DISSERTATION

CIGARETTE SMOKING AND ALCOHOL INGESTION AS RISK FACTORS FOR LARYNGEAL SQUAMOUS CELL CARCINOMA AT KENYATTA NATIONAL HOSPITAL.

A dissertation submitted in partial fulfilment of the requirements for the degree in Master of Medicine (M.Med) in Ear Nose and Throat, Head and Neck Surgery.

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DECLARATION

This dissertation is my original work and has not to the best of my knowledge, been presented for academic merit in any university.

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DEDICATION

I dedicate this work to my loving wife Michelle Kili Menach and daughter Chénang'at Menach for their patience, endless support, love and understanding throughout the period of my study.

I also dedicate it to my mother Emily and late father Peters Kamàtepón for their encouragement and the solid academic background they laid for us that has enabled me come this far in my endeavors.

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1. ACRONYMS AND ABBREVIATIONS

AA	Aromatic Amines
AJCC	American Joint Committee on Cancer
DNA	Deoxyribonucleic acid
ENT H & N	Ear Nose and Throat, Head and Neck
ETS	Environmental Tobacco Smoke
GSBSHS	Global School-Based Student Health Survey
GSTM1	Glutathione *S*- Transferase M1
GYTS	Global Youth Tobacco Survey
HNSCC	Head and Neck Squamous Cell Carcinoma
IARC	International Agency for Research on Cancer
INHANCE	International Head and Neck Cancer Epidemiology Consortium
KDHS	Kenya Demographic and Health Survey
KEMRI	Kenya Medical Research Institute
KNBS	Kenya National Bureau of Statistics
KNH	Kenyatta National Hospital
NIAAA	National Institute on Alcohol Abuse and Alcoholism
NCR	Nairobi Cancer Registry
NDMA	N-Nitrosodimethylamine
NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
NNN	*N'*-nitrosonornicotine
PAH	Polycyclic Aromatic Hydrocarbons
PAR	Population Attributable Risk
PET-CT	Positron Emission Tomography – Computerised Tomography
RNA	Ribonucleic acid
ROS	Reactive Oxygen Species
SCC	Squamous Cell Carcinoma.
UICC	International Union against Cancer
WHO	World Health Organization

2. ABSTRACT

Background: Laryngeal squamous cell carcinoma is strongly linked to tobacco inhalation as a cause. It is estimated to account for more than 70% of laryngeal squamous cell carcinomas and up to 89% when combined with alcohol.

Objective: To determine the prevalence of cigarette smoking and alcohol ingestion among patients with laryngeal squamous cell carcinoma and estimate the odds attributed to cigarette smoking and alcohol ingestion.

Methods: Fifty cases and fifty controls were recruited of matching age, sex and region of residence. History of smoking and alcohol intake was taken and analyzed to estimate the relative strengths of these exposures.

Results: 33 (66%) of the cases and 3 (6%) among controls smoked cigarettes. 74% had smoked for more than 30 years p <0.0001 OR 21.3 (95% CI: 2.6- 176.1). Cessation of smoking was associated with reduced risk (stopped <10yrs OR 19.5 (95% CI: 2.0-191). An ever smoker had increased risk compared to controls p< 0.001 OR 6 (95% CI 2.1- 18.023). Those who smoked only had glottic cancer p< 0.001 OR 19.75 (95% CI 2.0-188.55). Those who smoked and drank alcohol had supraglottic cancer p< 0.0001 OR 10.5 (95%CI 2.6-42). Being a current smoker and long duration of smoking were independent risk factors p= 0.002 OR 14.6 (95%CI 2.6- 80) and p <0.01 OR 7.3 (95%CI 1.6-33) respectively.

PAR for cigarette smoking is 62%.

Conclusion: Being a current smoker and long duration of smoking are important variables in assessment of cigarette smoking as a risk factor for laryngeal squamous cell carcinoma.

3. INTRODUCTION.

3.1 History of smoking

In human history, tobacco inhalation dates as far back as 5000BC when it was used for various religious, medicinal and later recreational purposes though no specific mention is made about it in the bible. (1)

Tobacco was introduced to Europe in the sixteenth and seventeenth centuries by traders and explorers who found it being used in the South Americas. (1) It was later cultivated commercially and then subsequently disseminated to the rest of the world through trade and colonization. (1)

Nicotine is the addictive compound to many cigarette smokers. (2) It causes increased release of neurotransmitters in nerve endings and increased production of dopamine and endorphins. (3) These compounds lead to increased alertness and are associated with pleasure. (3)

The commonest mode of tobacco inhalation is cigarette smoking. (4)This mode of consumption has been shown in USA and various developed countries to be more deleterious to health than other modes of inhalation since its introduction a few hundred years ago. (4)

At the moment, about a fifth of the world's population smoke cigarettes. (4) This figure is increasing exponentially due to extensive and aggressive marketing done by cigarette manufacturing companies. (4)

Tobacco is an important cause of human cancer and in some populations it is responsible for half of all cancer mortality among males and a lesser but rapidly rising percentage among females. (4) Globally, it is the leading known cause of cancer. (5) It should also be emphasized that tobacco inhalation is responsible for more mortality from cardiovascular and respiratory diseases than neoplasia in the developed world. (5-7)

Reviews by the IARC (4) have causally linked tobacco inhalation to development of upper aerodigestive tract including the larynx. The pancreas and the urinary bladder are also predisposed to tobacco smoke induced carcinogenesis. (4)

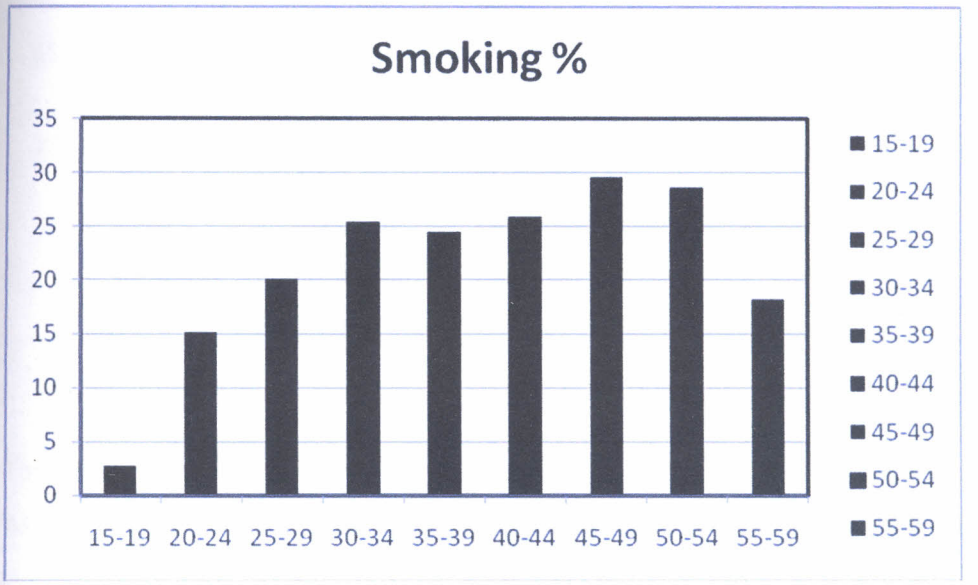
Among other exposures, alcohol ingestion has been found to interact in a multiplicative manner with tobacco in the genesis of laryngeal SCC. (8)

3.2 Prevalence of cigarette smoking in Kenya

In the Kenyan population, 23% of men and 1% of women were found to smoke cigarettes. (9) On the other hand, an average of 13% of schooling children smoked cigarettes and the

prevalence was higher among boys than girls. (10) The WHO GSBSHS survey showed similar findings. (11) The highest prevalence of smoking among Kenyan men is in the 45-49 age bracket. (Figure 1)

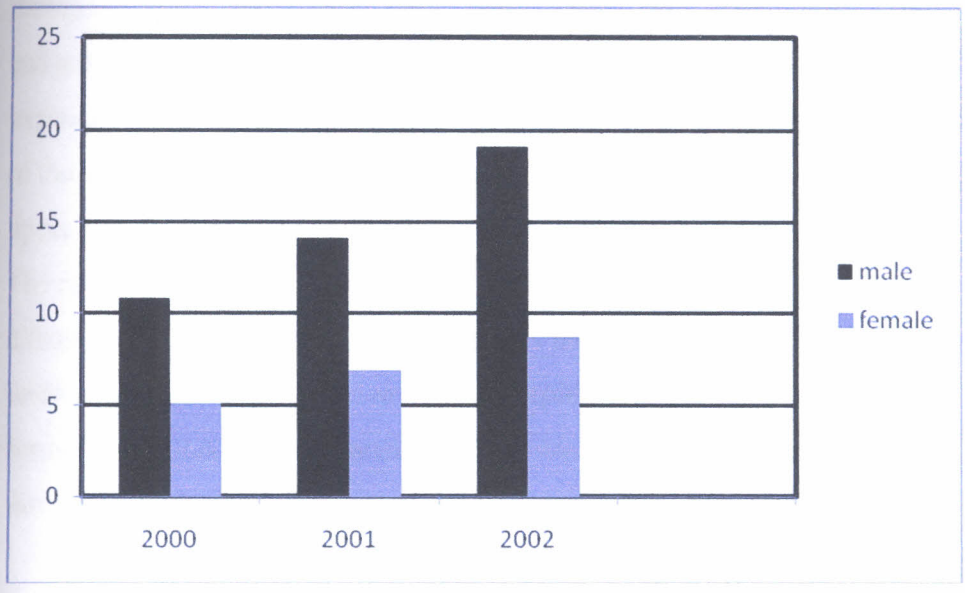
Figure 1: Age group prevalence of smoking in Kenya (9)



3.3 Cancer Burden in Kenya

In Kenya, cancer as a disease ranks third as a cause of death after infectious and cardiovascular diseases. (12) Currently Kenya has no reliable cancer registry and data availability is scanty and mainly hospital based. Laryngeal cancer is the fourteenth commonest cancer worldwide and according to WHO (4) it accounts for 1.8% of all new cancers diagnosed. Mutuma et al (12) found that head and neck cancer, of which laryngeal cancer is the commonest, is the leading cancer in males at 14.8% in Kenya and is third among females after cancer of the breast and cervix. There seems to be a steady rise in the incidence of head and neck cancer as evidenced by the trends documented at the NCR (Figure 2). (12) This cancer registry is however not thought to depict the accurate situation on the ground because cancer notification from health institutions is not as meticulous as desirable.

Figure 2: Rise in prevalence of head and neck cancer in Kenya (12)



The rates in males rose steadily from 10.8% in 2000 to 14.1% in 2001 then 19.1% in 2002 whereas the rates in females rose as follows: 5.1% in 2000, 6.9% in 2001 and 8.7% in 2002. Onyango et al (13) reported a 39% prevalence of laryngeal cancer among patients with head and neck cancer over the period between January to December 2004 of which 65% were associated with tobacco inhalation and alcohol ingestion. Among 793 head and neck cancer cases seen at KNH, Onyango et al (14) found that laryngeal cancer was leading then cancers of the tongue, mouth and nasopharynx in that order while SCC was the most common malignancy. Later, Nyandusi (15) showed that laryngeal cancer still topped the list of head and neck with a prevalence of 25.7%. 49% were cigarette smokers but specific and detailed account in relation to laryngeal cancer was not carried out. There were no controls in that study.

3.4 Applied anatomy of the larynx

The complex structure of the larynx consists of various folds and crevices on its mucosal surface and includes the narrowest part of the upper airway at the glottis. (16) The epiglottis, aryepiglottic folds, ventricular folds and true vocal cords project to the laryngeal airway whereas the laryngeal ventricle forms a lateral crevice above the true vocal cords. (16) The trachea is slightly angled in relation to the long axis of the larynx. (16) These unique features of the larynx cause turbulence to inflowing aerosols such as cigarette smoke therefore increasing deposition of particles onto the mucosa as demonstrated by Jinxiang et al (17).

The larger particles in cigarette smoke are mainly deposited onto laryngeal mucosa and lung during inhalation whereas the finer particles have been shown to be deposited during exhalation and secondary flows generated by turbulence. (17) Martonen et al (18) found that the higher exposure to inhaled aerosol particles predisposed the larynx to cancer compared to other regions of the airway. This is supported by a study by Yang (19) which showed a 3,000 times chance for upper airway cancer compared to the lower airway.

The epiglottic epithelium is stratified squamous on both surfaces but progressively changes to ciliated pseudostratified columnar towards the false vocal cords. (20) The ciliated epithelium undergoes another transition to stratified epithelium at the true vocal cords then reverts to the former lining. (20) These transitions have been shown in animal models to influence the distribution of metaplastic lesions in the larynx. (21, 22)

4. CANCER OF THE LARYNX

4.1 Background

Majority of laryngeal tumors are malignant and are summarized below (Table 1). (23)

Table 1: Malignant tumors of the larynx

Histology	Percentage
Squamous cell carcinoma (SCC)	85-95%
Verrucous carcinoma	1-4%%
Undifferentiated carcinoma	<1%
Adenocarcinoma	<1%
Miscellaneous carcinoma (adenoid cystic, spindle cell)	<1%
Sarcomas	<1%

Topographically, SCC is classified according to subsite involved. (23) Glottic cancer involves the true vocal cords, supraglottic cancer when it is confined to the supraglottis and subglottic cancer when it arises 1cm below the free edge of the true vocal cords. (23) Transglottic cancers cross the laryngeal ventricle vertically to involve an adjacent laryngeal subsite. (23)

The commonest subsite involved by cancer is the glottis (up to 60%) then supraglottis (up to 40%). (23) Haque et al (24) found that involvement of more than one subsite was commoner than single subsite involvement. In his study, the single most involved subsite was the supraglottis then glottis. (24) In both studies, the subglottis is the least involved subsite. (23, 24)

4.2 Diagnosis

It is made based on a full history and physical examination which includes specific ENT H&N clinical examination and indirect laryngoscopy and or stroboscopy. (23)

Persistent hoarseness of voice is the most consistent symptom and occurs in a majority of the patients. (24) Difficulty in breathing, noisy breathing, persistent cough, bloody sputum, neck swelling, ear and neck pain as well as difficulty swallowing are other symptoms encountered. (23, 24)

General ENT H&N examination is carried out with special attention to the neck for any swellings, widening of the laryngeal framework and presence or absence of laryngeal crepitus. Indirect mirror laryngoscopy, flexible nasopharyngoscopy or videostroboscopy is done to visualize the larynx, assess the vocal cord mobility and visualize lesions of the larynx. (25)

Imaging Studies

CT or MRI scanning with contrast enhancement is done to assess extension to adjacent tissues. (25) These are utilized in staging of the tumor and planning for surgery.

Staging

Staging of cancer of the larynx is based on UICC classification of malignant tumors. (25)

4.3 Management

Stage I and II tumors are generally treated by voice conservation surgery or radiotherapy. (23)

Stage III cancer is generally treated by combined total laryngectomy with or without neck dissection, and radiotherapy with or without chemotherapy. (23) Other centers offer concurrent chemo-radiation therapy as the only mode for stage III disease in order to achieve preservation of function. (23)

Operable stage IV tumors are treated by total laryngectomy, neck dissection, reconstruction and postoperative chemoradiation therapy. (23) Inoperable stage IV tumors are treated by chemoradiation. (23)

5. TOBACCO SMOKE CARCINOGENESIS

5.1 Neoplastic Mechanisms

Cigarette smoke is known to have multiple chemical compounds that are carcinogenic in the upper aerodigestive tract and lungs. (26) These chemicals are classified according to size and the most harmful and carcinogenic in respect to cancers of the lung, pharynx and larynx are found in the tar phase. (27) These chemicals include PAHs such as benzo (a) pyrene and dibenz (a) anthracene. (27) Consensus in IARC 1986b (28) demonstrated in hamster models that exposure to tar phase consistently lead to development of laryngeal cancer. The neutral portion of the tar phase has been extensively studied in relation to carcinogenesis in the aerodigestive tract while the basic fraction which contains nicotine has been shown to be strongly carcinogenic as well. (29, 30)

Other chemicals present in tobacco smoke such as polonium, phenols, nickel are thought to be cocarcinogens or promoters whereas catechols and phenols are known to generate oxygen free radicals that may cause DNA damage. (27)

PAH such as benzo (a) pyrene, which has been studied widely, is liberated from tobacco after burning and is absorbed through the epithelium after being dissolved in saliva. (29) This absorption is further enhanced in the presence of alcohol. (28) It is thereafter broken down by intracellular mechanisms to by products such as benzo (a) pyrene -7, -8 oxide and finally to benzo (a) pyrene-7, -8 dihydro-7,-8 diol, conjugated to form harmless products and then secreted through urine. (29) This activation within laryngeal mucosal cells is attributed to cytochrome P 450 2C, 3A4 and 1A1. (30)

In the event that conjugation does not occur, the dihydrodiol thus formed may be further metabolized by hydroxylation to benzo (a) pyrene -7, 8-dihydrodiol-9, 10-epoxide that reacts with DNA and RNA to form DNA adducts. (29)

Similar activation of aromatic amines such as 2- nathylamine found in tobacco smoke by microsomal enzymes and subsequent bonding to DNA has also been documented. (29) On the other hand, nicotine is broken down to nitrosamines such as NNN, NNA and NNK which are further modified through hydroxylation to metabolically active methyl diazohydroxide that interacts with DNA in a similar manner. (31, 32)

Work by Church et al (33) showed that tobacco smoke exhibited reactive oxygen species capable of binding DNA leading to mutations in various regions of the genome. Jaloszynski et al (34)

later showed that most of the damage seen in laryngeal SCC is attributed to ROS rather than PAH though this postulate is not conclusive.

The local immunosuppressive properties of cigarette smoke were demonstrated Rees et al. (35) He showed a significant reduction in T cell populations in the human larynx and hypothesized that this may predispose to laryngeal SCC.

5.2 Effect of alcohol on tobacco carcinogenesis

It is now well known that alcohol potentiates the carcinogenic effects of tobacco smoke. (8) Various mechanisms have been described but the most accepted is the reduced liver breakdown of nitrosamine metabolite NDMA which is a known carcinogen through competitive inhibition of the enzyme cytochrome P- 450 E21. (36) This reduced breakdown leads to accumulation of NDMA in the body tissues including the larynx where it is broken down to active metabolites that bind DNA. (36) On the other hand, chronic alcohol ingestion induces liver cytochrome P-450 2E1 enzyme leading to increased production of carcinogenic tobacco metabolites. (37) The metabolites produced are conjugated by GSTM1 and excreted as harmless products. (29) Increased risk for tobacco carcinogenesis is seen in individuals who have reduced activity of GSTM1 due to genetic polymorphism. (37)

Deficiencies of folic acid and zinc have been shown to contribute to enhanced effects of tobacco carcinogenesis among chronic alcoholics due to loss folic transmethylation activity that regulates gene expression. (38)

Alcohol is a known solvent of tobacco carcinogens leading to increased absorption of tobacco carcinogens in the upper aerodigestive tract. (39) The solvent effect is also true especially for the supraglottic larynx that is exposed to both inhaled and ingested material. (8)

The upper aerodigestive tract is known to have a low repair ability for PAH induced DNA damage. (31) These DNA adducts therefore persist setting stage for mutations, translocations and or deletions hence altered gene expression. (40)

The role of tobacco smoke in initiating preneoplastic changes in the epithelium of the larynx which eventually lead to invasive cancer has been documented. (41, 42) Molecular studies have shown corresponding progressive chromosomal losses in various loci in these lesions. (42) These genetic and epigenetic changes affect key regions of the genome that are responsible for normal growth regulation as shown in head and neck cancer models. (43) Cigarette smoke hence causes disruption of the normal cellular regulatory pathways leading to increased stimulation of

growth via amplification of cell cycle regulatory factors such as cyclin D1, amplification of transcription factors such as myc and ras, up regulation of intracellular transducers such as STAT proteins and disruption of apoptosis as well as genomic stability via loss of p53 function. (43) This leads to uncontrolled replication and immortalization of mucosal cells which are prerequisites of cancer development. (43- 46)

6. LITERATURE REVIEW

According to the IARC Monographs (4, 28), tobacco inhalation is causally linked to laryngeal cancer. More research has been done in various regions of the world to solidify this conclusion. (4) Other causes of laryngeal cancer include alcohol consumption, gastro esophageal reflux disease, infection with human papilloma virus & helicobacter pylori, some occupational exposures like sulfuric acid and possibly some dietary habits as outlined by Cattaruza et al. (47) The risks associated with cigarette smoking are also modified by alcohol consumption in an additive or multiplicative manner thus the cigarette smoking risk is examined within strata of alcohol consumption. (8) It has been shown that about seventy percent of laryngeal SCCs are causally linked to tobacco inhalation while 26% are linked to alcohol ingestion. (48) Hashibe et al (49) conducted a metaanalysis and found that these two exposures together accounted for 89% of laryngeal cancers. This study was carried out on pooled data from the INHANCE consortium which introduces inter observer bias. Furthermore, the types of control subjects differ in each of the studies. Some of the studies utilized hospital based control subjects whereas others used population based controls at a different time compared with the cases. (49) These weaknesses are acknowledged by the authors. (49)The strength of this study is in assembling of a very large number of head and neck cancer cases which allowed the authors to examine in detail the interaction between cigarette smoking and alcohol ingestion.

Risks conferred by current smoking vary and tend to increase significantly with increased intensity and duration (Table 2). (48, 50-52)

Table 2: Duration of smoking, sample size and corresponding odds ratios

Study	Sample size	Duration of smoking (yrs)	Odds ratio
Francheschi et al (48)	162	40	15.6
Hashibe et al (50)	89	20	6.76
Talamini et al (51)	191	40	16.2
Lee et al (52)	49	20	2.13

The above studies were carried out in the European and Indian populations and excluded African and Asian populations citing poor representation for estimation of odds ratios and population attributable risks in the studies carried in these regions. (49)

Several studies (Table 3) have demonstrated higher risks for laryngeal SCC with increased number of cigarettes smoked. (48, 51, 53)

Table 3: Number of cigarettes and corresponding odds ratios

Study	Number of cigarettes	Odds ratio
Francheschi et al (48)	>25	7.1
Talamani et al (51)	>25	42.9
Hashibe et al (53)	>20	12.8

It is has been established that there is a consistent reduction in risk for laryngeal SCC among former smokers compared to current smokers. (51, 52, 54, 55, 56) The duration required to appreciate the benefit of smoking cessation varied widely from 6 to 20 years. (51, 54, 55) Other studies have shown similar risks to never smokers among those who stopped smoking for over 20 years or quit before 35 years of age. (55, 56)

The combined risks of tobacco inhalation and alcohol ingestion have been shown to be multiplicative in a metaanalysis with 35 fold increased risk in the highest category of both exposures. (8) Francheschi et al (48) and Talamini et al (51) found even higher risks when compared to controls.

As regards subsite distribution, various authors in Turkey and France have shown that cigarette smokers were twice more likely to develop supraglottic cancer than glottic cancer. (57-59) Similar findings were shown by Talamini et al in Italy. (51)

As regards alcohol as a risk factor, the relative risk ratios for developing supraglottic cancer are slightly higher than glottic carcinoma in various studies (47, 51, 59, 60) although one study by Hashibe et al (50) showed similar risks for the two subsites. The same author found significantly higher risks for glottic cancer from alcohol consumption in a separate study he conducted at a later date. (53)

ETS has been thought to confer some marginal risk for laryngeal SCC depending on the areas of exposure. (61, 62) However, other studies and reviews published later did not convincingly show significant risk from ETS. (52, 63)

The literature reviewed above are mostly based on hospital records and few of the authors actually interviewed all cases on their own thereby introducing inter observer bias. Other handicaps to these studies include the fact that cases diagnosed at a different time were controlled against subjects at a different time altogether and were not regionally controlled. (49) Some studies in one of the metaanalysis did not obtain histological information from their records. (49)

This study was designed to analyze the risk of developing laryngeal SCC attributed to cigarette smoking when abused either alone or alongside alcohol in a Kenyan population and relate with the laryngeal subsites involved. The investigator recruited and obtained history from both case and control groups so as to eliminate inter observer bias.

7. JUSTIFICATION OF THE STUDY

Cancer of the larynx is the leading head and neck cancer among men in Kenya and is third commonest cancer among females. It has been strongly linked to cigarette smoking and alcohol intake in many studies. Local data is scanty and where available are not specific for cancer of the larynx. To the best of my knowledge, no case-control study has been carried out in the Kenyan population to evaluate cancer of the larynx risks regarding tobacco inhalation and alcohol intake. This study therefore sought to estimate the risks attributed to cigarette smoking and alcohol ingestion in this population. This will form a basis for future planning strategies aimed at reducing the burden of this cancer through reduction of cigarette smoking.

8. REASEARCH QUESTION

What is the prevalence of cigarette smoking and alcohol ingestion among patients who have been histologically diagnosed with SCC of the larynx?

09. OBJECTIVES

09.1. Broad objective

Determine the prevalence of cigarette smoking among patients histologically diagnosed with SCC of the larynx and estimate the risk of developing laryngeal SCC attributed to cigarette smoking.

09.2. Specific objectives

1. Determine the demographic characteristics of patients diagnosed with laryngeal SCC.
2. Determine and compare the prevalence of cigarette smoking among patients diagnosed with laryngeal SCC with sub sites involved and tumor characteristics.
3. Determine the type, amount and duration of cigarette smoking among patients with cancer of the larynx and estimate the risk for developing laryngeal SCC attributable to cigarette smoking.
4. Determine the prevalence of alcohol intake among patients diagnosed with SCC larynx and estimate its effect on cigarette smoking as a risk factor for laryngeal cancer.

10. HYPOTHESIS

10.1 Null Hypothesis

Cigarette smoking is not an important risk factor for development of laryngeal SCC in this set up and its effect differs from findings of studies done elsewhere.

10.2 Alternate hypothesis

Cigarette smoking is an important risk factor for laryngeal SCC in this set up and findings here are similar to studies done elsewhere.

11. STUDY DESIGN

This was a hospital based case- control study.

12. SETTING

Cases were recruited from the departments of ENT H & N and radiation- oncology whereas controls were recruited from the department of orthopedics.

13. STUDY POPULATION

Sequential incident and prevalent cases of histologically proven laryngeal SCC in the above departments as well as patients admitted in the orthopedic wards (controls) of matching age, sex and region who were clinically assessed and found not to have cancer of the larynx, were recruited for this study.

13.1 Inclusion criteria

A. Study group

All patients with histological diagnosis of SCC of the larynx, both prevalent (diagnosed within the last 3years) and incident who gave informed consent.

B. Control group

1. Orthopedic patients in whom cancer of the larynx has been excluded clinically.

2. Those who gave informed consent.

13.2. Exclusion criteria

1. Those who declined informed consent.
2. Patients in the control group suspected to have cancer.
3. Non SCC histologies and those not reported by a qualified pathologist.

14. SAMPLE SIZE CALCULATION

The sample size was calculated using the formula of comparing two proportions as outlined below. (Table 4)

For unequal groups of size n_1 and n_2, where $r = n_2/n_1$, is

$$n_1' = \frac{\left\{z_{\alpha/2}\sqrt{(r+1)\bar{p}\,\bar{q}} + z_\beta\sqrt{rp_1q_1 + p_2q_2}\right\}^2}{rd^2}$$

where $\bar{p} = \frac{p_1 + rp_2}{r+1}$ and $n_2 = rn_1$.

For small samples, employ a "continuity correction"

$$n_1 = \frac{n_1'}{4}\left(1 + \sqrt{1 + \frac{2(r+1)}{n_1' r|d|}}\right)^2.$$

Adapted from American Journal of Epidemiology 1974.

Table 4: Sample size calculation format

Factor under consideration		"Smoking"
1ST GROUP		"Cancer patients"
2ND GROUP		"Non-Cancer patients"
Parameter	**Symbol**	**Value**
Probability of "Smoking" in "Cancer patients" group	p_1	60.0%
Probability of "Smoking" in "Non-Cancer patients" group	p_2	30.0%
$p_1 - p_2$	d	0.3
Odds Ratio	OR	3.50
Proportion of participants expected in "Cancer patients" group	m_1	50.0%
Proportion of participants expected in "Non-Cancer patients" group	m_2	50.0%
Ratio of ("Cancer patients":"Non-Cancer patients") sizes	r	1.00
P corrected	p-bar	0.450
Power	1-β	80%
	z-β	0.84
Confidence level	1-α	95%
	z-α	1.96
Number of subjects required for "Cancer patients" group	n_1'	42
Number of subjects required for "Non-Cancer patients" group	n_2'	42
Continuity correction for n_1'	n_1	50
Continuity correction for n_2'	n_2	50
Sample size		**100**

A prevalence of 60% was arrived at as the probability of smoking among cancer patients based on studies done locally and an estimate of 30% prevalence among non- cancer patients which corresponds to the prevalence of smoking among adults of the age bracket 45-49 according to KDHS (8). With continuity correction of the sample size, statistical power of 80% and

confidence interval of 95%, a total of 100 patients were obtained of which 50 were cases and 50 were controls.

15. METHODOLOGY

The study was conducted between March 2011 and May 2011.

15.1 Methods

Recruitment of study patients

Sequential patients (cases) of all ages presenting in the ENT H & N Surgery department, Radiation-oncology departments of KNH with histologically proven SCC larynx were recruited for the study. The histologies were confirmed from the patient files and tumor board attendance register among those recruited from the ENT H&N department. A total of 39 cases were recruited from the ENT H&N department whereas 11 were recruited from radiation-oncology department. All the cases had been diagnosed histologically with SCC of the larynx and staged clinically, endoscopically, radiologically (CT scanning) and finally discussed in the ENT H&N tumor board. Eight cases were excluded from the study. Two had verrucous carcinoma whereas four had spindle cell carcinoma. Two other patients declined and were also excluded from the study.

Recruitment of controls

Controls were recruited from patients in the orthopedics department (inpatient and outpatient) and matched for age, sex and region with the cases to control for confounding factors. A total of 50 controls were recruited. 36 were recruited from the wards whereas 14 were recruited from the orthopedic clinic. Among the controls, three patients were found to have hoarseness of voice whereas one had a neck swelling and were therefore referred to the ENT H&N clinic for further evaluation. 31 (62%) of the controls were diagnosed with traumatic orthopedic conditions (fractures and or dislocations), 25% had benign orthopedic ailments such as sciatica and prolapsed intervertebral discs while the remaining 13% had osteoarthritis and acute infections such as septic arthritis and cellulitis. None of the recruited patients had been diagnosed with a neoplastic condition.

Controls were matched with the cases within a range of five years of the cases.

Informed consent

Initial explanation on the nature and scope as well as anticipated benefits and adverse effects of the study was made to all recruited cases and controls. Consent was then obtained from the

participants and a study number assigned where-upon demographic data was entered in a questionnaire.

Establishment of the presence and nature of smoking habit and alcohol ingestion

A medical history with primary focus on laryngeal malignancy was obtained to include onset of symptoms, severity, duration and involvement of other regions among the controls. An indirect laryngoscopy was then performed on all patients in the control group and those found to have any laryngeal lesion suspected to be malignant based on history, examination and indirect laryngoscopy were excluded from the study and referred to the ENT H&N consultant clinic for further evaluation. Those in whom malignancy has been ruled out in this manner were then recruited as controls for the study.

History of smoking and alcohol intake was then obtained to include whether they were current smokers, how long they stopped smoking if they were former smokers, age of onset of cigarette smoking, duration, type (filtered or non-filtered cigarettes), number of sticks in pack years and whether they lived with anyone who smokes.

History of alcohol intake was obtained to include whether they drank alcohol or not, age of onset of alcohol intake, duration, number of days they drank per week, type of alcohol taken and number of alcoholic drinks taken per week. A drink constituted 125mls of wine, 330mls of ale beer and 30mls of hard liquor. (64)

The respondents were then classified as "no drinkers, light drinkers, moderate drinkers, heavy drinkers and very heavy drinkers" as per the NIAAA (64) guidelines. (Table 5)

Table 5: NIAAA classification of alcohol drinking patterns

Classification	Women: Drinks per week	Men: Drinks per week
No drinkers	Nil	Nil
Light Drinkers	< 3 drinks	< 3 drinks
Moderate drinking	3-7 drinks	3-14 drinks
Heavy drinking	7-14 drinks	14-21 drinks
Very heavy drinking	>14 drinks	>21 drinks

15.2 Quality control

The proforma was pretested prior to commencement of the study and appropriate changes made. The principal investigator took the relevant medical history and conducted the necessary physical examinations, file perusal in both case and control groups to eliminate inter observer bias.

15.3 Data Management and analysis

All the information was recorded in a data collection form.

The data was thereafter entered into a computer and analysed by SPSS 17.0 statistical package with the help of a statistician. Descriptive statistics such as means, frequency and standard deviation were used for most of the data.

Results are presented in tables, graphs and pie charts. Mean, median and standard deviations were computed.

Risks for laryngeal SCC were estimated by calculating Odds ratios.

Attributable Fraction/Risk was estimated by the following formula and presented in percentages.

The formula below was applied since cases and controls were equal in number.

$$\text{Attributable Risk} = 1 - \frac{\text{(Non exposed cases)}}{\text{(Non- exposed controls)}} \times 100$$

Adapted from Basic epidemiology 2nd edition by R. Bonita

Associations between tobacco smoking and alcohol intake as risk factors for SCC of the larynx were analyzed by multiple linear regression models to estimate the relative strengths of the independent and joint effects of the exposures.

16. ETHICAL CONSIDERATIONS

Informed consent was obtained from the participating patients or their guardians and those who declined to participate in the study were excluded and given the same treatment as those who consented.

Costs incurred were borne by the principal investigator and no extra cost was passed to the patients.

Confidentiality of the participating patients was maintained at all times. Names and numbers obtained on the questionnaire were used only for purposes of follow-up where need arose.

Approval by the ethics and research committee of the KNH was sought before commencement of the study.

Results obtained from this study will be published and made available for use by the members of the medical fraternity.

17. RESULTS

In this study, 50 cases of histologically proven SCC of the larynx were recruited from the departments of ENT H&N and radiation-oncology as well as 50 controls of matching age, sex and region from the department of orthopedics. Matching by region in some situations was difficult due to unavailability of suitable controls. Other suitable respondents were therefore recruited.

Age distribution

The youngest age for the cases was 42 and while the oldest 84 years with a mean age 63 years. There was no significant difference between the case and control group (p= 0.297) although the mean age for cases was slightly lower (Table 6 and 7).

Table 6: Mean ages for cases and controls.

	arm	n	Mean	Std. Deviation	P value
Age	Controls	50	63.7	10.587	**0.297**
	Cases	50	61.36	11.717	

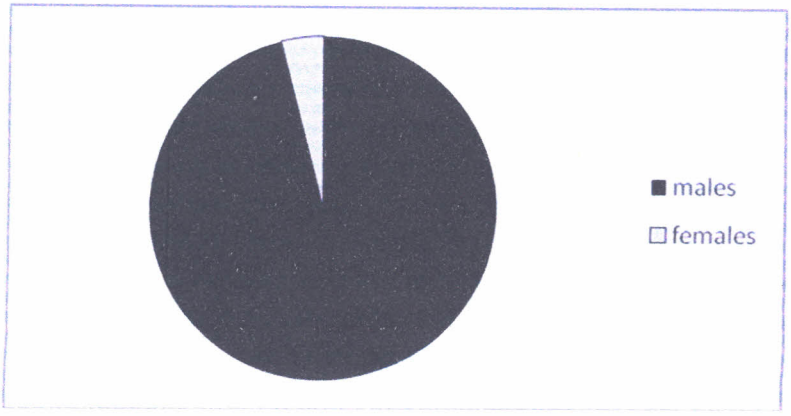
Table 7: Age distribution for cases and controls

		Arm			
		Controls		Cases	
		Count	Column N %	Count	Column N %
Age groups	40-44	3	6.1%	1	2.1%
	45-49	2	4.1%	4	8.5%
	50-54	4	8.2%	4	8.5%
	55-59	4	8.2%	9	19.1%
	60-64	13	26.5%	13	27.7%
	65-69	10	20.4%	6	12.8%
	70-74	7	14.3%	4	8.5%
	75-79	5	10.2%	4	8.5%
	80-84	1	2.0%	2	4.3%

Sex distribution

Out of the 50 cases, only 2 were female (4%) whereas the rest were male (96%) suggesting that this is a predominantly a male disease (Fig. 3).

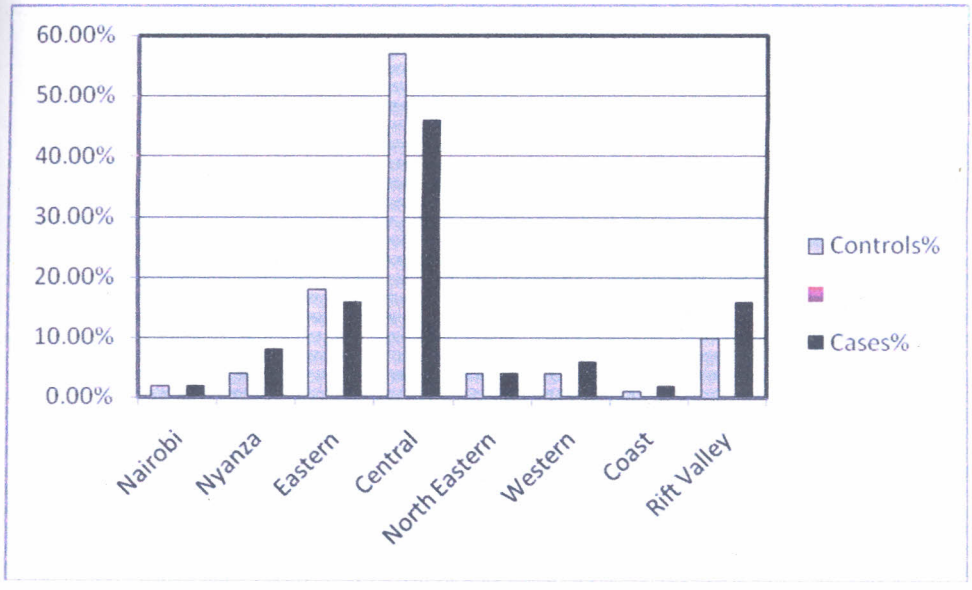
Figure 3: Pie chart showing sex distribution.



Region of origin

Out of the 50 cases, majority of them came from Central Kenya (46%) followed by Eastern province (16%) then Rift Valley 16%. Nyanza and Western provinces followed with 8% and 6% respectively (Figure 4). The least number of cases was seen in Nairobi province ($p = 0.281$). Some cases from Nyanza and coast province were controlled against controls recruited from other regions due to unavailability of the suitable age and sex.

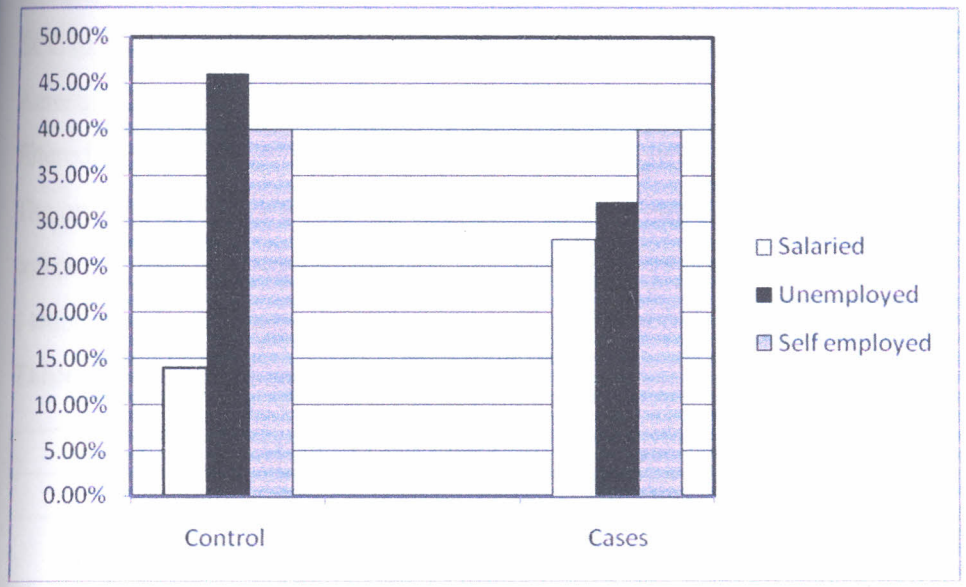
Figure 4: Distribution of cases and controls by region



Occupation

An equal proportion of cases and controls were self employed whereas more of the controls were unemployed compared to the cases. Twice as much of the cases were salaried compared to the controls although these findings were not statistically significant (p = 0.166). (Figure 5)

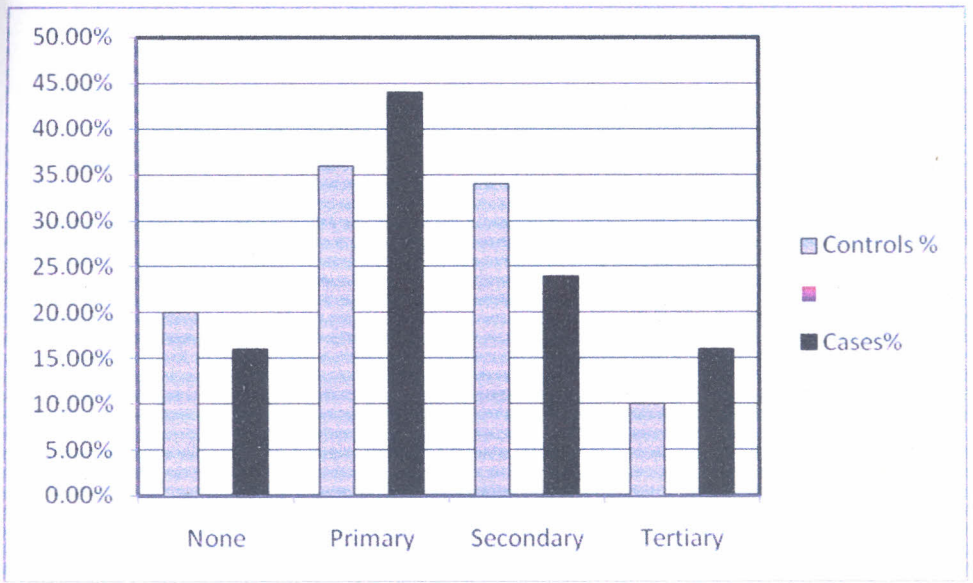
Figure 5: Occupation among cases and controls



Education

Majority of the cases had attained primary education compared to controls whereas other levels of education were comparable (p = 0.57). (Figure 6)

Figure 6: Levels of education among cases and controls



In general, the characteristics of cases in terms of sex, age, region of origin, occupation and level of education were similar with the distribution among controls.

Smoking History

33 of the 50 cases (66%) had a positive history of current cigarette smoking compared to controls that had a low prevalence of smoking of less than 6%. The odds ratio of developing laryngeal SCC in patients who are current smokers was 30.4 p value <0.0001 (95% CI: 8.2- 112.2).

Among the cases who smoked, 69.8% smoked filtered cigarettes whereas 30.20% smoked non-filtered cigarettes (p = 0.198). This was not statistically significant (Table 8).

The prevalence of the respondents who lived with someone who smoked in the house was comparable between cases and controls at 3% and 2% respectively. The p values for living with a smoker and smoking in the house were 0.558 and 0.307 respectively.

Table 8: Smoking history and habits

		Arm						
		Controls		Cases		Total		
		Count	Column N %	Count	Column N %	Count	Column N %	P value
Smoker	No	47	94.00%	17	34.00%	64	64.00%	**<0.0001**
	Yes	3	6.00%	33	66.00%	36	36.00%	
Cigarette Type Smoked	Filtered	13	86.70%	30	69.80%	43	74.10%	0.198
	Non-filtered	2	13.30%	13	30.20%	15	25.90%	
Lives with Smoker	No	49	98.00%	48	96.00%	97	97.00%	0.558
	Yes	1	2.00%	2	4.00%	3	3.00%	
Smokes in House	No	49	98.00%	47	94.00%	96	96.00%	0.307
	Yes	1	2.00%	3	6.00%	4	4.00%	

The duration since stopping smoking among controls was found to be significantly longer (24.57 years) compared to cases (12.13years) p = 0.029. Those who had stopped smoking for <10years had a reduced risk of developing laryngeal SCC OR 19.5 (95% CI: 2.0-190.9) as opposed to current smokers OR 30.4 (95% CI: 8.2- 112.2). (Table 10)

Similarly, the mean age of starting cigarette smoking among the cases was 20.18 whereas it was 25 among the controls (p 0.044). Those who started smoking below the age of 20 years had p <0.0001 OR of 31.733 (95% CI: 8.74-115.04) whereas those who started smoking between 21-

40 years of age had p < 0.001 OR 7.727(95% CI: 2.409- 24.787). P value for those who began smoking after 40 years was 1.00 (Table 9)

Table 9: Age of smoking debut

Age of debut cigarette smoking	B	P value	OR	95% C.I	
				Lower	Upper
Never smoked		.000			
<=20 years	3.457	**<0.001**	**31.733**	8.754	115.040
21-40 years	2.045	**0.001**	**7.727**	2.409	24.787
>40 years	22.937	1.000	9.154E+09	.000	.

There was a significant difference in the mean of the total number of pack years among the cases 31.40405 compared to the controls 5.4235 p < 0.0001 OR 21.3 (95% CI: 2.6- 176.1). This shows a strong association between cumulative cigarette smoking and laryngeal SCC.

Duration of smoking was also found to be significantly different among cases and controls p<0.0001 OR 12.7 (95% CI: 3.4- 47.5). The cases had smoked an average of 38.36 years compared to controls who had smoked for an average of 14.88 years. The short duration of smoking among the controls is explained by the fact that 92% of controls who had smoked stopped smoking at various times before being recruited for the study. (Table 10)

Table 10: Smoking characteristics

	arm	N	Mean	Std. Deviation	P value
Duration since stopping smoking <10yrs	Controls	14	24.57	12.364	**0.029**
	Cases	15	12.13	16.173	
Age of debut to smoking	Controls	16	25	5.797	**0.044**
	Cases	44	20.18	8.633	
Cumulative Pack Years	Controls	17	5.4235	6.57536	**<0.0001**
	Cases	42	31.0405	23.83292	
Duration of smoking	Controls	17	14.88	9.44	**<0.0001**
	Cases	44	38.36	15.716	

Ever smokers

From the results of this study, having smoked in one's lifetime has a significant positive relationship for developing laryngeal SCC. The highest risk was noted for supraglottis p= 0.003 OR 6.7778 (95% CI 1.7- 27.021) followed by glottis p <0.0001 OR 6.151 (95% CI 2.1- 18.023) and lastly transglottic SCC p 0.012 OR 3.5 (95% CI 1.269- 9.652) (Table 11).

Table 11: Ever smokers

	Yes	No	p value	OR	Lower CI	Upper CI
Supraglottis	9	3	**0.003**	**6.778**	1.7	27.021
Glottis	14	6	**< 0.0001**	**6.152**	2.1	18.023
Subglottis	1	0	0.18			
Transglottic	12	8	**0.012**	**3.5**	1.269	9.652

Alcohol consumption

Amongst the cases recruited, 38 (76%) gave a positive history of alcohol intake compared to the controls among whom 29 out of 50 drank alcohol corresponding to 58% p = 0.05 OR 2.3 (95% CI: 1.0-5.4) showing elevated alcohol related risks for laryngeal SCC. When stratified into the various categories of alcohol drinking as outlined by NIAAA (62), only those who were very heavy drinkers had significant p value 0.002 OR 6.0 (95% CI: 11.957- 18.398). The sample size in this study was designed to assess cigarette smoking in relation to laryngeal SCC and therefore the history of alcohol intake is included so that we can be able to control for its effects as it is a major confounder in regards to tobacco induced carcinogenesis.

Comparison between risk factors and sub sites involved

Cigarette smoking as the only exposure

Among patients who smoked only, 4 of them (20%) had glottic cancer p=0.001 OR 19.75(2.069-188.552) which was statistically significant. Other laryngeal sub sites did not have any patients who smoked only. In this study, glottic SCC seems to be the only subsite associated with cigarette smoking only (Table 12).

Table 12: Cigarette smoking in relation to subsites involved.

		Smoking only							
		Yes		No					
		Count	Row N %	Count	Row N %	p value	OR	Lower CI	Upper CI
Supraglottis	Yes	0	.0%	13	100.0%	0.375	-		
	No	5	5.7%	82	94.3%				
	Total	5	5.0%	95	95.0%				
Glottis	Yes	4	20.0%	16	80.0%	**0.001**	**19.750**	**2.069**	**188.552**
	No	1	1.3%	79	98.8%				
	Total	5	5.0%	95	95.0%				
Subglottic	Yes	0	.0%	1	100.0%	0.818	-		
	No	5	5.1%	94	94.9%				
	Total	5	5.0%	95	95.0%				
Transglottic	Yes	0	.0%	20	100.0%	0.251	-		
	No	5	6.3%	75	93.8%				
	Total	5	5.0%	95	95.0%				

Cigarette smoking and alcohol intake as joint exposures

Compared to controls, combined consumption of cigarette smoke and alcohol had significant effect on the risk of supraglottic, glottic and transglottic SCC. The highest significant risk was observed for supraglottic SCC (OR 10.476) whereas there was no significant increased risk for glottic SCC (Table 13)

Table 13: Cigarette smoking and alcohol intake as joint exposures

		Smoking & drinking alcohol							
		Yes		No					
		Count	Row N %	Count	Row N %	p value	OR	Lower CI	Upper CI
Supraglottis	Yes	10	76.9%	3	23.1%	**<0.0001**	**10.476**	**2.634**	**41.662**
	No	21	24.1%	66	75.9%				
	Total	31	31.0%	69	69.0%				
Glottis	Yes	10	50.0%	10	50.0%	**0.04**	**2.810**	**1.025**	**7.699**
	No	21	26.3%	59	73.8%				
	Total	31	31.0%	69	69.0%				
Subglottic	Yes	1	100.0%	0	.0%	0.134	-		
	No	30	30.3%	69	69.7%				
	Total	31	31.0%	69	69.0%				
Transglottic	Yes	12	60.0%	8	40.0%	**0.002**	**4.816**	**1.715**	**13.519**
	No	19	23.8%	61	76.3%				
	Total	31	31.0%	69	69.0%				

Tumor characteristics

In this study, majority of the cases had histological diagnosis of well differentiated (G1) squamous cell carcinoma followed by moderately differentiated (G2), poorly differentiated (G3) and the least was undifferentiated carcinoma (G4).

Majority of the cases presented in stages 3 and 4 of the disease.

The most involved sub sites were the glottis, followed by transglottic, supraglottic and lastly subglottic tumors (Table 14).

Table 14: Tumor characteristics

		Count	Column N %
Differentiation	Well differentiated (G1)	19	38.00%
	Moderately differentiated (G2)	17	34.00%
	Poorly differentiated (G3)	10	20.00%
	Undifferentiated (G4)	4	8.00%
Stage	1	3	6.40%
	2	10	20%
	3	24	46%
	4	13	27.60%
Supraglottis	Yes	12	24%
Glottis	Yes	20	40%
Subglottic	Yes	1	2%
Transglottic	Yes	17	34%

Smoking Only

On analyzing the histological grades against the risk exposures being studied, only 4 cases smoked only and had developed G1 carcinoma. Of note is that none of those who smoked only had the other histological grades as outlined below. This relation was of statistical significance p value <0.0001 OR 21.333 (95% CI 2.227- 204.364) when compared to controls (Table 15).

Table 15: Histological grade among smokers only

	Yes	No	p value	Lower OR	Upper CI	Upper CI
G1	4	15	**<0.0001**	**21.333**	**2.227**	**204.364**
G2	0	17	0.299			
G3	0	10	0.444			
G4	0	4	0.64			

Combined smoking and alcohol

There is a statistically significant increased risk for laryngeal SCC on all histological grades except for G1 tumors among those who smoked cigarettes and consumed alcohol concurrently (Table 16).

Table 16: Histological grade for concurrent smoking and alcohol intake

	Yes	No	p value	OR	Lower CI	Upper CI
G1	8	11	0.245			
G2	10	7	**0.006**	**4.218**	**1.425**	**12.285**
G3	8	2	**<0.001**	**11.652**	**2.305**	**58.895**
G4	3	1	**0.052**	**7.286**	**0.726**	**73.075**

Multivariate analysis

Multivariate logistic regression was performed involving variables that had significant p values. The following variables were therefore included: Current smokers (p=<0.0001), Duration of stopping smoking (p=0.029), Age of smoking debut (p=0.044), cumulative pack years (p<0.0001), duration of smoking (p<0.0001), prevalence of alcohol intake (p=0.05), drinks taken per week (p=0.028), G1 tumors (p <0.001) and G2 tumors (p 0.022).

On multivariate logistic regression, only two variables were independently associated with increased risk for laryngeal SCC. These were Current smokers whose OR was 14.576 (95% CI 2.624- 80.979) and Duration of smoking OR 7.312(95% CI 1.619- 33.024). Being a current smoker and prolonged duration of smoking are the most important independent factors contributing to development of laryngeal SCC (Table 17).

Table 17: Independent factors on multivariate analysis

	p value	**OR**	Lower	Upper
Current smoker	0.002	**14.576**	2.624	80.979
Smoking duration	0.01	**7.312**	1.619	33.024

Lastly, based on the prevalence of smoking among cases and controls, the population attributable risks associated with cigarette smoking was found to be 62%. This is the proportion by which laryngeal SCC would be reduced if cigarette smoking were to be eliminated.

18. DISCUSSION

The recruited patients were aged between 42 and 84 years whereas the mean age was 63 years for controls and 61 years for the cases. There was no statistically significant difference between the two groups in terms of age distribution ($p = 0.297$). It is however known that laryngeal SCC is a predominantly male disease possibly because of the fact that men tend consume more alcohol and smoke more tobacco than females in this set up as is found in other parts of the world. (4)

In terms of sex distribution, both groups had an equal number of males and females and there was no statistically significant difference. The male to female ratio in this series was 24:1 confirming the strong association with the male sex. This is comparable to what has been found elsewhere and indeed some studies in a systematic review carried out by Farhad et al (65) have shown 100% male prevalence while the rest showed male predominance. The reason for this distribution was cited to be due to higher prevalence of smoking and alcohol intake among males compared to females which is true in this set up as depicted by KDHS. (66) This fact is further supported by case control study carried out by Sylvano et al (65) among female patients diagnosed with laryngeal SCC ($n= 68$) which showed cigarette smoking as the most important risk factor associated with laryngeal SCC followed by alcohol ingestion. This is similar to what has been seen among males in various parts of the world. (48- 51) As a conclusion in Sylvano's study (67), sex was not shown to have a significant causal or protective effect. Cigarette smoking still accounted for more than 70% of laryngeal SCC as shown in other studies involving both sexes. (48-52)

This study is in agreement with KDHS (9, 66) which shows that cigarette smoking among Kenyan females is low. In the Kenyan survey, about 2% of women said they used tobacco of any kind whereas 1% said they smoked a cigarette which is consistent with the low smoking prevalence of laryngeal SCC.

Of the 50 cases recruited, majority of them came from Kenya's Central province (46%) followed by Eastern province (16%), Rift valley (16%), Nyanza (8%), Western (6%), North Eastern (4%) and lastly Nairobi and Coast province at 2% each. An attempt was made to match the cases with controls according to geographical location but a larger percentage was derived from central Kenya (57%). This was due to lack of suitable controls but this did not affect the results obtained statistically ($p = 0.281$). This distribution shows that a majority of the cases came from Central

province followed by Eastern province and thirdly Rift Valley province which is in keeping with work done by Onyango. (13) This may be due to the fact that central and eastern provinces of Kenya are closer in proximity to KNH compared to other provinces. On the other hand, the prevalence of cigarette smoking has been shown to be highest in these provinces. (9, 66) Central province has the highest prevalence of 30.4% followed by Eastern province at 26.0%. (9, 66) This correlates quite well with higher laryngeal SCC in these provinces seen in this study. The other provincial estimates of cigarette smoking rates are as follows: Coast province (22.6%), Nairobi province (17.1%), North Eastern (15.6%), Rift Valley province (14.3%), Western (11.2%) and lastly Nyanza province (7.9%) which on both surveys, has had the least prevalence of cigarette smoking. This corresponds to lower risks in these regions. We can therefore postulate in this study that the higher rates of cigarette smoking in Central and Eastern province may be responsible for the higher prevalence of laryngeal SCC encountered. Of note is the lower prevalence of laryngeal SCC cases among Nairobi province residents. Majority of the patients in this study (59.6%) were aged between 55- 69 years. The low prevalence of cancer patients in this region may be explained by the fact that most of them may have retired to their respective provinces of origin.

With regard to occupation, majority of the cases were self employed (20%), 16% unemployed and 28% were on salaried employment. This was comparable to the controls ($p = 0.166$) and was therefore not statistically significant during analysis of this data. These findings are similar to KDHS (66) estimates when stratified for age. The large group of unemployed and self employed groups may be explained by the fact that most of the patients are elderly and may therefore have retired from employment. The KDHS (66) did not include respondents of the ages older than 49 years which would include the retirees and also fails to capture the age brackets where majority of our cases and controls are categorized.

Majority of cases in this study had attained at least primary or secondary education whereas only 16% had attained tertiary level of education. These findings when compared with controls were comparable ($p = 0.57$). This finding may have had an impact among the cases in the sense that low levels of education have been associated with development of head and neck cancer in general as shown by Onyango et al. (12) Raitiola et al (68) found increased risk for laryngeal SCC among respondents in the low social and economic status. This is in agreement with other studies done elsewhere. (45, 46, 67) Kapil et al (69) in India showed a similar correlation

between low socio economic status and laryngeal SCC. This is related to higher prevalence of cigarette smoking among patients of low socioeconomic status who also smoke cheaper hand rolled non filter cigarettes which have higher levels of carcinogens. There was no significant difference between filtered and non filtered cigarettes in this study (p = 0.198).

Out of the 50 cases in this study, 33 of them (66%) were present smokers compared to controls where only 3 (6%) smoked. Patients who were current smokers had a significant risk for laryngeal SCC in general compared to controls (OR 30.4) regardless whether they drank alcohol or not. This result is comparable to those found by Francheschi et al (48) in Northern Italy where ORs ranged from 2 to 15.6 for the shortest and greatest duration respectively. Metaanalysis carried out by Hashibe in central Europe (49, 50) showed similar findings OR= 12.83 for cigarette smokers only and OR 36.7 for those who also took alcohol. Findings in this study may have been affected by the much lower prevalence among the controls whereby the rate was 6% compared to estimates of 7% in Nyanza province who had the lowest prevalence according to KDHS (9,66). This may be explained by the fact that KDHS survey's maximum age range was 45-49 years whereas the average age of controls in this study is 61 years. Furthermore, the controls were hospital based and therefore their characteristics may not compare well with a population based group since the hospital group has been educated about the harmful effects of cigarette smoking and alcohol intake during their clinic attendance. Most patients in this study smoked filtered cigarettes. This was however not statistically significant (p=0.198).

ETS has been shown in many studies across the world to be an important risk for laryngeal SCC. (61, 62) Lee et al (51) and Secretan et al (63) in later studies showed no significant risk from ETS for laryngeal SCC. In this study, we did not find any significant increased risk for laryngeal SCC from ETS as the p values among cases and controls were 0.558 and 0.307 respectively. It should be pointed out that the respondents were few in both categories (2 among cases and 1 among the controls) and therefore further analysis could not be done.

14 (28%) of the controls were former smokers and 13 of them (26%) had stopped smoking for more than 10 years with a p value of 0.029 suggesting an inverse association with laryngeal SCC. Various studies done before have shown that there is reduced risk of developing laryngeal SCC after quitting smoking although the degree of reduced risk differs greatly depending on the age and duration. (51, 52, 54, 55, 56) Results in this study show that there is a progressive drop in risk after smoking cessation which was evident within 10 years of stopping (OR 19.5).

Furthermore, ever smokers still had a higher risk for developing laryngeal SCC in this study especially for supraglottic SCC (p=0.003, OR= 6.788).

Maier et al (70) in the Heidelberg case control study found that those who started smoking at a younger age and smoked for longer had a higher risk association with development of laryngeal SCC OR 9.7. This positive risk association is in keeping to the findings in this study where the OR 31.733 for those who began before the age of 20 years. Those who began smoking between the age of 21-40 years had OR 7.727 ($p < 0.001$, 95% CI: 2.409-24.787). This risk is higher than that published by Francheschi et al (48) who also found increased risk in other subsites in head and neck where cigarette smoking was a risk factor. It has also been documented by Wiencke et al (71) that there is molecular epidemiologic evidence showing that early age of onset of smoking produces biologic changes that enhance susceptibility to the effects of cigarette smoke carcinogens. He showed that among SCC cases, early onset of smoking predisposed to higher incidence of loss of heterozygosity of 3p21. This study similarly shows that early age of onset (under 20 years) is a significant risk factor for development of SCC larynx ($p < 0.001$ OR 31 95%CI: 8.75-115).

The mean pack years among cases were 31. When compared to controls there was an OR of 21.3 positive risk for laryngeal SCC which is in keeping with various studies done across the world. (48, 50- 53) Dosemeci et al (58) found OR of 6.0 (n=197) for those who had >21 pack years whereas Hashibe (49) found lower OR of 12.8 for the highest rank of mean pack years. These studies did not provide ORs for pack years of more than 30 but rather grouped them as >20 years. This may explain the relatively higher estimates in the current study although over reporting among cases may be a factor.

Francheschi et al (48), Hashibe et al (50, 53), Talamini et al (51) and Lee et al (52) in their studies showed that duration of smoking was one of the most important risk factors for laryngeal SCC. The highest stratification of duration of smoking of 40 years showed greater risk for laryngeal SCC of 15.6 as seen in Francheschi's study (46) whereas lower durations (<20 years) showed lesser ORs 2.13 as seen in Lee's study. The present study showed similar findings especially among those who smoked for >20 years (OR 12.7). 92% of the controls in the present study had quit smoking cigarettes at various time intervals during the study.

Alcohol consumption has been positively associated with laryngeal SCC mostly as a co factor and also as an independent factor. (8, 39) In this study there is an overall increased risk for

laryngeal SCC OR 2.3 ($p \leq 0.005$, 95% CI1.0-5.4). Dosemeci et al (58) in Turkey showed that those who ever drunk alcohol had OR of 1.7 in general with a maximum OR of 2.3 among patients who drank 35 drinks per week. The mean average drinks per week in this study were 58 which correlate quite well with the Italian study. Other studies performed in Europe showed similar findings. (51, 55)

Duration of alcohol intake has been found to be a significant positive risk factor for laryngeal SCC in many multicenter studies. This is particularly true for those who are heavy drinkers who have been found to have elevated risk for laryngeal SCC. (50, 53, 57, 59, 60) Findings in this study are similar although the risk seems higher compared to other regions of the world quoted above. It must be noted that each of these studies classified alcohol ingestion differently. On the other hand, the societal biases and stigmata differ from countries and may have altered how the study participants responded. From this study, those who were very heavy drinkers were found to have a higher risk for laryngeal SCC compared to controls OR 6.0 ($p \leq 0.002$ 95% CI 1.957-18.398). We may not read so much into these findings on alcohol intake due to the sample size since it was geared towards assessing the risk associated with cigarette smoking.

Subsite distribution of laryngeal SCC is known to be risk factor dependent. (57, 58, 59) Various studies have shown increased risk for glottic cancer among those patients who smoked only whereas those who smoked and drank alcohol developed supraglottic cancer more than glottic cancer. (57, 55, 58, 59) Other studies have however disputed these findings as shown by Hashibe et al (50) who found similar risks between supraglottic and glottic cancer. This current study is in agreement with earlier studies showing a strong risk for glottic cancer among patients who smoked only compared to controls ($p=0.001$, OR 19.75). Furthermore, being an ever smoker just like being a former smoker, is a well known risk factor for laryngeal SCC. (54)The results in this present study suggest that being an ever smoker confers a positive risk for laryngeal SCC across all laryngeal subsites; the highest being for supraglottic cancer p 0.003 OR 6.778 which is comparable to glottic SCC OR 6.1. Lewis et al (21) and other authors (17, 18, 19) showed that the glottis is anatomically the narrowest part of the upper airway and is therefore more susceptible to deposition of inhaled carcinogens found in cigarette smoke. This anatomic region also exhibits the transition zone from squamous epithelium to pseudo stratified columnar epithelium and is at a greater risk of cigarette smoke induced carcinogenesis. (22)

This study shows a significant risk (OR 10.476) for supraglottic cancer among those who inhale tobacco and consume alcohol. The supraglottis is unique from the other sub sites of the larynx since it is exposed to both inhaled and ingested agents. As is known from published studies, alcohol is a topical mucosal solvent for cigarette smoke carcinogens and therefore enhances their absorption. (8, 39) A possible chemical synergism has also been postulated. (36, 37, 38, 39, 40, 51) There was an overall increased risk for all sub sites when the two are consumed concurrently (Table 13). (48, 51, 57, 58, 59)

Cigarette smoking and alcohol ingestion have been shown to predispose to the development of less differentiated tumors compared to HPV which is known to predispose to the well differentiated (G1) SCC in the head and neck region. (72,73) In this study, majority of the patients who smoked only developed G1 carcinoma which is not in keeping with published data. (73) The number involved in this study is small and therefore we may not make statistical inference based on this. On the other hand, confounding factors such as HPV may have played a role despite this being a controlled study as it is now an acknowledged risk for head and neck SCC in general. In this study, alcohol consumption predisposed to G2 carcinoma while those who were alcohol drinkers and cigarette smokers as well developed the less differentiated G3 and G4 SCC (Tables 15 and 16). There was a higher chance for developing G3 carcinoma (OR 11.652), followed by G4 carcinoma (OR 7.286) and lastly G2 carcinoma (OR 4.218). This is in keeping to various studies done globally. (72, 73) Many molecular epidemiologic studies (43) have shown that alcohol intake and cigarette smoking were risk factors for mutation of p53 genes, p16 genes and over expression of cyclin D1. (43, 73) P53 gene mutation as well as cyclin D1 over expression among others, predispose to development of less differentiated SCC in the head and neck region. (43) Similar findings are seen in the present study. These tumors are aggressive, present late at an advanced stage and have high recurrence rate although in this set up various other factors come into play as regards to late presentation as shown by Oburra H.O (74) and later by Onyango et al. (13) In future, these molecular markers need to be studied in this population so that we can understand further what predicts the development of these poorly differentiated tumors in this set up.

Logistic regression showed that those who were current smokers had the highest risk for laryngeal SCC (OR 14.576) followed by duration of cigarette smoking (OR 7.312) as the only independent risk factors.

In this study, it is apparent that the duration of smoking and whether one is a current smoker show more consistent association with laryngeal SCC development than other measures of cigarette smoking. Those who stopped smoking cigarettes had a significantly reduced risk for laryngeal SCC although those who were previous smokers still had a higher risk compared to controls. There was an increased risk among those who smoked and drank alcohol across all laryngeal sub sites.

The PAR attributed to cigarette smoking was found to be 62%. This therefore means that the prevalence of laryngeal SCC would reduce by 62% if cigarette smoking was eliminated in this setting.

The findings in this study are similar to those found in other studies and therefore the null hypothesis is rejected and alternate hypothesis was found to be true.

19. CONCLUSION

Cigarette smoking is an important risk factor for laryngeal SCC in this set up. Those who smoked only had the highest risk for glottic cancer whereas those who smoked and drank alcohol had higher risks across all sub sites but mainly the supraglottis. On the other hand, smoking only had higher associations with well differentiated carcinoma whereas concurrent cigarette smoking and alcohol intake predisposed more to the less differentiated SCC of the larynx. The results of this study provide epidemiologic evidence that cigarette smoking and alcohol intake are strongly associated with laryngeal SCC in this set up.

20. LIMITATIONS

1. A few cases were controlled for by age and sex but not for geographical region of origin. This may have had an impact on the study although to a small extent since the p values obtained when the two groups were compared were not significant.
2. This was a hospital based study and therefore it may not reflect the true picture of the general population considering Berksonian selection bias where hospital based respondents tend to participate more readily in research projects compared to controls.
3. Being a case control study, recall bias may have impacted on the responses we got from the research subjects despite the fact that only the principal researcher obtained history and examined all patients.

4. The orthopedic trauma patients, some of whom were used in this study, may have had alcohol related accidents thus may have not been ideal controls.

21. RECOMMENDATIONS

1. Develop and standardize a clinical risk assessment form that will be used by every clinician in ENT H&N to document history, capture data on the head and neck risk factors with specific emphasis on the various aspects of cigarette smoking and alcohol consumption, family history of cancer and record clinical and endoscopic findings.
2. Explore the possibilities of setting up a head and neck cancer database (as those in other centers around the world) based at KNH where all head and neck cancer cases will be recorded so as improve our patient care through research and improved teaching.
3. Implement public education on the adverse consequences of cigarette smoking and alcohol consumption in relation to the development of head and neck cancer based on the findings of previously published and current research.
4. Formulate elaborate public health policies aimed at reducing the prevalence of cigarette smoking and alcohol consumption across the country.
5. Conduct studies on head and neck cancer preferably with larger sample sizes so as to conclusively elucidate other risk factors for head and neck cancers.
6. Conduct molecular and genetic studies so as to outline molecular events that occur in this population.

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23. APPENDIX I

GENERAL PATIENT INFORMATION AND CONSENT FORM

General patient information

We would like to seek your consent to participate in a study aimed at understanding the various aspects of the role of cigarette smoking and alcohol intake in the development of cancer of the larynx. This will include the age of onset of cigarette smoking, duration, and number of cigarettes smoked in pack years, sites of the larynx involved and effects of concomitant alcohol intake. This will enable us to elucidate the role of cigarette smoking in development of cancer of the larynx in our set- up.

How to participate

1. We will ask you questions seeking to know your age, region of origin, smoking habits as and your alcohol habits as outlined above.
2. We shall record all findings of examinations done and data obtained from the interview conducted among all participants in regards to cigarette smoking and alcohol intake.
3. Information regarding the results of tissue taken from the voice box to diagnose your disease will be obtained from your medical records and used for the purpose of this study (applies to those with cancer of the larynx only).
4. Similar findings from all participants in this study will be obtained and compared with data obtained from patients who do not have cancer of the larynx. The above data will be used to determine the chances of developing cancer of the larynx attributed to cigarette smoking.

How does your participation affect you?

It does not affect you adversely in any way because:

1. You will receive the same treatment even if you did not enroll for the study.
2. No treatment will be given to you in addition to what you require and neither will you be denied any treatment that you require.
3. All information obtained from you will be accorded confidential treatment.

Are there any hidden dangers?

1. None at all except for discomfort in the throat that will be experienced after spraying the throat with a topical spray anesthetic agent to allow for indirect laryngoscopy among the control patients. This should wear away in about an hour after completing the procedure.
2. Refusing to consent will not affect the management you will receive.

How does your participation help us?

1. The findings of this study will help us determine the risk attributed to cigarette smoking in development of cancer of the larynx in our set up. The findings will be presented in scientific conferences and published in journals without divulging specific patient information.
2. Cigarette smoking is a major public health concern as it has been linked to various diseases apart from cancer. The findings of this study will be useful in formulating specific public health policies and strategies aimed at reducing cigarette smoke related diseases.
3. You are free to discuss this with family members and we shall be ready to answer any questions raised. If you have understood everything that has been mentioned above and accepted to participate in this study, you may sign the consent form provided.

CONSENT FORM

Patient number……

Consent by the patient.

I……..of…… hereby give consent to be included in this study. The nature of the study has been explained to be by Dr…… He has NEITHER coerced me NOR has he forced me to be part of this study. I understand that there will be NO monetary gain in return.

Date……Signed……..

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

Date…… Signed……

MAELEZO YA UTAFITI KWA MGONJWA NA KIBALI CHA UTAFITI

Tungependa kukuomba ruhusa ya kukuhusisha kwenye utafiti huu ambao unanuia kufichua jinsi uvutaji wa sigara unavyochangia kusababisha saratani ya koo. Tungependa kujua umri ambao ulianza kuvuta sigara, muda ambao umevuta sigara na sigara ngapi unavuta au umekuwa ukivuta kwa siku. Tungependa kujua pia kama unakunywa pombe na vile unavyoongezea madhara ya uvutaji sigara.

Jinsi ya Kushiriki

1. Tutakuuliza maswali tukinuia kujua umri wako, sehemu unapotoka, na jinsi umekuwa ukitumia sigara na pombe kama vile umeelezwa hapo awali.
2. Tutarekodi matokeo yote ya maelezo ambazo utatueleza pamoja na yale tutafichua wakati wa kukupima. Tutarekodi pia matokeo kuhusu utumizi wa sigara na pombe kutoka washiriki wote wa utafiti huu.
3. Matokeo ya kipande cha nyama uliotolewa kwenye koo lako yatatumiwa kwenye utafiti huu (ambao wana saratani pekee).
4. Matokeo kutoka wanaojihusisha na utafiti huu ambao wana saratani yatalinganishwa na matokeo ya wale ambao hawana saratani ili kufichua kiwango ya saratani ya koo ambayo ulitokana na matumizi ya sigara.

Kushiriki unakudhuru vipi?

Haikudhuru kwa njia yoyote kwa sababu:

1. Utapewa matibabu sambamba na wale ambao hawatashiriki.
2. Hakuna chochote ambacho utapewa kukusawishi kushiriki kwenye utafiti huu na hautanyimwa matibabu yeyote unayohitaji usiposhiriki.
3. Habari zozote utakazotoa zitawekwa kwa siri.

Kuna madhara zozote ambazo zimefichwa yanayoweza kutokana na utafiti huu?

1. Hakuna wala tu usumbufu au kuwashwa koo ambao utatokea baada ya kuwekwa dawa kwenye koo ili tuweze kuchunguza kutumia kioo kwa wale hawana saratani. Usumbufu huu utaisha baada ya takriban saa moja.
2. Kutoshiriki hautabadili matibabu unayohitaji.

Kushiriki kwako kutafaidi vipi?

1. Matokeo ya utafiti huu yatasaidia kukadiri kiwango ya saratani ya koo ambayo inasababishwa na uvutaji sigara. Haya matokeo yatatumika kwa elimu ya sayansi na kwenye vitabu ili kuelimisha madaktari na wafanya kazi wengine wa afya.
2. Uvutaji sigara ni mojawapo ya vitu ambavyo yanadhuru afya ya umma na husababisha magonjwa mengi mbali na saratani. Matokeo ya utafiti huu yatasaidia serikali kupitia wizara ya afya kuunda mikakati na sera za kupunguza magonjwa yanayohusishwa na uvutaji sigara.
3. Una huru kujadiliana na familia yako kabla ya kujihuzisha na utafiti huu. Tuko tayari kujibu waswali yote utakayouliza. Iwapo umeelewa na kuridhika na maelezo hayo na umekubali kushiriki kwenye utafiti huu, utatia sahihi kwenye kibali cha utafiti unaofuatia.

KIBALI CHA UTAFITI

Nambari.....

Mimi.....kutoka.....ninakubali kushirikishwa katika utafiti huu. Nimeelezwa kuhusu utafiti huu na Daktari.....

SIJALAZIMISHWA kujihuzisha na utafiti huu. Naelewa ya kuwa utafiti huu hautanifaidi kifedha.

Tarehe.....Sahihi.....

Mimi Dr.....nahakikisha ya kuwa nimemweleza mgonjwa kuhusu utafiti huu.

Tarehe.....Sahihi.

24. APPENDIX II

SMOKING INFORMATION QUESTIONNAIRE.

1. Research Number ☐

2. Age Years ☐

3. Sex Male ☐ Female. ☐

4. Region/ Province
   - Nairobi ☐ North eastern ☐
   - Nyanza ☐ Western ☐
   - Eastern ☐ Coast ☐
   - Central ☐ Rift Valley ☐

5. What is your occupation? Salaried ☐ Unemployed ☐ Self Employed ☐ Student ☐

6. Education Level None ☐ Primary ☐ Secondary ☐ Tertiary ☐

7. Are you a Current Smoker? YES ☐ NO ☐

8. If you are a former smoker, how long ago did you stop smoking? Years ☐

9. What type of cigarette do you smoke? FILTERED ☐ NON FILTER ☐

10. At what age did you begin to smoke cigarettes? YEARS ☐

11. How long have you been smoking? YEARS ☐

12. What is/was the total number of cigarette packs you use/used per day? ☐

13. Do you live with someone who smokes? YES ☐ NO ☐

14. How long have you lived with the person who smokes? YEARS ☐

15. Does he/she smoke inside the house? YES ☐ NO ☐

16. Do you drink alcohol? YES ☐ NO ☐

17. How long have you drunk alcohol? YEARS. ☐

18. What kind of alcohol do you take?

Traditional brew ☐ Bottled beer ☐ Spirits ☐ Wine ☐ Any ☐

19. How many times do you drink in a week? DAYS ☐

20. How many alcoholic drinks do you take in a week? DRINKS ☐

21. At what age did you start drinking alcohol? ☐

22. Tumour Characteristics

1. Histological grade:

Well Differentiated (G1) ☐ Moderately differentiated (G2) ☐

Poorly differentiated (G3 ☐

Undifferentiated (G4) ☐

2. Stage

i ☐ ii ☐ iii ☐

iv ☐

3. Laryngeal subsite

Supraglottis ☐ Glottis ☐ Subglottic ☐

Transglottic ☐

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2nd March, 2011

Ref: KNH-ERC/ A/37

Dr. Owen Pyeko Menach
Dept. of Surgery
School of Medicine
University of Nairobi

Dear Dr. Pyeko

RESEARCH PROPOSAL: "CIGARETTE SMOKING AS A RISK FACTOR FOR LARYNGEAL SQUAMOUS CELL CARCINOMA AT KENYATTA NATIONAL HOSPITAL " (P8/01/2011)

This is to inform you that the KNH/UON-Ethics & Research Committee has reviewed and **approved** your above revised research proposal for the period 2nd March 2011 – 1st March 2012.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimens must also be obtained from KNH/UON-Ethics & Research Committee for each batch.

On behalf of the Committee, I wish you a fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely,

PROF A N GUANTAI
SECRETARY, KNH/UON-ERC

c.c. The Deputy Director CS, KNH
The HOD, Records, KNH
The Chairman: Dept. of Surgery, UON
Supervisors: Prof. H.O. Obura, Dept of Surgery, UON
Dr. Asmeeta Patel, Dept. of Surgery, KNH