C	C	G	C	G
27	69.0	F	G	A	TC (pathogeni	C	G	C	G
28	65.0	M	AG (Pathoge	A	TC (pathoger	C	G	C	G
29	62.0	F	G	A	TC (pathogeni	C	G	C	G
30	52.0	M	G	A	C	C	G	C	G
31	68.0	M	AT (Pathoger	No call	TC (pathoger	C	G	C	G
32	66.0	F	AG (Pathogen	A	C	C	G	C	G
33	60.0	F	G	A	C	C	G	C	G
34	43.0	M	No call	No call	C	C	G	C	G

Sheet1

Plate 2. Sample of P53 SNP data

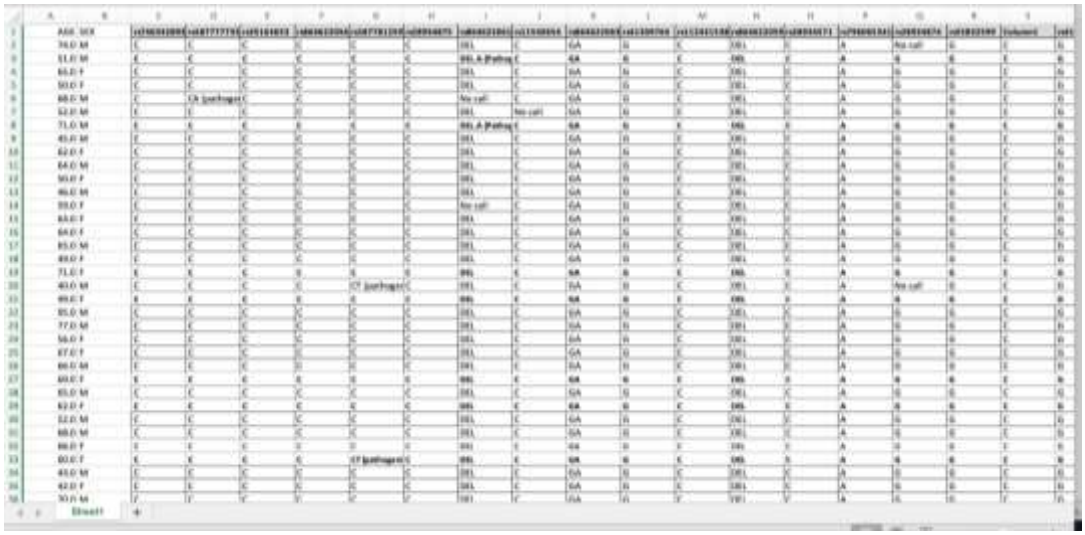


Plate 3. Sample of Notch1 SNP data

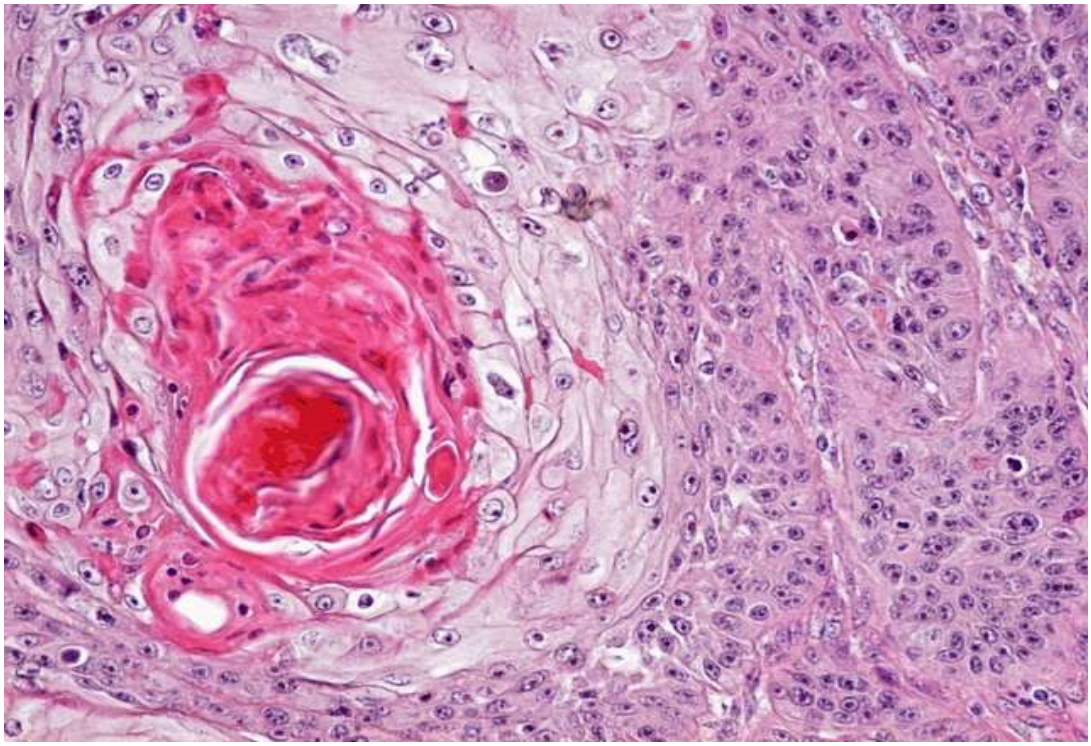


Plate 4. A photomicrograph of Well differentiated squamous cell carcinoma (H&E Stain x200)

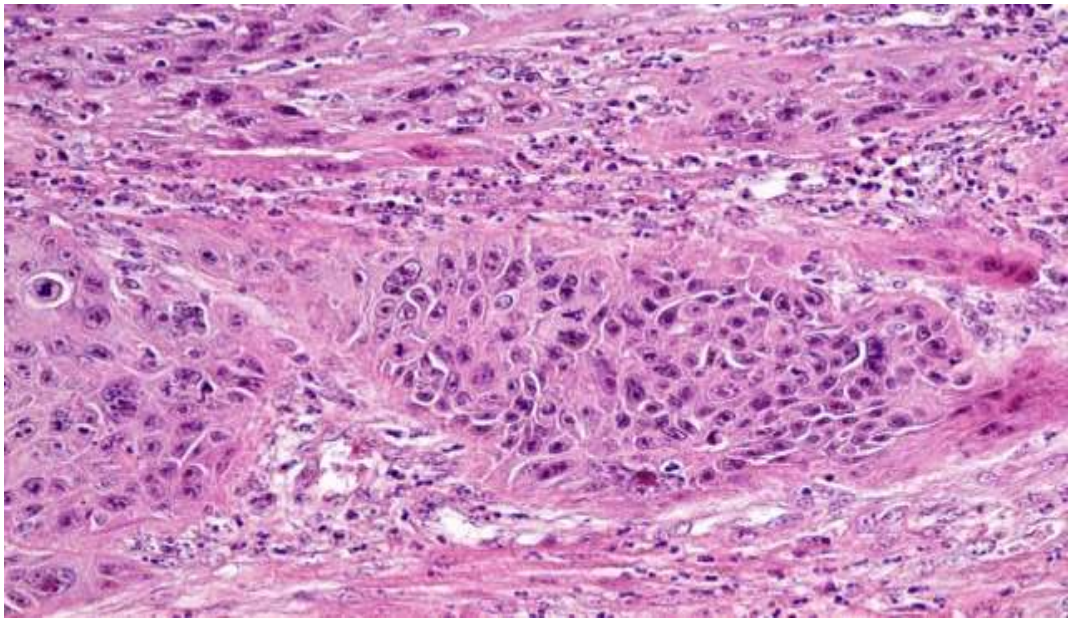


Plate 5. A photomicrograph of moderately differentiated squamous cell carcinoma (H&E Stain x200)

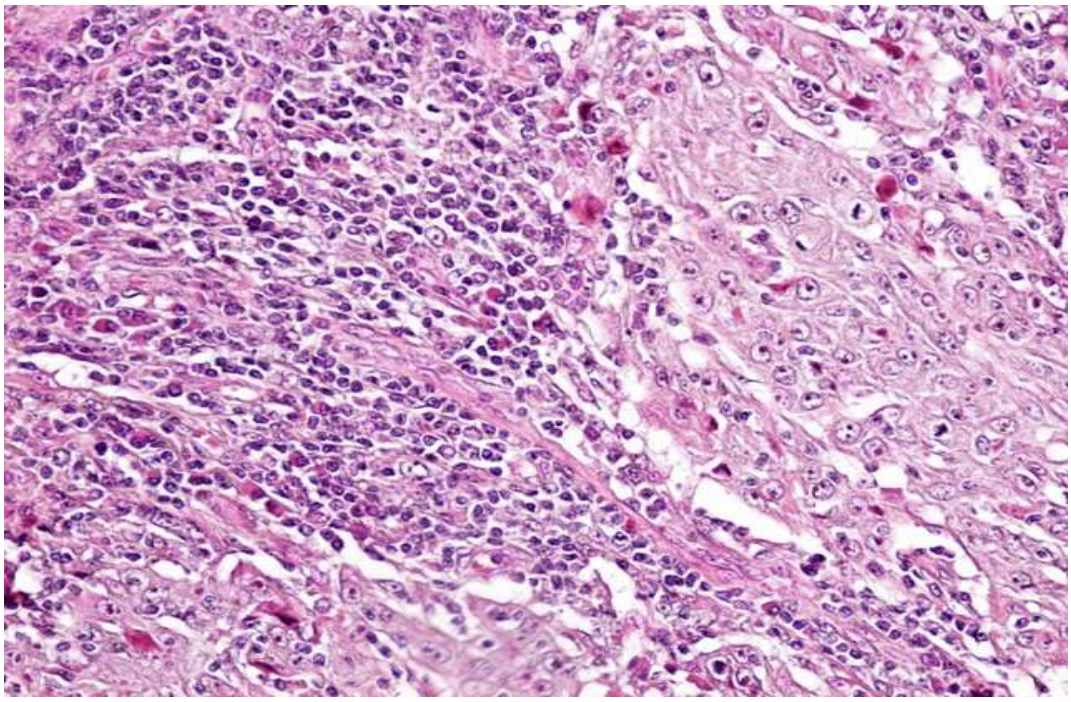


Plate 6. A photomicrograph of poorly differentiated squamous cell carcinoma (H&E Stain x200)

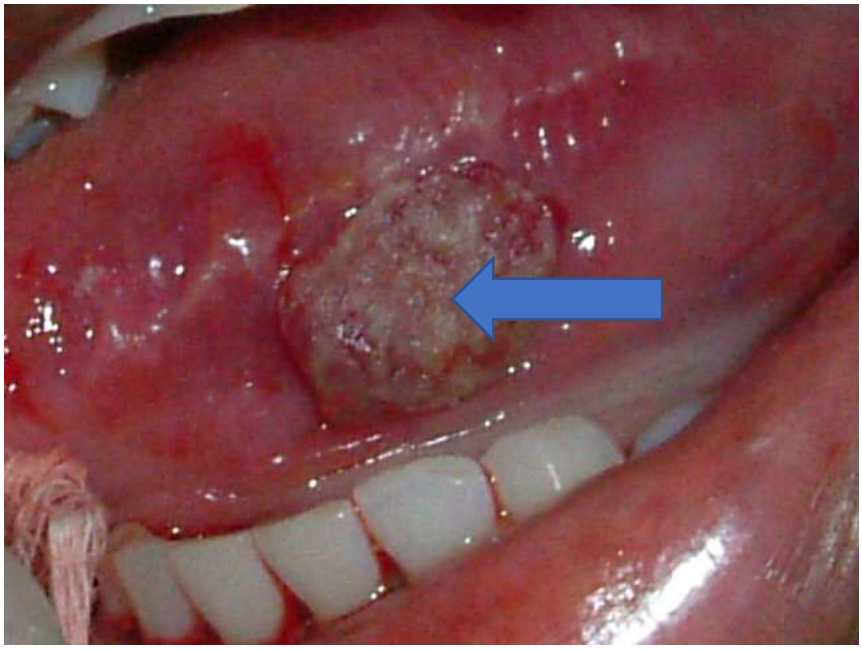


Plate 7. Squamous cell carcinoma of the tongue

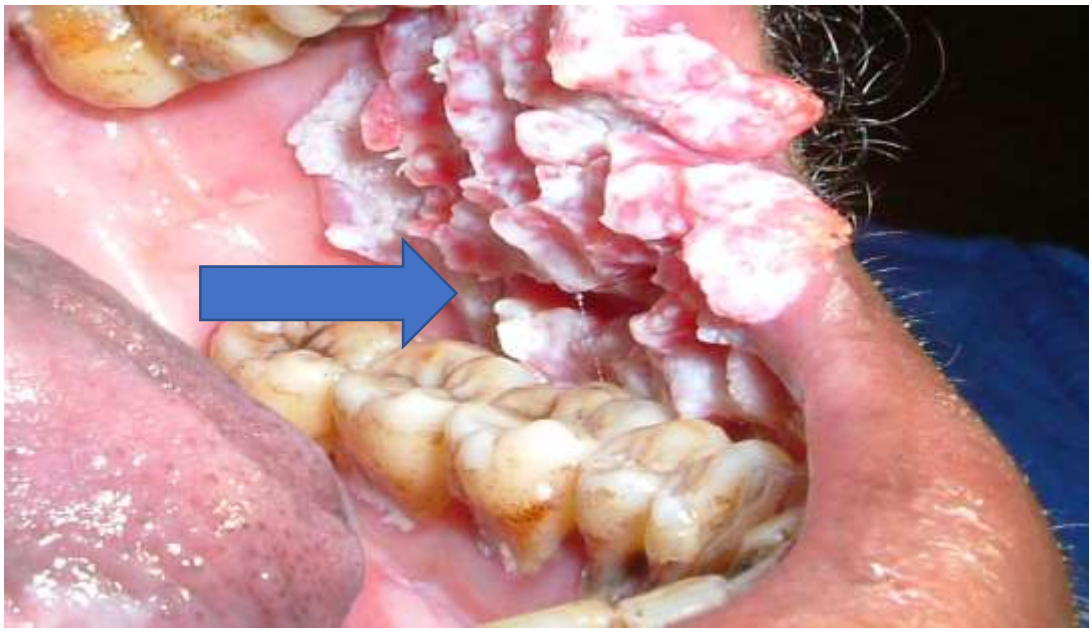


Plate 8. Squamous cell carcinoma of the buccal mucosa

Plate 9. Squamous cell carcinoma of the lower lip

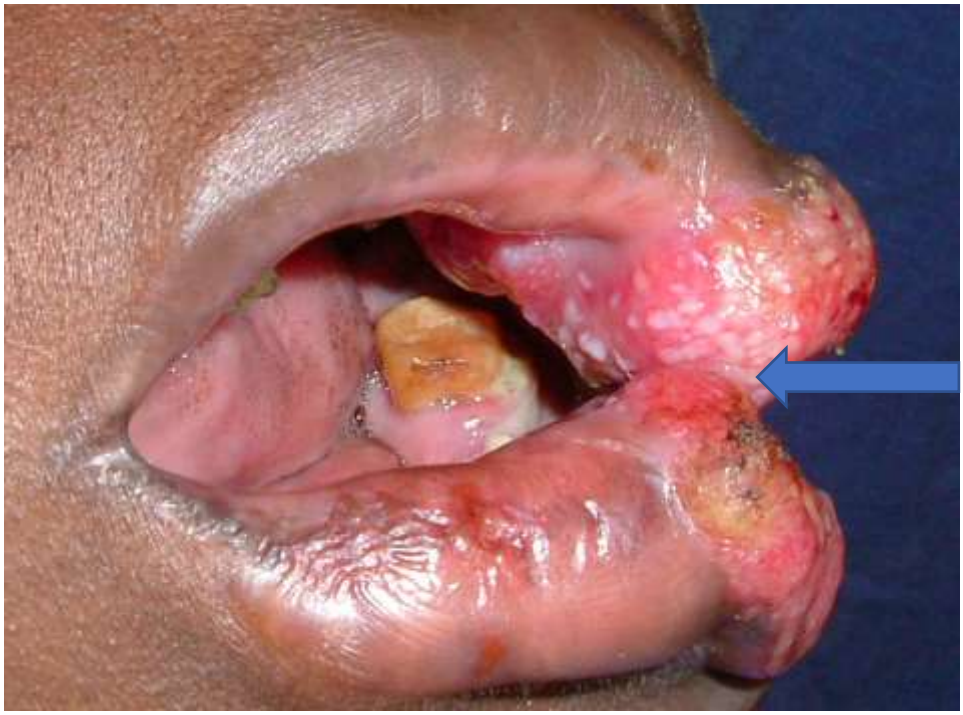


Plate 10. Squamous cell carcinoma of the buccal mucosa, oral commissure with extension to the skin.

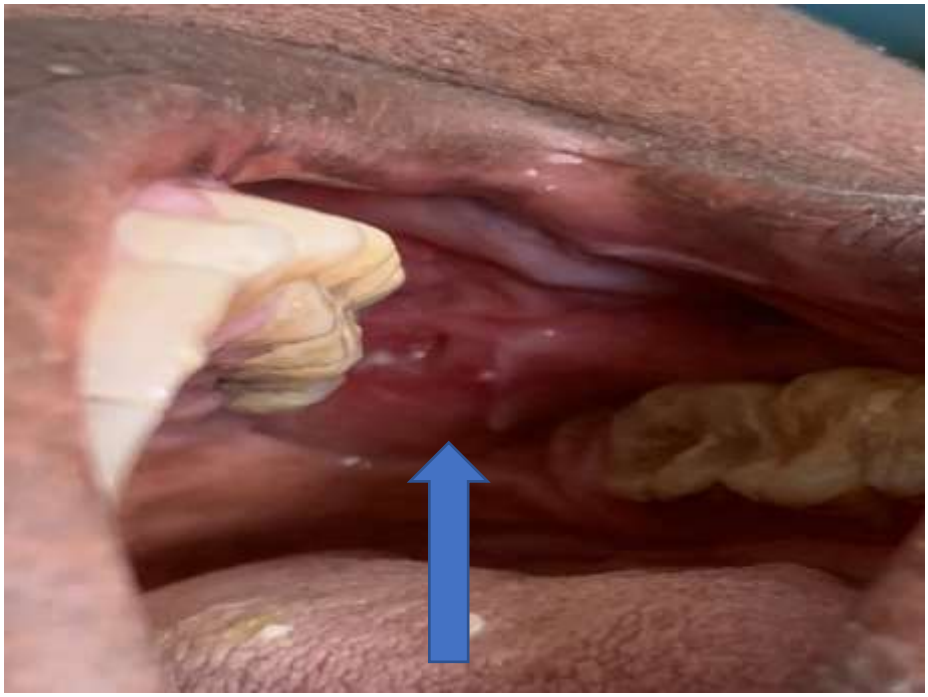


Plate 11. Squamous cell carcinoma buccal and retromolar area

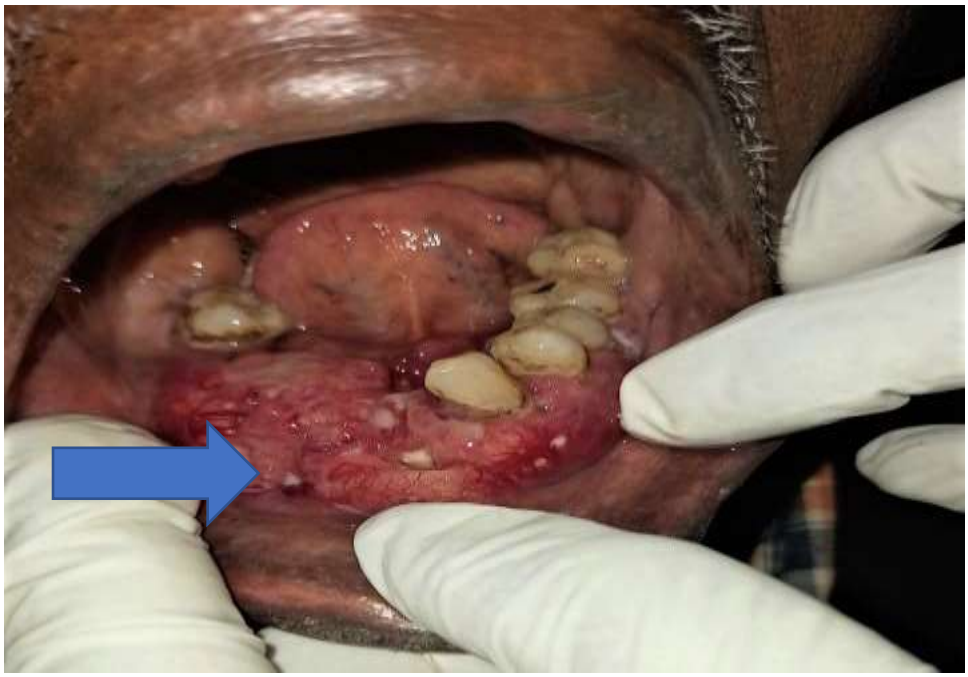


Plate 12. Squamous cell carcinoma of the mandibular gingiva

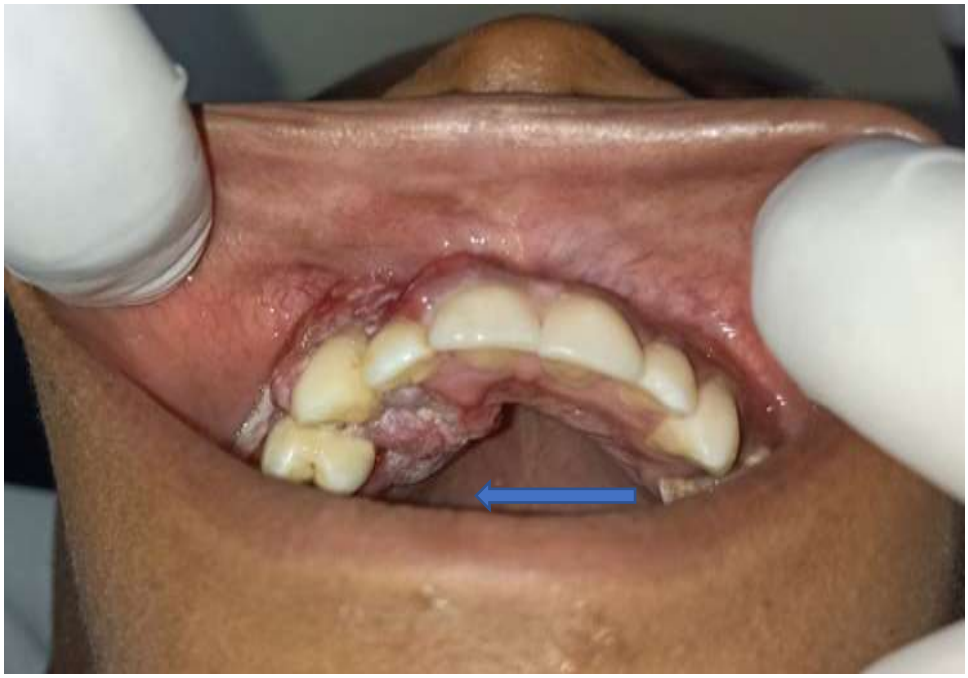


Plate 13. Squamous cell carcinoma of the maxillary gingiva

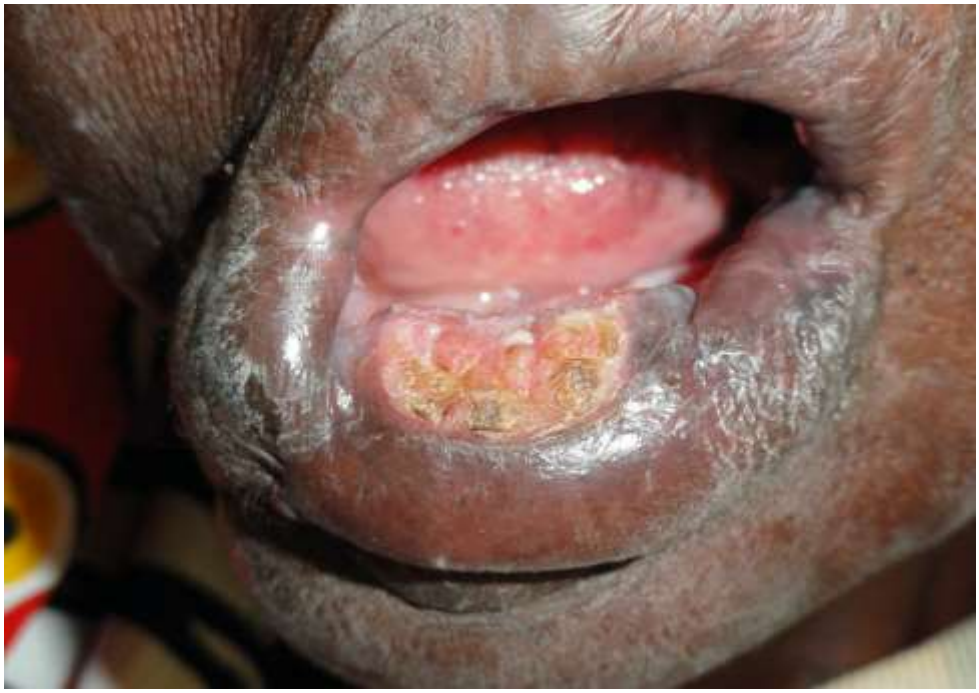


Plate 14. Recurrent squamous cell carcinoma after radiotherapy

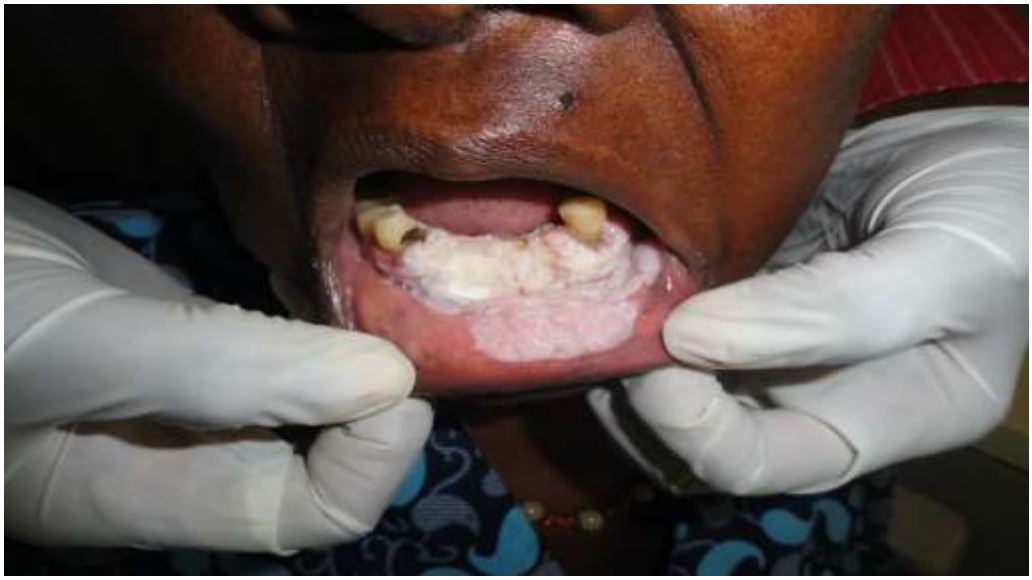


Plate 15.Squamous cell carcinoma mandibular gingiva and labial vestibule

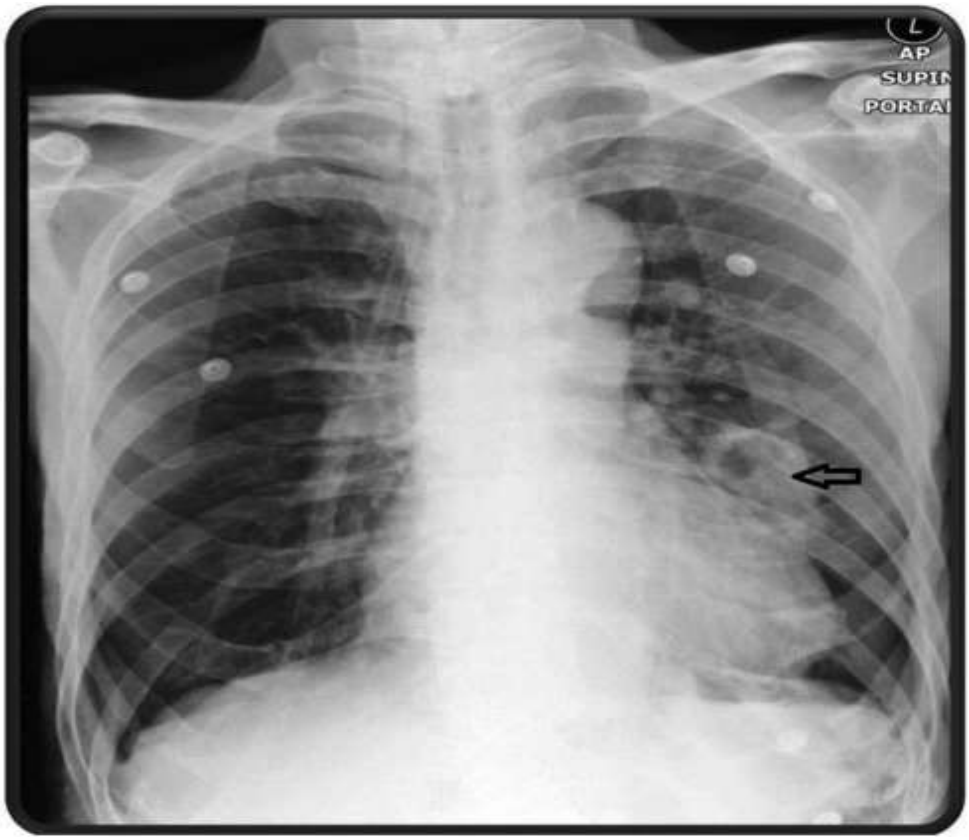
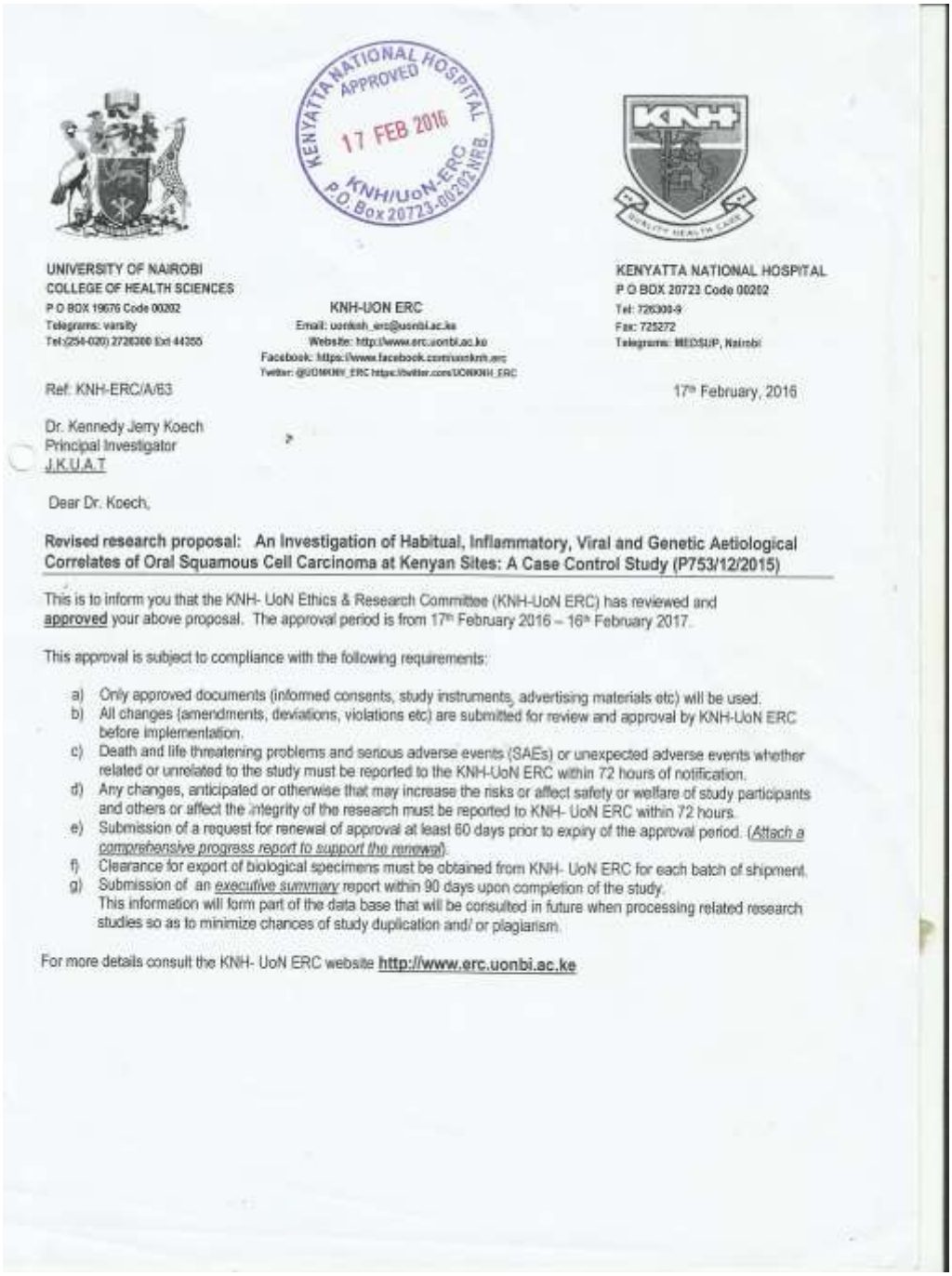


Plate 16. Metastatic squamous cell carcinoma of the lungs

Appendix II: ERC Approvals.



KENYATTA NATIONAL HOSPITAL APPROVED 17 FEB 2016 KNH/UoN-ERC P.O Box 20723-00202 NRB

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17th February, 2016

Dr. Kennedy Jerry Koech
Principal Investigator
J.K.U.A.T

Dear Dr. Koech,

Revised research proposal: An Investigation of Habitual, Inflammatory, Viral and Genetic Aetiological Correlates of Oral Squamous Cell Carcinoma at Kenyan Sites: A Case Control Study (P753/12/2015)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH-UoN ERC) has reviewed and approved your above proposal. The approval period is from 17th February 2016 – 16th February 2017.

This approval is subject to compliance with the following requirements:

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

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

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

Yours sincerely,

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

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

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Ref: KNH-ERC/ Mod&SAE217

18th August 2020

Dr. Kennedy Jerry Koech
PhD Candidate
School of Public Health
J.K.U.A.T

Dear Dr. Koech

Re: Approval of Modification- Study titled, 'An Investigation of Habitual, Inflammatory, Viral and Genetic Etiological Correlates of Oral Squamous cell carcinoma at a Kenyan site: A case control study' (P753/12/ 2015)

Your response dated 21st July 2020 refers.

Upon review, the KNH-UoN ERC has **approved** change of study title from 'An investigation of Habitual, Inflammatory, Viral and Genetic Aetiological correlates of Oral Squamous cell carcinoma at Kenya Sites: A case control study' to '**An investigation of Habitual, Inflammatory, Viral and Genetic Aetiological correlates of oral Squamous cell carcinoma at a Kenya site: A case control study**'.

Noted that these changes will not alter the study outcomes as previously approved and are therefore acceptable.

Yours sincerely,

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

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

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Ref. No.KNH/ERC/R/116

20th June 2018

Dr. Kennedy Jerry Koech
Principal Investigator
J.K.U.A.T

Dear Dr. Koech,

Re: Approval of Annual Renewal –"An Investigation of Habitual, Inflammatory, Viral and Genetic Aetiological Correlates of Oral Squamous Cell Carcinoma at Kenyan Sites; A Case Control Study (P753/12/2015)

Refer to your communication dated 6th June, 2018.

This is to acknowledge receipt of your study progress report and hereby grant you annual extension approval for ethics research protocol P753/12/2015.

The approval dates are 17th February 2018 – 16th February 2019.

This approval is subject to compliance with the following requirements:

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

g) Submission of an *executive summary* report within 90 days upon completion of the study This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

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

Yours sincerely,

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SECRETARY, KNH-UON ERC

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

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Ref. No.KNH/ERC/R/149

19th August 2020

Dr. Kennedy Jerry Koech
PhD Candidate
School of Public Health
J.K.U.A.T

Dear Dr. Koech

Re: Approval of Annual Renewal – An investigation of Habitual, Inflammatory, Viral and Genetic Etiological Correlates of Oral Squamous cell carcinoma at a Kenyan site: A case control study (P753/ 12/2015)

Refer to your communication dated 21st July 2020.

This is to acknowledge receipt of the study progress report and hereby grant annual extension of approval for ethical research protocol P753/ 12/2015.

The approval dates are 17th February 2020 – 16th February 2021.

This approval is subject to compliance with the following requirements:

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

Yours sincerely,

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

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director, CS, KNH
The Chair, KNH-UoN ERC
The Assistant Director, Health Information, KNH
Supervisors: Prof. Wallace Bulimo, Prof. Simon Karanja, Dr. Peter Wanzala

Appendix III: Publications

East African Medical Journal Vol. 98 No. 11 November 2021
SOCIODEMOGRAPHIC, CLINICAL AND PATHOLOGICAL FEATURES OF ORAL SQUAMOUS CELL CARCINOMA IN A KENYAN CENTRE
Dr Kennedy Jerry Koech. BDS, MDS(OMFS), Oral and Maxillofacial Surgeon, Kenyatta National Hospital, PO Box 19552-00202, Nairobi, Prof. Wallace Bulimo. BSc,Msc, PhD, Associate professor, Department of Biochemistry, University of Nairobi, PO Box 30197-01000,Nairobi, Prof. Simon Karanja. BVM, MSC, PhD., Associate Professor, School of Public Health, Jomo Kenyatta University of Agriculture and Technology, PO box 62000-00200, Nairobi, Dr. Peter Wanzala. BDS, MSC, PhD, Research Scientist, Centre for Public Health Research, Kenya Medical Research Institute, PO Box 54840-00200, Nairobi.

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SOCIODEMOGRAPHIC, CLINICAL AND PATHOLOGICAL FEATURES OF ORAL SQUAMOUS CELL CARCINOMA IN A KENYAN CENTRE

K. J. Koech, W. Bulimo, S. Karanja and P. Wanzala

ABSTRACT

Background: **Oral squamous cell carcinoma is the sixth most common cancer in the world and despite advances in medical care the survival trends for OSCC have not significantly improved over the years . Information about the sociodemographic, clinical and pathological presentation of OSCC in Kenya is scanty in the published literature. In order to further understand the disease this study aimed to determine the sociodemographic and clinicopathological features of OSCC in a Kenyan hospital population.**

Objectives: **To determine the sociodemographic and Clinicopathological features of Oral Squamous cell Carcinoma (OSCC) at a Kenyan Centre.**

Study design: **This was a descriptive prospective study.**

Study participants: **Patients who presented with oral squamous cell carcinoma at the Kenyatta National Hospital, Nairobi.**

Results: **Out of the 157 cases 61.8% and 38.2% were males and females respectively. Mean age of 58 Years. Majority were of cases were farmers while most the controls were in the informal sector. Pain was the most common symptom among the cases with 68.15% complaining of moderate to severe pain. Palpable submandibular and cervical lymph nodes were more common among those with lymphadenopathy. The well differentiated histological type was prevalent. Tongue was the most common site among all cases although OSCC had a predilection buccal mucosa among females.**

Conclusion: **This study reveals that OSCC is predominantly a disease of the older male, pain a major symptom and tongue the most common site. Well differentiated carcinoma was predominant.**

We recommend further studies to determine the prognostic value of these features in this population.

INTRODUCTION

Oral squamous cell carcinoma is the sixth most common cancer in the world[1]. A review by Jonson(2003) found that females were less likely to have Oral Squamous cell Carcinoma (OSCC) owing to their lower exposure to traditional risk factor of alcohol and tobacco [2]. Statistics from industrialized countries including those in the European union and North America show that men are affected two to three times more than women [2]. Regarding age distribution, the incidence of OSCC is reported to be significantly higher among those above the age of 45 years and has continued to increase in developed countries where the life expectancy has been rising[2].

Symptoms of OSCC are variable and depend on the location and extent of the primary tumour, ranging from non-healing ulcers with varying degrees of pain, an exophytic lesion with several contiguous loose teeth, a non-healing extraction socket to excessive salivation with reduced mobility of the tongue and severe pain. Halitosis secondary to a fungating necrotic tumour is present in some patients with massive lesions [3].Tongue remains the most common site for intraoral cancer between European and the US populations, amounting to 40–50% of oral cancers. Buccal cancer is more common among Asian populations due to betel quid/tobacco chewing habits. In Sri Lanka, 40% of oral cavity cancers are found on the buccal mucosa[4]. In contrast, a study from south Africa found that tongue was the most common site among blacks followed by the floor of the mouth[4].

It is estimated that the current and future estimated burden of OSCC is shifting to the less developed countries which are poorly equipped to handle the increasing burden [5]. Regarding prognosis, the survival trends for OSCC has not significantly improved over the years and according to a study by Chen (2018), the average 5-year survival for all oral sites was 51.3% with females having a slightly better outlook than males[6]. As such data on; sociodemographic, clinical and pathological presentation of OSCC in Kenya will further understand the disease in a Kenyan hospital population.

MATERIALS AND METHODS

Following approved by the KNH/UON ERC (approval number P753/12/2015) this prospective clinical study was carried out at the department of oral and maxillofacial surgery of the Kenyatta national hospital in Nairobi, Kenya over a 1-year period. Sequential sampling method was used to select the participants from among patients seeking treatment in the department. Those with confirmed histopathology diagnosis of Squamous cell carcinoma and who consented to participate in the study were assessed. Sociodemographic information was obtained from the patients including age, gender and occupation. In addition, the symptoms of pain were measured using the numerical rating scale (NRS) with ranges of 1 to 10 representing none to excruciating pain and recorded. Recent unintentional weight loss as noted from poorly fitting clothes or actual reported reduction in weight was recorded. Additionally, body mass index (BMI) was calculated after measuring the weights in kilograms and heights in metres. Thereafter a clinical examination was carried out to determine the sites and sizes of the lesions as well as cervical lymph node status. In addition, their previous histopathological diagnosis were reviewed by one pathologist and those that were equivocal subjected to immunohistochemistry. Thereafter, the patients underwent chest radiographic examination to identify any pulmonary involvement. The data was inputted into SPSS version 25 (IBM Corp, Armonk, NY, USA) for analysis. Descriptive statistics were used to determine frequencies while continuous variables were analyzed for means and standards deviations.

Independent sample t-tests were used to compare means while categorical variables were analyzed using chi-square tests for differences, homogeneity or associations. The levels of significance was determined at P values of less than 0.05 at 95%confidence intervals. The data has been presented using narratives and tables.

RESULTS

Out of the 157 patients there were 97 (61.8%) males and 60(38.2%) females with age range of 28 to 96 years (mean age = 58, SD 13.2 years). There was no significant deference between the mean ages of the males and females.

Majority of the patients 78(48.4%) were farmers while 74 (47%) were in the informal sector in the towns ranging from small scale traders to casual workers. The rest were employed in the formal sector. A higher percentage of females (68%) than males (36%) were engaged in farming of vegetables. Among the farmers majority 45 (65.2%) used chemical pesticide on their crops while 22(31%) used chemical fertilizer. Table 1 shows the sociodemographic presentation of the participants.

Table 1

Sociodemographic features of the participants

	Male (%)	Female (%)	Total(%)
Mean age (years)	56.5	60	58
Gender	61.8	38.2	
Occupation			
Farmer	36	68.3	48.4
Formal	5.2	33.3	47
Informal	58.8	28.3	4.6

Among the patients 38.2%(n=60) had the tongue involved, followed by buccal mucosa 41(26.1%), mandibular gingiva 21(13.4%) and maxilla 10(6.4%). While the tongue was the most common site among males at 42.3%, OSCC showed a higher predilection for buccal mucosa among females at 36.7%. These differences were however not statistically significant. All the cases presented with ulcerated lesions, which had a mean longest diameter of 4.6cm and a median of 5cm. There was no statistically significant difference in ulcer sizes between males and females. Figure 1 shows the distribution of the patients according to sites of the lesions.

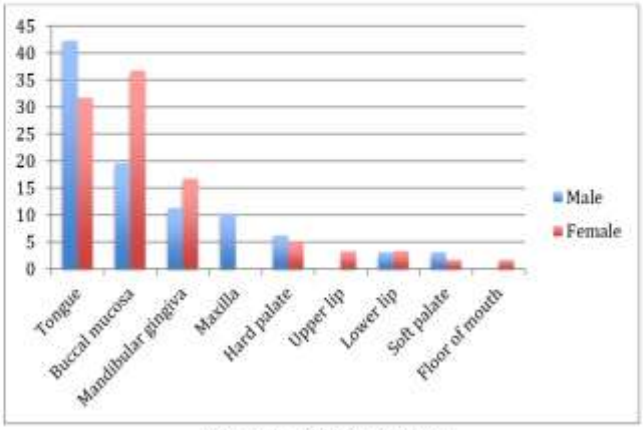

Fig.1. Site predilection of OSCC

In this study most of the patients (93.4%, n=147) presented with symptoms of pain with severities ranging from 1 to 10 on the numerical rating scale (mean=5) with 68.15% complaining of moderate to severe pain of 5 and above. Other symptoms included odynophagia (29.3%(n=46)) and weight loss [56.1%(n=88] with 28.2% being cachexic (BMI less than 18.5kg/M^2). In addition, 30.6%(n=48) had clinical involvement of cervical lymph nodes. The submandibular lymph nodes were more clinically palpable (21.3%, n=42) with sizes ranging from 2cm to 6cm. These were followed by level 2 nodes (15.3%, n=23) with most of the nodes not exceeding 3cm, submental nodes (9.6%, n=5) and level 3 nodes.

During the study period 30 patients underwent surgery including neck dissection and out of these, 50% were found to have lymph nodes that were positive for OSCC on histopathology. There was no significant relationship between the sites and size of the primary lesions with the lymph node status. Chest X-rays were done as part of the investigations and 4.5% of the patients had chest metastasis. The distribution of the patients according to symptoms and cervical lymph node involvement is depicted in table 2.

Table 2

Distribution of participants according to symptoms and cervical node involvement

	Male (%)	Female (%)	Total(%)
Nodal involvement			
Level 1	22.6	30	30.9
Level 2	18.5	18.4	15.3
Level 3	5.2	3.3	3.7
Chest metastasis			4.5
Pain>5			68.5
Weight loss (self reported)			56.1

Histopathology results showed that majority of the cases (73.2%) had well differentiated squamous cell carcinoma while 22.9% had moderately differentiated squamous cell carcinoma. The rest (4.9%) had poorly differentiated squamous cell carcinoma. Figure 2 shows the site predilection of OSCC.

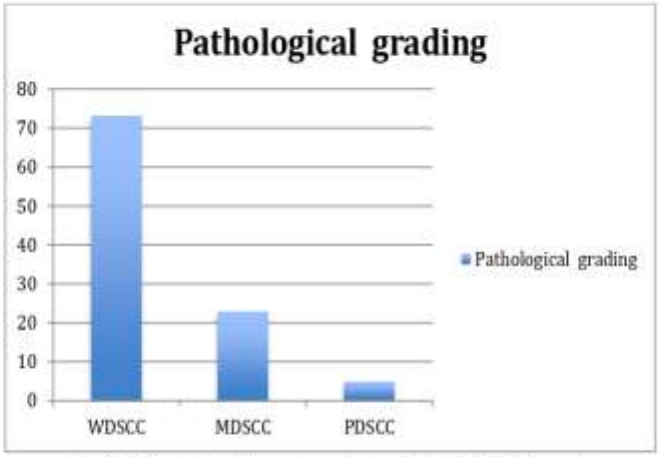

Fig.2. Distribution of the cases according to pathological staging.

DISCUSSION

Generally, OSCC is disease that predominantly affects older males. This study determined that there were more males than females, whose mean ages was 58 years in agreement with global literature which generally report almost twice as much OSCC in males than in females [7]. In addition, a large multicenter study placed the peak age of presentation at 50-59 years but with a significantly lower age among the Asian patients than European [8]

These observations are possibly due to factors including higher exposure of males to the suspected risk factors including tobacco and alcohol [7] and the age associated vulnerability to cancer[9]. However, there are also possibilities of genetic vulnerability as seen in studies from Thailand and Malaysia, neighboring countries which share some cultural habits showed a slightly higher female to female ratio[10, 11] .Among the patients slightly more (48%) were farmers while 47% were in the informal sector. This finding seemed to suggest that farming might have occupational risk factors, including the use of toxic chemicals. In the published literature there is paucity of studies that implicate farming in the disease. One study from the Nordic countries only associated lip cancer to prolonged solar exposure among farmers[12]. In addition, the current study found that among the farmers there were more females (68%) than males (36%) who engaged in vegetable farming and that the majority (65.2%) used chemical pesticides while the rest used only fertilizer. Although Chemical pesticides were not investigated as risk factors in this study it is possible that some of the chemicals might have carcinogenic properties. An in vitro toxicological study on Cypermethrin, a common type-II pyrethroid pesticide demonstrated cytotoxicity of macrophages [13].This pesticide might also be used by our farmers and would require further investigations. In addition, studies should be carried out in order to determine the possible

carcinogenic properties of the different chemical pesticides.

Depending on site and stage of the disease the symptoms of OSCC are varied including non-healing ulcers and varying degrees of pain[3]. In this study 93.4% of the cases complained of pain with a mean severity of 5 (range 1-10) on the numerical rating scale, associated with a non-healing ulcer in the mouth. Pain is a subjective symptom which has measured using several methods including visual analogue scale (VAS), numerical rating scale (NRS) and verbal rating scale (VRS)[14]. In this study we chose the NRS due to its simplicity and the fact that it is easy to administer. This is supported by Hjermstad et al. who reported that compared to VAS and VRS, NRS is widely preferred due to its ease of use and good applicability [15].

Pain has been reported in all stage of OSCC, from precancerous to advanced disease with no correlation between the size of the tumour and intensity of the pain[16].An extensive systematic review found that up to 50.7% of cancer patients have pain ranging from moderate to severe in all stages of the disease[17]. The fact that most cases in the current study had pain is possibly a reflection of the advanced stage of the disease at presentation, which on average was T4. It is suggested that OSCC pain is caused by the secretion of mediators into the cancer microenvironment including Endothelin-1(ET-1), proteases and nerve growth factor (NGF). Significantly, it has been shown that patients with OSCC have high levels of ET-1 in the cancer microenvironment. It is theorized that Proteases activate cell surface receptors on afferent nerves while NGF is secreted to promote local growth and survival of nociceptive nerves[18]. In addition, NGF has also been shown to regulate body weight through lipid and glucose metabolism as well as feeding behaviour and may induce cachexia[19]. Not surprisingly, 56% of our patients reported weight loss and 28.2% could be described as being cachexic with BMI of less than 18.5kg/M^2. This finding is close to previously reported prevalence of up to 46% of weight loss in head and neck squamous cell carcinoma [20].

Regarding site predilection the tongue was the most commonly affected site, in agreement with other reports[21-23]. The explanation for propensity of OSCC to involve the tongue is not known but one can only speculate that pooling of toxins in the floor of the mouth and trauma from the teeth might be responsible. However, findings from other studies that mandibular gingiva is the most common site puts to question the role of dental friction [10, 24].

Most of our patients presented to the hospital late with a mean ulcer diameter of 4.6cm, which by TNM stage puts the lesions at T4. This delay in seeking care can be explained by several factors including age, socio-economic conditions and gender of the patients which have been identified as contributing towards [25]. It has also been reported that many patients are not aware of cancer while others thought that it was a minor condition that would resolve on its own[25, 26].These factors might also be responsible in our population.

The OSCC metastasizes mainly through lymphatic channels and involvement of the cervical lymph nodes can occur early in the disease[27]. Results of this study show that 30.6% of the cases had clinically positive cervical lymph nodes detected in Levels 1-3, which have also been described as the most commonly involved lymphatic sites in OSCC [28, 29]. The spread to the lymph nodes is confirmed through histopathological examination following neck dissection and the positive rate has been found to vary widely depending on the stage of the disease [27, 30, 31]. Out of all the cases in our study 30 patients underwent surgery including neck dissection and 50% had positive cervical lymph nodes at histopathology, which had no relationship with site of OSCC. This

finding demonstrates the need to consider each site as having the potential for lymph node spread.

Compared to other sites in the body the incidence of distant metastasis in head and neck squamous cell carcinoma is relatively small, with pulmonary spread accounting for up to 66 % of the metastasis[32]. Although there was no attempt to look for all distant metastasis the current study established that 4.5% of the cases had chest radiographic features that were suggestive of metastasis. This was close to the findings of Hazegawa et al [33] in a case series which established that 80% of the 6.7% of the OSCC patients with distant metastasis had pulmonary involvement. In the same study, there was a relationship between the stage of the disease as well as the histological grading of the tumour and the risk of pulmonary spread.

Turning to pathological grading of the tumours, majority of our cases (73.2%) had well-differentiated tumors, compared to moderately differentiated and poorly-differentiated which were reported in 22.9% and 4.9% respectively. These findings disagreed with other studies which have reported higher prevalence of moderately and poorly differentiated and tumors[34, 35], differences could be due several factors including genetic variations and environmental factors. These studies have also described the positive relationship between the pathological grade of the tumour, recurrence and cancer specific survival, with poorly differentiated and moderately differentiated tumors having worse prognosis than well differentiated counterparts.

CONCLUSION

This study has shown that OSCC predominantly affects the older men more than women. In addition, farming where women are more involved seems to be associated with and the disease and would require more investigations. We have also established that our patients generally present late with advanced tumours and that pain and weight loss are common features in the disease. Although the tongue was the most commonly involved site it did not have a relationship with the spread of the disease to cervical lymph nodes and thus every site should be considered important.

We recommend that further studies should be done to determine the possible risks posed by farming chemicals and the prognostic significance of the different pathological grades of the disease in our population.

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THE RELATIONSHIP BETWEEN C- REACTIVE PROTEIN AND ORAL SQUAMOUS CELL CARCINOMA IN A KENYAN CENTRE: A CASE CONTROL STUDY

K. J. Koech, W. Bulimo, S. Karanja and P. Wanzala

ABSTRACT

Background: **Oral squamous cell carcinoma (OSCC) is the sixth most common cancer in the world with several aetiological factors including inflammation having been associated with the disease. Inflammatory markers including interleukin 6(IL-6) and C-reactive protein (CRP), have been shown to be elevated in OSCC and wide geographical variations have been reported. Information about the association between CRP and OSCC in Kenya is scanty in the published literature and therefore this study aimed to determine the relationship between the disease and CRP in a Kenyan population.**

Objectives: **To determine the relationship between C-reactive protein and Oral Squamous cell carcinoma in a Kenyan centre.**

Study design: **This was a case control study**

Study participants: **Cases were patients who presented to the Kenyatta National Hospital with OSCC while age and gender matched controls were patients in the same hospital who did not have the disease.**

Results: **Of the 157 cases 61.8% and 38.2% were males and females respectively, with mean age of 58 Years (SD 13.2 years). The mean CRP levels were 10.55mg/l and 20.92mg/l for cases and controls respectively and this difference was significant (critical ratio 3.91, $p<0.01$). After setting up different cut-off points of 3mg/l, 5mg/l and 10mg/l patients with OSCC had a significant likelihood of having CRP levels of greater than 5mg/l (OR=19.98, 95% CI 6.65-60.05)**

Conclusion: **This study reveals that patients with OSCC are more likely to have systemic inflammation resulting in production of C-reactive proteins. Assessment of CRP would be valuable in the management of this disease**

INTRODUCTION

Oral squamous cell carcinoma is the sixth most common cancer in the world and affects more males than females [1]. A review by Jonson(2003) found that almost males were twice more likely than females to have as Oral Squamous cell Carcinoma (OSCC) possible due to exposure of traditional risk factors including tobacco and alcohol, and that the incidence of OSCC is significantly higher among those above the age of 45 years [2]. Aetiology of OSCC is linked to several factors including inflammation[3] with the observation that inflammatory agents including viruses , bacteria and chemicals will in some cases elicit a prolonged low-grade immune response that in the case of infections does not result in the clearance of the pathogens, but to a state of continuous low-grade inflammation[4]. This state of chronic inflammation may predispose diversity of diseases, including malignant neoplasia [5]. According to the WHO, associated bacterial infections cause up to 20% of cancer deaths in low- and middle-income countries and 9% of cancer deaths in high-income countries, and chronic inflammation constitutes an important component of these infections (http://www.who.int/mediacentre/factsheets/fs297/en/) [6].

At present clinico-pathological role of Interleukin 6(IL-6) is not clear however , a pro-inflammatory cytokine, is consistently found to be elevated in patients with OSCC [7]. This cytokine is produced at inflammatory sites and secreted into the blood, thereby initiating systemic inflammatory reaction and production by hepatocytes of acute phase proteins including C-reactive protein (CRP), fibrinogen and a1-antitrypsin[3]. In a case control study done using an ELISA method to measure IL-6 levels in patients with OSCC Chang and colleagues found statistically higher serum levels of IL-6 in patients with OSCC tumours compared with those of healthy controls and among those with the oral premalignant lesions. They also found that the higher IL-6 serum levels were associated with higher T status, overall stage, and deeper tumour depth and bone invasion [8].

The CRP is a classical acute-phase protein which displays a rapid and pronounced rise of its plasma concentration in response to acute inflammation, infection, and tissue damage [3]. Genetic and environmental factors both have an influence on an individual's basal CRP concentration, with circulating levels varying from 0.1 to 10 mg/l in apparently healthy people [9, 10]. Increased CRP concentrations have been reported in many diseases, including cardiovascular diseases, type 2 diabetes, arthritis and many types of cancers [9, 11]. As a prognostic biomarker CRP has been found to predict recurrence, tumour destruction including bone invasion, lymph node metastasis as well as overall survival[12, 13]. When measured by high-sensitivity assays CRP has been described as not just a marker of prevalent cancer but also a predictor of increased risk of cancer in apparently healthy individuals [14-16].

Several possible mechanisms could explain the association between circulating levels of CRP and cancer. The first one is causality, where elevated levels of CRP could play a causal role in development of cancer. The second mechanism is reverse causality where existing cancer causes elevated levels of CRP and thirdly a confounder causes both elevated CRP and the risk of cancer. The link between chronic inflammation and cancer appears to be bidirectional: inflammation can precede and promote tumour development and progression, but tumour development and progression can also induce inflammation through elaboration of various cytokines. Therapies, including non-steroidal anti-inflammatory drugs (NSAIDS) may thus have a role to play in reducing progression of tumours and the associated symptoms [17].

There is paucity of published data linking inflammation with OSCC in Kenya and therefore this study aimed to determine the relationship between CRP as an inflammatory marker and the disease and to generate baseline data that can be used for future research.

MATERIALS AND METHODS

This study was approved by the KNH-UON ERC (approval number P753/12/2015) and consents obtained from the participants. Over a 1-year period 157 confirmed cases of OSCC were prospectively recruited at the department of Oral and Maxillofacial Surgery of the Kenyatta National Hospital in Nairobi, Kenya. Demographic characteristics of the patients including age and gender were recorded. Clinical and pathologic data including oral sites of cancer, cervical lymph nodes involvements and the histological types of the disease were recorded. Thereafter, blood samples were collected for CRP serum. Cases who had previously been treated for OSCC were excluded from the study. Age and gender matched controls were recruited from among patients who were undergoing haematological tests for other conditions not related to OSCC at the same hospital. Blood samples were taken, and the serum used to determine CRP levels using an automatic biochemistry machine, BiOLiS 50i superior® (Tokyo Boeki Medisys Inc) and the results reported in mg/l.

The data was inputted into SPSS version 25 (IBM Corp, Armonk, NY, USA) and analysed. Independent sample t-tests was used to analyse continuous variables while Chi-square statistics determined the relationship between the categorical data. In addition, Logistical regression was used to determine the Odds ratios. Statistical significance was set at p values of 0.5 at 95% confidence intervals. The data has been presented in narratives and tables.

RESULTS

Of the 157 cases there were 97 (61.8%) males and 60(38.2%) females and age range of 28 to 96 years (mean age = 58 years, SD 13.2 years). There was no significant deference between the mean ages of the males and females.

The mean and median CRP measurements for the cases were 10.55mg/l and 5.8mg/l respectively. On the hand the controls had a mean of 20.92mg/l and a median of 8.2 mg/l. There were no differences of CRP levels between male and female subjects. Considering that the parameters were continuous, cut off points of 3mg/l, 5mg/l and 10mg/l were used in order to compare cases against those of controls, with odds ratios of 1.09, 19.98 and 0.022 being determined (Table 1). Among the cases most (93.4%, n= 147) presented with symptoms of pain with severities ranging from 1 to 10 on the numerical rating scale (mean=5) with 68.15% complaining of moderate to severe pain of 5 and above. The submandibular lymph nodes were the most clinically palpable (21.3%, n=42) with sizes ranging from 2cm to 6cm. These were followed by level 2 nodes (15.3%, n=23) with most of the nodes not exceeding 3cm, submental nodes (9.6%, n=5) and level 3 nodes.

Regarding pathology, histopathology results showed that majority of the cases (73.2%) had well differentiated squamous cell carcinoma while 22.9% had moderately differentiated squamous cell carcinoma. The rest (4.9%) had poorly differentiated squamous cell carcinoma .There was no significant relationship between the CRP levels and cervical lymph node status ($X2=0.634, p=0.426$), severity of pain($X^2=4.5$, $p=0.03$) , site ($X^2=6.05, p=0.735$) and histopathological types; well differentiated ($X^2=0.56, p=0.75$), moderately differentiated ($X^2=0.22$, $p=0.64$) and poorly differentiated ($X^2= 0.215, p=0.64$) [table 2] .

Table 1

Association between CRP Levels and OSCC

CRP level(mg/l)	Odds Ratio	Std. err. z	P>z	95%	CI
CRP>3	1.09	0.29	0.33	0.738	0.64 1.84
CRP>5	19.98	11.21	5.33	0.0	6.65 60.05
CRP>10	0.022	0.016	-5.08	0.00	0.005 0.096

Table 2

Relationship between Clinicopathological features and CRP levels

	CRP>5 mg/l	**CRP<5mg/l**	**X^2**	**P**
Pain score >5 (%)	49.7	17.2	4.5	0.03
Lymph Nodes(%)	25.4	5.7	0.63	0.426
WDSCC (%)	56.7	17.2	0.56	0.755
MDSCC (%)	18.5	4.5	0.22	0.64
PDSCC (%)	1.9	1.2	0.22	0.64

DISCUSSION

In general, OSCC is a disease that predominantly affects older males, as was observed in this study which determined that there were more males than females, whose mean ages was 58 years. This was in agreement with global literature which generally report almost twice as much OSCC in males than in females [18]. In addition, a large multicentre study placed the peak age of presentation at 50-59 years but with a significantly lower age among the Asian patients than European [19], underscoring the fact that there are wide geographical variations in the epidemiology of the disease. These observations are possibly due to factors including higher exposure of males to the suspected risk factors including tobacco and alcohol [18] and the age associated vulnerability to cancer[20]. However, there are also possibilities of genetic vulnerability as seen in studies from Thailand and Malaysia, neighbouring countries which share some cultural habits showed a slightly higher female to female ratio[21, 22].

Regarding inflammation as an aetiologic factor, two theories namely induction and response hypothesis could be associated with increased inflammation in cancer. The induction hypothesis suggests that chronic inflammation results in excessive cell proliferation and activation of a cascade of cellular actions, leading to induction of irreversible DNA damage. On the other hand the response hypothesis states that the immune response of the host is a consequence of tumor growth itself and could be the reason for the elevation in levels of the inflammation marker CRP [17]. Although the role of CRP in OSCC is controversial some case series have demonstrated the prognostic value of the cytokine in OSCC[23-26]. In the current study the means and medians CRP for cases were 10.55mg/l and 5.8mg respectively while those of controls were 20.92mg/l and 8.2mg/l. The higher mean CRP levels of the controls was due to the fact this were hospital patients suffering from different diseases which also cause elevated CRP, as was explained by Berkson (1946) who observed that diseases that are independent in the population might share exposure factors in hospital based case control studies[27]. One study from Taiwan reported mean CRP levels of 5.9mg/l to 8.37mg/l in various oral sites of OSCC and also found that levels above 5mg/l had a

positive correlation with advanced clinicopathological stage of the disease [28]. For purposes of calculating the odds ratios CRP cut off points of 3mg/l, 5mg/l and 10mg/l were used with odds ratios of 4.96, 2.66 and 0.3 respectively being obtained. After comparing the cases and hospital-based controls the current study established that the odds of having a CRP level above 3mg/l and 5mg/l given disease was 4.96 and 2.66 times respectively, suggesting an association between CRP and OSCC. In this study 93.4% of the cases complained of pain with a mean severity of 5 (range 1-10) on the numerical rating scale, associated with a non-healing ulcer in the mouth. Pain has been reported in all stage of OSCC, from precancerous to advanced disease with no correlation between the size of the tumour and intensity of the pain[29].An extensive systematic review found that up to 50.7% of cancer patients have pain ranging from moderate to severe in all stages of the disease[29]. We found a positive relationship between moderate to severe pain and CRP levels above 5mg/l, indicating that pain is an important subjective marker of inflammation. This was in concurrence with a study by Laird et al (2010) involving a cohort of 718 cancer patients which demonstrated a strong correlation between pain and systemic inflammation[30]. In our study the other clinicopathological features including site and pathological grade were not significantly associated with the elevated inflammatory marker, possibly because of similar immune responses. Arising from these findings it can be postulated that OSCC might result from inflammation that is sufficient enough to cause elevated levels of CRP. On the other hand, OSCC might result in an elevation of the inflammatory marker and that inflammation is the driver of tumour growth.

CONCLUSION

This study has demonstrated that there is a link between inflammation and OSCC. In addition, the current study established that pain is associated with inflammation. The assessment of CRP serum levels is recommended in the management of OSCC.

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Appendix IV: Data Collection and Consent Forms

Data collection form for cases

Date;……..

Personal details

Record Number……

Age……Sex……..

Weight ……..Height……

Occupation……

Area of current residence……

Economic activities in the area.

1. Farming Activities
    a. Type of crops…….
    b. Chemicals used……

Habits.

1. Alcohol; Yes……. No…….
2. Cigarrete……
3. Chewed Tobacco……
4. Snuff……
5. Khat……

Symptoms.

1. Pain……..Score……..
2. Wound……Duration……
3. Swelling…….Duration……..

4. Odynophagia…… Duration……
5. Weight Loss; Yes……No……

Clinical findings.

1. Ulcer…… Location……
2. Mass……..Location……..
3. Mobile Teeth……..Location……..
4. Size Of Ulcer……
5. Induration; Yes……..No……
6. Tongue Mobile; Yes…….No……..
7. Foul Smell; Yes……..No……..
8. Wasting; Yes…….No…….

Status of lymphnodes.

1. Submental; yes……..no……
   Bilateral; yes……..no…….
2. Submandibular; yes……..no…….
   Bilateral; yes…….no……
3. Level 2; yes……size……..no……
   bilateral; yes……no……..
4. Level 3; yes……size……..no……
   Bilateral; yes……no……..
5. Level 4; yes……size…… .no……
   Bilateral; yes……no…….
6. Level 5; yes…… size…….no……
   Bilateral; yes…….no……
7. Level 6;yes……size…...no…….
   Bilateral ; yes……no…….

Chest lesions on chest radiograph.

1. Metastasis; yes……no……

2. Pleural effusions; yes…….no……

Histopathological findings.

1. Well differentiated…….
2. Moderately differentiated……
3. Poorly differentiated……..
4. Undifferentiated…….

Lymphnodes histopathology

Positive…….negative…….

Molecular studies.

1.Biopsy; P53 Mutation; present……....absent……

variants…….

Notch1; present…….absent……..

Variants…….

2.Oral brushes;

P53 Mutation; present……absent……...

Variants…….

Notch1mutation ; present……..absent……..

Variants……..

HPV studies

Biopsy material.

1. Present……..absent…….

2. Subtype……quantities……

Swabs.

1. Present……..absent…….
2. Subtype……quantities……

Biochemistry studies.

CRP levels……

Data collection form for controls

Date;…….

Personal details

Record Number……

Age……Sex……..

Weight ……..Height……

Occupation……

Area of current residence……

Economic activities in the area.

3. Farming activities
    c. Type of crops…….
    d. Chemicals used……

Habits.

6. Alcohol; Yes……No……..
7. Cigarrete……..Duration……..

8. Chewed Tobacco……Duration……
9. Snuff…… Duration……..
10. Khat…….Duration……

Clinical details.

Diagnosis of illness……..

Duration of illness…….

Molecular studies from oral brushes.

P53 Mutation; present……absent……...

Variants……..

Notch1; present……..absent……..

HPV studies from swabs.

4. Present……..absent……
5. Subtype……quantities……

CRP levels……..

Consent information

Consent information for cases

Introduction and purpose

I am Dr Kennedy Koech, a PHD student at the Jomo Kenyatta University of Agriculture and Technology, department of Public Health. I am inviting you to participate in a research that I am conducting whose purpose is to study **Pattern of presentation and correlates of squamous cell carcinoma in selected sites in kenya**. The disease is a form of cancer, which affects parts of the mouth and is generally known to be the 8th most common cancer in the world with incompletely

understood cause. Although there are studies that have been done on the subject in other parts of the world very few have been carried out in Africa and particularly In Kenya. . There is reason to believe that our data would be different due to our genetic and other environmental factors.

Benefits

1. The study will not be of immediate benefit to you but in the long run the findings may be used to formulate policies and treatments for patients with similar illnesses.
2. Participating in the study will not result in any financial benefit and neither will you incur any extra financial cost.
3. Some results of the investigations done on you will be used in your treatment.
4. The questions that you will be asked and the subsequent examination is part of routine diagnosis of your disease.
5. Some of the specimen that will be obtained including blood and biopsy forms part of your routine investigations. However, additional tests will be carried out on the samples as part of this study.

Inconvenience, risks and right of withdrawal.

1. Your involvement in the study is purely voluntary and you are under no obligation to participate and you are free to withdraw at any stage..
2. There is no extra danger or risk associated with participating in the study.
3. All standard precautions will be taken while taking your specimen and discomfort will be minimized as much as possible.
4. Your refusal to participate of withdrawal from the study will not in any way affect the quality of the treatment offered to you.

Type and amounts of specimen

1. Swabs will be obtained from the lesion using a special brush.
2. Approximately 1cm of Tissue will be obtained from the site of the disease via an incisional biopsy procedure. The specimen will be obtained under local

anaesthetic. There will be small discomfort during injection of the anaesthetic and the area will be sore for a few days. To minimize the soreness you shall be put on medications as part of the standard protocol .The specimen will be used to make a diagnosis of the disease and to carry out other tests as part of the study. The reports will be shared with your regular caregivers so that addition specimen will not be required for the same diagnosis.

3. Approximately 6 ml of blood will be obtained by laboratory staff using a sterile needle and syringe from your veins. The sample will be used for your routine tests and also for part of the study. Importantly, No extra blood will be taken because of the study. You will experience small discomfort from the needle and the site will be sore for a few days.

No samples will be taken out of the country and where the specimen will be stored for further analysis after the study permission shall be sought from the ethics and standards committee.

Duration

The interview and examination will be carried out by a qualified doctor and will take approximately 30 minutes. The biopsy procedure will take 30 minutes while drawing of blood samples, which will be done by laboratory staff will take another 10 minutes.

Statement of confidentiality.

- You will be assigned a serial number that will be used throughout the study and your name will not used. Only the investigators will know your identity.
- Where photo graphs of the lesions will be taken your identity will be completely hidden
- Like all other scientific studies the findings will be shared with other scholars. We may therefore publish the findings in journals or present them in scientific meetings. In any case, no information that can identify you will be shared.

Tafsiri kwa Kiswahili

Habari kuhusu utafiti huu

Utangulizi na madhumuni

Mimi ni Dr Kennedy Koech, mwanafunzi wa PhD katika Chuo Kikuu cha Jomo Kenyatta cha Kilimo na Teknolojia, idara ya Afya ya Umma. Nakukaribisha kushiriki katika utafiti ambayo nina fanya yenye lengo la kujifunza kuhusu saratani ya mdomo. Ingawa kuna tafiti zimefanyika juu ya somo katika maeneo mengine ya dunia chache sana yamefanywa katika Afrika na hasa hapa Kenya. . Kuna sababu ya kuamini kwamba takwimu zetu itakuwa tofauti kutokana na sababu maumbile yetu na mengine ya mazingira.

Faida

1. Utafiti haitakuwa ya faida kwakao kwa wakati huu lakini katika muda mrefu matokeo inaweza kutumika kuunda sera na matibabu ya wagonjwa weneye magonjwa hayo.

2. Kushiriki katika utafiti haitasababisha faida yoyote ya kifedha na wala wewe hautingia kwenya gharama yoyote ya ziada ya fedha.

3. Baadhi ya matokeo ya uchunguzi yata tumika katika matibabu yako.

4. Maswali yenye utaulizwa na uchunguzi baadae ni sehemu ya utambuzi wa kawaida mara kwa mara katika ugonjwa wako.

5. vipande via vya mwili wako itakayo tolewa itakuwa sehemu ya uchunguzi kawaida yako. Hata hivyo, vipimo vya ziada utafanywa kwenye tishu kama sehemu ya utafiti huu.

Usumbufu, hatari na haki ya kujitoa.

1. Kuhusika kwako katika utafiti ni kwa hiari na wewe ni hauna wajibu kushiriki na wewe ni uko na haki wa kujiondoa katika hatua yoyote ..

2. Hakuna hatari au madhara zinazohusiana na kushiriki katika utafiti.

3.Tahadhari za kawaida zitachukuliwa wakati kuchukua vipande vya nyama yako na usumbufu itapunguzwa kadri iwezekanavyo.

4. kukataa kwako kushiriki au kujitoa kutoka utafiti hakut athiri ubora wa matibabu inayotolewa na wewe kwa njia yoyote.

Aina na kiasi cha nyama na damu

1. utaulizwa kutema kiasi kidogo ya mate kwenye chupa.
2. Miosho ya mdomo itatolewa kwa kutumia aina ya mswaki. Utasikia vile anavyosikia ukitumia mswaki wa meno kwenye ulimi wako
3. Kadiri 1cm ya nyama itatolewa kutoka sehemu ya ugonjwa kupitia biopsy . hiyo nyama itatolewa baada ya kudungwa dawa ya kugandisha (anesthetic). Kutakuwa na uchungu ndogo wakati wa sindano ya anesthetic na eneo itakuwa na maumivu kwa siku chache. Ili kupunguza maumivu kwa kidonda utapewa dawa inoyotumika kwa kawaida. Hiyo nyama itatumika kufanya uchunguzi wa ugonjwa na kutekeleza vipimo vingine kama sehemu ya utafiti.
4. Takriban 6 ml damu itachukuliwa kwa kutumia sindano kutoka mishipa yako. sampuli zitatumika kama sehemu ya utafiti na pia kwa uchunguzi huu. Damu zadi haita chukuliwa kwa ajili ya utafiti huu. Kutakuwa na uchungu kidogo ndogo kutokana na sindano na sehemu ya kutolewa dama itakuwa na maumivu chache .

Hakuna sampuli zitachukuliwa nje ya nchi na ambapo vipaned vya nyama au damu itahifadhiwa kwa uchambuzi zaidi baada ya utafiti huu ruhusa utatafutwa kutoka kwa kamati ya utafiti.

Muda wa uchunguzi

Mahojiano na uchunguzi utafanywa na daktari waliohitimu na itachukua takriban dakika 30.

Kutowa nyama na sampuli ya damu itachukua dakika 30.

Taarifa ya usiri.

• Utapewa nambari yenya itatumika katika utafiti na jina lako halitatumika. wapelelezi peke yao watajua utambulisho wako.

• Pale ambapo picha yaugonjwa zitachukuliwa utambulisho wako itakuwa kabisa siri

• Kama masomo mengine yote ya kisayansi matokeo itakuwa itaongelewa na wasomi wengine. Tunaweza kwa hiyo kuchapisha matokeo ya utafiti katika majarida au kuyawasilisha katika mikutano ya kisayansi. Katika hali yoyote, hakuna unaweza kutambulika. ukiwa na jambo lolote ama maswali yeyote kuhisiana na utafiti huu tafadhali wasiliana na wafuatao;

1. Dr Kennedy Koech. Tel. 0722788251

2. Dr Peter Wanzala Tel. 0721624374

3. Prof. Wallace Bulimo Tel. 0733524141

4. Prof. Simon Karanja . Tel. 0726424669

Kama kwa sababu yoyote ungependa kuwasiliana kamati tafadhali kutumia nambari ya simu zifuatazo; 0733606400 or 0722829501 na extension 44102

Consent information for controls

<u>Introduction and purpose</u>

I am Dr Kennedy Koech, a PHD student at the Jomo Kenyatta University of Agriculture and Technology, department of Public Health. I am inviting you to participate in a research that I am conducting whose purpose is to study **pattern of presentation and correlates of squamous cell carcinoma in selected sites in Kenya** . The disease is a form of cancer, which affects parts of the mouth and is generally known to be the 8th most common cancer in the world with incompletely understood cause. Although there are studies that have been done on the subject in

other parts of the world very few have been carried out in Africa and particularly In Kenya. . There is reason to believe that our data would be different due to our genetic and other environmental factors. As a person who is free from the disease you have been selected for comparison with patients afflicted by oral cancer.

Benefits

1. The study will not be of immediate benefit to you but in the long run the findings may be used to formulate policies and treatments for patients with similar illnesses.
2. Participating in the study will not result in any financial benefit and neither will you incur any extra financial cost.
3. The questions that you will be asked will assist in trying to get the cause of oral cancer among those afflicted.

Inconvenience, risks and right of withdrawal.

1. Your involvement in the study is purely voluntary and you are under no obligation to participate and you are free to withdraw at any stage.
2. There is no extra danger or risk associated with participating in the study.
3. All standard precautions will be taken while taking your specimen and discomfort will be minimized as much as possible.
4. Your refusal to participate of withdrawal from the study will not in any way affect the quality of the treatment offered to you in the hospital.

Type and amounts of specimen

1. Swabs will be obtained from your mouth using a sterile brush. The procedure is painless and is similar to what you feel when you use your toothbrush on your tongue

2. Approximately 5 ml of blood will be obtained by the laboratory staff for your routine tests using a sterile needle and syringe from your veins. The sample will be used for investigation of your current problem and also for part of the study. No

additional bllod sample will be obtained for the study. You will experience small discomfort from the needle and the site will be sore for a few days.

No samples will be taken out of the country and where the specimen will be stored for further analysis after the study permission shall be sought from the ethics and standards committee.

Duration

The interview will be carried out by a qualified doctor and will take approximately 10 minutes. The process of obtaining oral swabs will take approximately 5 minutes and blood samples will take another 10 minutes.

Statement of confidentiality.

- You will be assigned a serial number that will be used throughout the study and your name will not used. Only the investigators will know your identity.
- Like all other scientific studies the findings will be shared with other scholars. We may therefore publish the findings in journals or present them in scientific meetings. In any case, no information that can identify you will be shared.

In the event that you may need any further information in relation to this study please contact the following;

1. Dr Kennedy Koech:Tel. 0722788251
2. Dr Peter Wanzala: Tel. 0721624374
3. Prof. Wallace Bulimo: Tel. 0733524141
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Tafsiri kwa Kiswahili kwa wasiyo na saratani

Habari kuhusu utafiti huu

Utangulizi na madhumuni

Mimi ni Dr Kennedy Koech, mwanafunzi wa PhD katika Chuo Kikuu cha Jomo Kenyatta cha Kilimo na Teknolojia, idara ya Afya ya Umma. Nakukaribisha kushiriki katika utafiti ambayo nina fanya yenye lengo la kujifunza kuhusu saratani ya mdomo. Ingawa kuna tafiti zimefanyika juu ya somo katika maeneo mengine ya dunia chache sana yamefanywa katika Afrika na hasa hapa Kenya. . Kuna sababu ya kuamini kwamba takwimu zetu itakuwa tofauti kutokana na sababu maumbile yetu na mengine ya mazingira.

Faida

1. Utafiti haitakuwa ya faida kwakao kwa wakati huu lakini katika muda mrefu matokeo inaweza kutumika kuunda sera na matibabu ya wagonjwa weneye magonjwa hayo.

2. Kushiriki katika utafiti haitasababisha faida yoyote ya kifedha na wala wewe hautingia kwenya gharama yoyote ya ziada ya fedha.

3. Maswali yenye utaulizwa yatusaidia kwa uchunguzi wa saratani ya mdomo

Usumbufu, hatari na haki ya kujitoa.

1. Kuhusika kwako katika utafiti ni kwa hiari na wewe ni hauna wajibu kushiriki na wewe ni uko na haki wa kujiondoa katika hatua yoyote ..

2. Hakuna hatari au madhara zinazohusiana na kushiriki katika utafiti.

3.Tahadhari za kawaida zitachukuliwa wakati kuchukua miosho ya mdomo na damu yako na usumbufu itapunguzwa kadri iwezekanavyo.

4. kukataa kwako kushiriki au kujitoa kutoka utafiti hakuta athiri ubora wa matibabu inayotolewa na wewe kwa njia yoyote.

Aina na kiasi cha miosho na damu

1. Miosho ya mdomo itatolewa kwa kutumia aina ya mswaki. Utasikia vile anavyosikia ukitumia mswaki wa meno kwenye ulimi wako
2. Utaulizwa kutowa mate kwenye chupa
3. Takriban 5 ml damu itachukuliwa kwa kutumia sindano kutoka mishipa yako. sampuli zitatumika kama sehemu ya utafiti. Hauta tolewa damu zaidi kwa ajili ya utafiti huu. Kutakuwa na uchungu kidogo kutokana na sindano na sehemu ya kutolewa dama itakuwa na maumivu kidogo kwa siku chache.

Hakuna sampuli zitachukuliwa nje ya nchi na ambapo sampuli itahifadhiwa kwa uchambuzi zaidi baada ya utafiti huu ruhusa utatafutwa kutoka kwa kamati ya utafiti.

Muda wa uchunguzi

Mahojiano utafanywa na daktari waliohitimu na itachukua takriban dakika 10.

Kutowa miosho ya mdomo itachukua dakika 5 ilhali sampuli ya damu itachukua dakika 10 zingine.

Taarifa ya usiri.

• Utapewa nambari yenya itatumika katika utafiti na jina lako halitatumika. wapelelezi peke yao watajua utambulisho wako.

• Kama masomo mengine yote ya kisayansi matokeo itakuwa itaongelewa na wasomi wengine. Tunaweza kwa hiyo kuchapisha matokeo ya utafiti katika majarida au kuyawasilisha katika mikutano ya kisayansi. Katika hali yoyote, hakuna unaweza kutambulika.

Consent certificate

Patient number……

I……Of……

Give consent to participate in this study.

The nature of the study has been explained to me by Dr……..

Date……..signed/ thumb print……(patients signature)

Witness.

Name……signed……date……

I , Dr……..confirm that I have explained to the patient the nature of the study.

Signature of the investigator……

In the event that you may need any further information in relation to this study please contact the following;

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2. Dr Peter Wanzala: Tel. 0721624374
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Fomu ya idhini

Nambari ya utafiti.....

Mimi.....kutoka.....

Nina peana idhini ya kushiriki katika hii utafiti.

Asili ya utafiti ime elezwa kwangu na Dkt..... ..

Tarehe ....sahihi / kidole .... (mgonjwa)

Shahidi.

Jina……sahihi……tarehe……

Mimi Dr ....……nadhibitisha ya kwamba nimeelezea kwa mgonjwa asili ya utafiti.

Sahihi ya mchunguzi .....

Ukiwa na jambo lolote ama maswali yeyote kuhisiana na utafiti huu tafadhali wasiliana na wafuatao;

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Consent for biopsy procedure

Patient number……

I……Of……

Give consent to have a biopsy procedure done on me.

The nature and benefit of the procedure has been explained to me by Dr……..

Date……..signed/ thumb print……(patients signature)

Witness.

Name……signed……date……

I , Dr……..confirm that I have explained to the patient the nature of the biopsy procedure .

Signature of the investigator……

Fomu ya idhini ya kutolewa nyama

Nambari ya utafiti.....

Mimi.....kutoka.....

Nina peana idhini ya kutolewa kipande cha nyama kwenye ugonjwa.

Asili ya utafiti ime elezwa kwangu na Dkt..... ..

Tarehe ....sahihi / kidole .... (mgonjwa)

Shahidi.

Jina…….sahihi……tarehe……

Mimi Dr ....……nadhibitisha ya kwamba nimeelezea kwa mgonjwa asili ya utafiti.

Sahihi ya mchunguzi .....