CELLULAR MORPHOLOGICAL CHANGES ON ORAL MUCOSA AMONG PATIENTS PRESENTING IN TO THE DENTAL CLINICS IN TWO NAROK COUNTY REFERAL HOSPITALS

BY

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DECLARATION

I hereby declare that this is my original work and has not, to my knowledge been submitted for the award of degree in any other university or institution of higher learning

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DEDICATION

To My Daughters who have stood by me. When it was my lowest moment to quit in life you were there. My sister Tabitha Kerubo who made sure she was there any moment from the hard life in Trans-Mara to Kiambogo settlement scheme when mama needed her to being an MBA Graduate of Meredith college was encouraging. My daughters, Okenyuri, Mokobi, Gechemba and Nyaboke. My sons Mbaka and Makori. All the assignments and typing work of this project cannot be printed without you being beside my frequent nagging Irene Kwamboka Nyankira, be blessed always.

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TABLE OF CONTENT

LIST OF FIGURES

LIST OF PHOTOMICROGRAPHS

LIST OF TABLES

LIST OF ABBREVIATION

AIDS	Acquired Immunodeficiency syndrome
SOP	Standard operating procedures
TBS	The Bethesda system
UON	University of Nairobi
KNH	Kenyatta National Hospital
PAP	Papanicolaou
H&E	Haematoxylin and Eosin
WHO	World Health Organization
CDC	Centre for disease Control
ERC	Ethical and review Committee
HIV	Human immunodeficiency Syndrome
OSCC	Oral squamous cell carcinoma
NILM	Negative for Intraepithelial lesion or Malignancy
TMJ	Temporal mandibular Junction
PIN	Personal Identification Number
PI	Principal Investigator
HSV	Herpes simplex Virus
ACS	America Cancer society
AMS	America Medical society

Abstract

Cytological effects of chewing tobacco have been documented in India, Europe and North America. Use of cytology to investigate changes in cellular morphology associated with chewing tobacco has not been done in Kenya. This study utilized brush cytology for diagnosis of inflammatory, pre-malignant, malignant and other associated pathological conditions among the people who chew tobacco presenting at two Narok County level 4 hospitals.

Objectives: To describe clinical and cytological changes in the oral mucosa of patients who chew tobacco in two Narok County level 4 hospitals namely Kilgoris and Narok.

Setting: Recruitment was done in two level 4 hospitals Dental clinics with assistance from the county dentists and dental assistants. Half of the participants were recruited in Kilgoris and the other half in Narok.

Subjects: One hundred patients with a history of chewing tobacco for more than six years were recruited in total after consenting.

Design and Methodology: This study was a cross sectional descriptive study that in two level four hospitals in Narok county over a period of 6 months (March –September 2016) among

Patients presenting to two Narok County level 4 hospitals who were oral tobacco users. Cytobrush procedure was used to collect cellular material from the oral cavity of patients presenting to the dental clinics. Once the sample was taken, it was gently rolled on three microscope slides to make an imprint. Two slides were immediately fixed by immersion in 95% ethanol and stained with Pap stain and H&E. The third slide was air-dried and stained with a Romanowsky stain. After staining in KNH/UON cytology laboratory slides were examined by the principle investigator and two pathologists who are the supervising pathologists to the principal investigator. Face to face interview was used to collect the demographic data as well as risk factors for oral mucosa diseases.

Data management and statistical analysis

Frequency and percentages were used to describe the patterns of oral mucosa cytology including the occurrence of neoplastic lesions that were reported. Statistical analysis was done using STATA VERSION12

RESULTS: Most of the participants were male (76**%).** Majority were negative (95%) for oral mucosa neoplasia and (5%) had atypical squamous cells of undetermined significance. 9% had bacterial infection and 20% had Candida probably due to poor oral hygiene and tobacco chewing

or sharing. The duration of chewing tobacco among the subjects ranged from 6-65 years. The longer duration of chewing did not have significant association with oral lesions.

Conclusion: Candida and Bacterial infections were the most common cytological findings among the tobacco chewers in Narok County.

Oral mucosal neoplasia was least observed even among the elderly who were oral tobacco users for more than 40 years. Analysis of association of risk factors was performed and found not to be significant.

Recommendations.

1.0 Most of the findings could be attributed to poor oral hygiene of oral mucosa during the chewing processes. As a county health committee could educate the residents

2.0 The physical observation of Leukoplakia needs to be looked at to avoid development to full term neoplasia.

3.0 Candida and bacterial infections are the most common findings among the 100 subjects. This could be attributed to poor oral hygiene, mechanical biting during the chewing process as this could requires public education.

CHAPTER ONE

1.0 INTRODUCTION

Oral and pharyngeal cancers reportedly combined represent one of the most common type of cancers. Reported as the seventh cause of deaths worldwide, oral cancer are ranked more common than pharyngeal cancers and recorded as fifth most common in occurrences (1). These cancers combined oral and pharyngeal are reported as three times more common in males as in females2 who also use oral tobacco products (2). Factors such as alcohol, recently reported Human papiloma virus (HPV) infections3 and exposure to some chemical substances in some Asian countries have been associated with oral cancer as well as oral use of tobacco by chewing in countries such as China (4).

Tobacco chewing, is known to contain nicotine with harmful effects to various body systems .According to researchers in the united states of America, nicotine has been detected in some urine samples with other active ingredients or compounds found in tobacco (5).These compounds which are released in the process of chewing into saliva are mainly absorbed through the oral mucosa surfaces (5). The oral mucosa may be susceptible to toxic substances through the exposure of high doses of tobacco components, rendering it susceptible to potentially toxic effects. Tobacco chewing continuously induces oral lesions on the buccal mucosa surfaces. These lesions have been shown to occur specifically at the regions where the bolus is placed during chewing as this turns genotoxic to cells in the oral mucosa (6).Thus when these lesions are irritated at some point several studies suggesting strong association between tobacco use and oral lesions like keratosis, hyperkeratosis leukoplakia and oral cancer development.

Current reports show that the prevalence of oral cancer associated with tobacco chewing is on the rise in developing countries a shift from the previous increase in developed countries (7).

The carcinogens acquired from tobacco may lead to acquisition of genomic disorders that proceeds to malignancy. Two types of precancerous lesions in the oral cavity are identified, the principal precancerous lesion being white lesions leukoplakia and the less common red lesion erythroplakia (8). In a population with a high prevalence of oral leukoplakia in the oral cavity, this is a strong risk indicator of oral cancer or malignancy. In addition, the use of any form of tobacco has generally been accepted as the principal etiologic factor for oral leukoplakia that may lead to malignancies (9). However, in some cases the observation of these lesion in individuals who do not have oral leukoplakia (10), other factors may play role in individuals who do not use tobacco (11). Alcohol and tobacco are suspected as possible etiological co-factors for oral mucosal cancer, but their independent roles have not been established separately in most cases12.

In Kenya, in addition to chewing tobacco, the Maasai people widely use alcohol which is popular because of their socio-cultural values where the passage to adulthood where by tobacco use is an acceptable cultural way of life. This ethnic group is ranked high among communities that may be affected by both oral and naso-pharyngeal carcinomas because of mixed high tobacco use in the form of powder (snuff) that is taken through the nose besides chewing of loose leaves of tobacco (13).

The success of oral cancer prevention in this region will rely upon the understanding of the prevailing conditions, risk factors and identification of possible etiological factors involved in proper management so that precancerous may not progress to cancerous lesions (14). This study therefore sought to describe the oral mucosal cytological changes among patients who chew tobacco within Narok county, were attending the dental clinics presenting with dental problems

and other factors associated with oral mucosal. Although the Maasai people occupy much of Narok and Kajiado counties in Kenya, the majority are spread to Northern Tanzania.

2.0 LITERATURE REVIEW

2.1 BACKGROUND

Kenya is a country with an increase of cancer cases annually, but there is no central cancer registry (Data centre) with records which can be relied upon, with exception of a few cases of news papers reporting about cancer, Kenyatta national Hospital medical records, and Nairobi cancer registry. Unlike other countries with advanced cancer registry, such as German, Switzerland, the United States of America and most European countries that keep track cancers cases and types (14).

The most recent available literature focuses on tobacco as a cash crop and public health concerns about tobacco use among the youth as a drug of abuse. The cytological effects of oral tobacco use has never been researched in Kenya and recorded. Countries such German and Switzerland keep records on diagnosis, treatment, and follow-up on oral cancer patients, have also incorporated the international guidelines and standards that are comparable with (WHO) (15).Tobacco chewing habit is worldwide practice in many forms. In Kenya and especially Narok County, the habit of tobacco chewing is wide spread, especially among the older generation of men and women. In Asia Pacific quid and tobacco chewing have been shown to be a major risk factor of oral cancer when combined with alcohol consumption (16).

Cultural habits play a big role in Indians of South Africa who have 68% of cheek cancer attributed to tobacco and areca nuts chewing. The aerodigestive tract cancers rate follows in order of smoking, alcohol consumption, betel chewing (17)

Huron Indian myth has it that in ancient times, when the land was barren and the people were starving, the Great Spirit sent forth a woman to save humanity. As she traveled over the world, everywhere her right hand touched the soil, there grew potatoes. And everywhere her left hand touched the soil, there grew corn. And when the world was rich and fertile, she sat down and rested. When she arose, there grew tobacco (18)

Prehistory: Although small amounts of nicotine may be found in some old World plants, including belladonna and *Nicotiana Africana*, and nicotine metabolites which have been found in human remains and pipes in the Near East and Africa, there is no indication of habitual tobacco use in the Ancient Americas, as their experts believe that tobacco plant, as we know it today, began growing in the Americas(18).

The fresh mature cut leaves are fire cured or naturally dried under the sun , processed for use and transported to the market in some areas around Narok county, from areas such as Kuria in Migori County. However the majority of the loose leaves tobacco reaching Narok town is shipped from as far as Meru and Bungoma counties. The succulent stems and leaves are harvested regularly from the tobacco plants, and are highly valued for their stimulating properties. Nicotine is the major component of tobacco where chewers may also experience irritability and insomnia due to harmful effects of chemicals released into saliva during chewing process and absorbed through the oral mucosa (19). The oral mucosa is thus exposed to high doses of nicotine constituents, rendering it susceptible to some effects. Tobacco chewing is associated with oral cavity changes such as hyperkeratosis, epithelial hyperplasia, mild dysplasia and increased periodontal disease including oral cancer (11).

2.2. Cultivation of tobacco around Narok County

Tobacco product prepared from the leaves of the tobacco plant is part of genus in Nicotiana and of the Solanaceae (night shade) family in Kenya the evergreen shrub of the family Nicotiana (20). Scientifically first classified as part of Genus in Nicotiana because is group of herbs and shrubs in the nightshade family and cultivated in Migori , Meru , Bungoma Elgeyo Marakwet Counties which happen to be major suppliers to the consumers in Narok, Baringo, Kajiado Samburu and other counties where tobacco chewing is minimal.

2.3 Economic importance of Tobacco

Globally people who chew tobacco on a daily basis are many (23). In places where it is grown on a large scale such as in the North America continent most is processed into, cigarettes, cigars and also packaged in sachets or small tins sold in the various markets. In Kenya this is not a common trend cigarette manufacturer's control tobacco market from the small scale farmers to packaging and selling of the products. However there are few farmers who are not controlled by the cigarette companies that harvest their mature tobacco products and ship to various market places packed in small sachets for sale, where the common tobacco chewer can buy the dried leaves for chewing. Tobacco chewing has a long history as social custom involving men and women. Tobacco leaves business is source of income for many who would otherwise be jobless in Kenya as the economy has languished in the doldrums, cultivation and marketing of tobacco is at an increase due to competition between the two major cigarette manufacturers namely: BAT and Mastermind tobacco companies (24). Domestic consumption now extends beyond traditional tobacco-chewing communities, where by even ethnic groups with no history of oral tobacco use, the young people are chewing tobacco, since the price of tobacco leaves is cheaper than packaged cigarettes in many areas around Kenya, and tobacco chewing is on the rise (25).

2.4 Composition of Tobacco

The environment and climate dictate the chemical composition in tobacco such as nicotine phenols, nitrosamines, carbon monoxide nitrogen, oxides volatile aldehydes and others. (Table 1) profile of tobacco leaves. The taste of tobacco leaves varies from one type to another and depends on the tannicacid content. The leaves have an astringent taste and a characteristic aromatic odour (25). Various compounds (over 4000) have been identified in tobacco, including alkaloids, aldehydes (acrolein, formaldehyde), carbon monoxide, hydrogen cyanide, nitrogen

oxides, benzene, toluene, phenols (phenols, cresol), and aromatic amines (nicotine, ABP (4-Aminobiphenyl). The radioactive element polonium-210 is also known to occur in tobacco smoke. The chemical composition of smoke depends on puff frequency, intensity, volume, and durable at different stages of cigarette consumption

TUMORIGENIC AGENTS IN TOBACCO	
Compounds	**In processed tobacco, per gram**
Benzo (a) pyrene	0.1-90ng
Quinoline	1-2ug
N-Nittrosodimentylamine	0-215ng
N-Nitrosonornicotine	0.3-89ug
4-(Menthylnitrosamino)-1-(3-pyridyl)-1-butanone	0.2-7up
N-Nitrosoanabasine	0.01-1.9ug
N-Nitrosomorpholine	0.690ng
Forldehyde	1.6-7.4ug
Acetaldehyde	1.4-7.4ug
Crotonaldehyde	0.2-2.4ug
1,1Dimethylhydrazine	60-147ug
Ethyl carbamate	310-375ng
Hydrazine	14-51ng
Arsenic	500-900ng
Nickel	2000-6000ng
Chromium	1000-2000ng

Cadmium	1300-1600ng
Lead	8-10ug
Polonium-210	0.2-1.2 pci

Table 1 Composition of tobacco **(**from Balint et al (17)

2.5 Toxicology effects of tobacco in humans

Chewing tobacco (smokeless tobacco) has adverse effects associated with many health problems such as.

- Addiction to smokeless tobacco
- Smokeless tobacco and cancer
- Smokeless tobacco and oral diseases
- Reproductive and developmental risks.

Cardiovascular System	**Gastrointestinal System**	**Hepatobiliary System**
Tachycardia Myocardial infarction Cerebral haemorrhage Pulmonary edema) Heart attack and stroke	Dry mouth, dental caries Periodontal disease Paralytic ileus Weight loss Duodenal ulcer Malignancy	Fibrosis Cirrhosis **Oral cavity** White gray patches Leukoplakia Gum disease Tooth decay Tooth loss
Genitourinary system	**Respiratory system**	**Obstetric effects**
Urinary retention Malformations	Tachypnoea Bronchitis	Low birth weight Still births
Metabolic and endocrine effects	**Ocular effects**	**Central nervous system**
Hyperthermia Perspiration	Blurred vision, mydriasis	Dizziness Impaired cognitive Function Fine tremor

Table 2: Toxicity and side effects of smokeless tobacco in humans (from Balint et al (17)

Generally chewing tobacco has been shown to affect almost all human systems, organs and functions. The main toxic effects include increased blood pressure, tachycardia, insomnia anorexia, constipation, general malaise, irritability, migraine and impaired sexual potency in men (24). Further, tobacco affects the nervous system to some extent.

Tobacco induces cardiovascular complications by increasing episodes of acute myocardial infarction during or after tobacco chewing episodes (26). Tobacco chewing has also been reported to be a significant risk factor for a cute cerebral infarction (26). Chewing affects the oral cavity and certain parts of the digestive tract (27). The tannins present in the tobacco leaves are thought to be responsible for the gastritis that has been observed in tobacco chewers. Chewing tobacco reduces the absorption of ampicillin and to a lesser extent that of amoxicillin but the effects are minimal, lasting two hours after tobacco chewing stops (28). Detailed studies on the possible effects of tobacco chewing on human reproductive system are listed as low birth weight, brain damage to the fetus, lung problems during growth that exposes the infant to various infections. However, the available data suggest that chronic use may lead to decreased sexual function and importance (29).

2.6 Oral cancer

Oral (the lips, teeth, and gums, the front two-thirds of the tongue, buccal mucosa, under the tongue, hard palate, the small area behind the wisdom teeth and pharyngeal the backbone-third of the tongue, the soft palate, the tonsils and the back of the throat) cancers combined represent the fifth most common type of cancer and the seventh cause of deaths by cancer worldwide, although oral cancer is more common than pharyngeal cancer (28). Oral and pharyngeal cancers are three times more common in males than in females (30).Alcohol and tobacco are known risk factors for oral, pharyngeal, hypopharyngeal and nasopharyngeal cancers (29). There is evidence

of a dose-response relationship between their consumption, addiction and the occurrence of these cancers, as well as a strong interaction between them (31). It has also been reported that a diet low in fruits and vegetables, (HPV) infections and people who are excessively exposed to the sun rays are at risk oral cancers and others like pharyngeal, nasopharyngeal and cardiovascular diseases (5, 32).

2.6.1 Types of oral cancer

Table 2 shows the different types of oral neoplasm. Some oral tumors can be benign (non-cancerous), may be some condition such as precancerous (to become cancerous), while others may be cancerous upon diagnosis. There are different types of oral cancers which may develop in different areas of the mouth and throat at different times. The precancerous conditions in the mouth are grouped into the leukoplakia and erythroplakia. The leukoplakia condition is characterized by a whitish patches which at times develops inside the mouth and throat while erythroplakia is characterized by a red and raised patches also developing inside the mouth. In some studies (25) percent of Leukoplakia may develop into cancer while erythroplakia are found to be 70 percent of time cancerous (33) often caused by tobacco chewing ,or other forms. These initially benign conditions have been documented to occur anywhere in the mouth when properly followed. Dysplastic or cancer cells may be present in a leukoplakia or erythroplakia whereby only a biopsy can determine these precancerous cells.

Most common type of oral cancer happens to be squamous cell carcinoma, which also happens to be about 40% of the cancers that begin on the floor of the mouth or o the side and bottom of the tongue, and another 40% that may occur on the lower lip occasionally. The remainder begins on other parts of the mouth including tonsils and salivary glands. This hard lump may form cancers or ulcers that are firm-bordered may bleed intermittently as sores ((34). Areas affected may

appear as mixed white and red smooth or raised white, red patches. Another variant form of squamous cell carcinoma) is verrucous (warty) carcinoma that may appear white on grooved surface of the buccal mucosa surfaces (37). Melanoma can be the other type of cancer, Kaposi sarcoma are not common as malignant forms that predominate the oral mucosa. With a history of sunburns usually associated with malignant melanoma that occurs on the surface of the skin in most diagnosis occasionally

occurs in the roof mouth, most common records show spread from a skin site to the mouth, usually result in to malignant melanoma often has uneven, irregularly shaped borders with different ranges in colour from brown to black dark blue. Kaposi`s sarcoma found in people with AIDS, is cancer of the blood vessels lining near the skin of the mouth and throat. When this sarcoma occurs, it's usually on the roof of the mouth. The tumour is slightly raised and usually blue or purple in appearance (37).

Noncancerous growths of the salivary glands are much less common than salivary gland cancers which are mucoepidermoid carcinomas, which typically forms in a small minor salivary glands or roof of the mouth in most cases. The malignancies may occur as a lump in some of the larger glands related to salivary, either behind, lower, under or on the side jaw. Jawbone Cancers may include osteosarcomas and other metastatic tumours.

MALIGNANT TYPE	DESCRIPTION
Squamous cell carcinoma	originates in the squamous cell lining of the oral cavity and oropharynx. In the early stages, this cancer is present only in the lining (carcinoma in situ). When the cancer spreads beyond the lining, it is called invasive squamous cell cancer.
Verrucous carcinoma	Also considered a type of squamous cell carcinoma, this low-grade cancer rarely metastasizes. Comprising less than 5 percent of all diagnosed oral cancers, verrucous carcinoma can spread deeply into surrounding tissue, requiring surgical removal with a wide margin of surrounding tissue.
Minor salivary gland cancers	The lining of the oral cavity and oropharynx contains numerous salivary glands. Rarely cancer may originate in a salivary gland. Treatment depends on the type and location of the salivary gland cancer, as well as extent of spread.

Table3: Types of oral tumours

2.7 Loose leaves tobacco and oral cancer.

In Kenya, its mostly speculated of an association between chewing tobacco and oral malignancies (39). In other parts of the world, over 50% malignancies that develop in to oral mucosa are from people who use tobacco products as either chewing alone or combined with other substances (40). These lesions changes are considered a pre-cancerous and may finally develop into oral cancer without proper diagnosis and management. White lesions which are keratotic in nature are present in the mouths of small population of tobacco chewers (about one-fifth) cavity (41). Buccal cavity epithelial cells can experience genotoxic effects depending on dose related to oral dipping of tobacco as reported in the Indian study of 2011(43) . Duration of oral tobacco use, sometimes contributes to prevalence or severity of these lesions which

increases with frequent use of others substances (42). With continuous research which is important for determination of oral malignancies due to oral tobacco use within narok county. Relationship between oral malignancies and oral tobacco use is an important step on oral cancer and chewing tobacco in Kenya.

2.8 Epidemiology of tobacco induced oral cancer

Macigo et al (15) found leukoplakia among oral tobacco users examined in Kenya during his study, which was not significant in association between oral tobacco and leukoplakia that was compared then. Hill and Gibson, (1989) compared oral white lesions, among 50% of tobacco of oral tobacco users in Yemen resembling those of frictional keratosis of the mouth of oral tobacco users was noted by macigo etal. Prolonged Oral tobacco use for a long period, has been recorded as cause of stomatitis, pain and other effects, including stress and staining or teeth colouration, pre-malignancy or malignancy (42). However, no histopathological study was done to confirm the absence or presence of dysplasia or malignancies among the subjects. A recent study (2012) which

concluded that tobacco use is one of the risk factors that increases the chances of oral lesions such gingivitis, temporomandibular junction (TMJ) periodontitis and dryness of the mouth (43). The malignant lesion was observed to occur at exactly the same site as where the tobacco bolus was held (43). The authors came to conclusion that there was a strong correlation between oral tobacco use and oral cancer in this case. There is an increased risk of oral carcinoma amongst oral tobacco chewers (43), Other than tobacco use itself, mixing with alcohol consumption (43) tobacco has been found to be a significant factor in the high prevalence of

head and neck squamous cell carcinoma, dysplastic lesions and others among the Yemeni population (43).

2.9 Detection of oral pre-cancerous and cancerous lesions

Detection of oral cancer or other lesions among asymptomatic patients in early stage dramatically improves patient management process, thus reducing morbidity and mortality (44). It is difficult to clinically differentiate by visual detection or observations, oral cancers at early stages, the signs of premalignant and malignant lesions which are similar looking to benign lesions. Mostly diagnosis is made by scalpel biopsy which remains the most accurate, reliable method for diagnosis of many oral mucosal abnormalities in many parts of the world (45). However, the invasive nature of this method makes it not only patients, but also surgeons to be reluctant, and fearful of such an invasive surgical procedure but also the economic cost that come with the procedure. The patients` reluctance may be compounded by the clinicians` hesitance to perform surgical procedures in an unfamiliar setting or anatomical site since these areas requires specialized surgeons (38). The use of cytobrush has a wide acceptance in clinical application when assessment of surface oral and oropharyngeal mucosal abnormalities. Early assessment of such lesions may lead to prompt identification of dysplasia which may require minimal invasive procedure to remove lesions. This increases cure rates than subsequent scalpel (surgical or excision)) biopsy(45).

2.10 JUSTIFICATION

As is the evidence from the literature (review), oral cancer is a health problem which concerned nations cannot afford to ignore. Tobacco has been identified as one of the many risk factors that contribute to development of oral cancers and other cancers such as lung cancers mainly among cigarette smokers. The natural history of oral cancer gives an opportunity to make diagnosis at an early stage which if properly managed could lead to prevention of development to invasive stage. Fortunately, there are methods for early detection including oral brush cytology which is cheap, non-invasive, fast and reliable hence potentially helpful because of high chance of acceptability by both the clients and medical practitioners. Methods for treating these non-invasive lesions are also available. This method would obviate surgery with its attendant risk while achieving a similar goal.

Kenya is one the producers/consumers of tobacco products associated with development of oral cancer. Currently, there is limited data in Kenya on the relationship between tobacco chewing and oral diseases, cancer in particular, as this justify the need to conduct studies to provide data and find other risk factors in relation to neoplastic and non-neoplastic oral diseases.

2.11 Research Question

Are there cytological changes in oral mucosal cells of oral tobacco chewers in Narok County, Kenya?

2.12 Objectives

2.12.1 General objective

To describe the cytological changes of oral mucosal cells, among oral tobacco chewers in Narok County, Kenya.

2.12.2. Specific objectives:

a) Primary

1. To describe the pattern of oral mucosal cytological findings among habitual tobacco chewers.
2. To determine the prevalence of neoplastic lesions on oral mucosal cytology among tobacco chewers.

b) Secondary

3. To identify other potential risk factors, associated with oral lesions among oral tobacco chewers.

3.0 MATERIALS AND METHODS USED

3.1 Study site

This study was conducted at dental clinics at two Level 4 Hospitals in Narok County Kenya. (Kilgoris level 4 and Narok Level 4)

3.2 Study design

This was a cross-sectional descriptive study aimed at determining the prevalence and describing the pattern of oral mucosal cytological changes associated with oral tobacco chewing.

3.3 Study population

Consent and recruitment was done among adults both male and female aged 26 years and above, with a history of chewing tobacco for at least six years. The criteria of choosing adults above 26 years was arrived at due to culture that an adult is a person over 20years who has graduated from the manyatta moran seclusion that is when he can be allowed to use tobacco or alcohol.

3.4 Selection criteria

3.4.1 Inclusion criteria

1. Persons (male or female) with a history of chewing tobacco for over 6 years.
2. Adults 26 years and above

3.4.2 Exclusion criteria

1. Prior diagnosis of oral cancer or intraepithelial lesion.
2. Non consenting patients
3. Chewing tobacco and smoking cigarettes at the same time

3.5 Sample size determination

The sample size was calculated using Fisher`s formula. Oral white lesions prevalence of 7% was used having been obtained from a study in India conducted in 2011 which found the preference of oral mucosa (50)

$$n = \frac{Z^2P(1-P)}{d^2}$$

Where; n is the minimum sample size for proposed study

Z is the normal standard deviation corresponding to 95% confidence interval

P is the known prevalence

D is the margin of error of precision set at ± 5%

$$n=\frac{1.96^2 \times 0.07\ (1-0.07)}{0.05^2}$$

$$= 100.0352$$

$$= 100$$

3.6 Sampling method

The first phase of the study was to identify the dental clinics in Narok County where tobacco chewers visit when they have dental problems. A convenient sampling method was used to increase recruitment into the study. The study used convenient-consecutive sampling technique, with the help of research assistants, to randomly recruit all accessible and consenting oral tobacco chewers who were above the age of 26 years and had chewed tobacco for more than 5 years, and were not cigarette smokers. Data was collected from the participants until the target of the study was achieved. Those eligible for the study were allowed to get their dental work procedure done before samples for the study were taken. However the dentist and dental assistants gave them fast track priority to save time for sample collection. This went on until the required sample size was attained as requested by the PI.

3.7 Data and specimen collection

Data was obtained in the following manner. Socio-demographic information from consenting participants was filled into pretested questionnaires. Oral assessment and the findings were recorded on the laboratory form. Collection of oral samples was done at Kilgoris and Narok level IV Hospitals. Lastly, data pertaining to cytological findings was obtained following screening and signing out of the smears at KNH/UON cytology laboratory (Appendix V)

3.8 Laboratory investigations

3.8.1 Oral sample collection

Research assistants (who were trained dental technician in the dental unit in the two county level 4 hospitals) helped the principal investigator to collect cellular smears from participants' after dental work. A cytobrush was used to collect cells from surface oral

mucosa surfaces by vigorous abrasion. The preferred chewing site (where the tobacco bolus used to stay) was the target site for collection. The collected specimen was smeared onto three clean pre-labelled glass slides and two slides were fixed in 95% alcohol immediately for at least 15 minutes. The third slide was air dried

3.9 Biosafety measures

Aseptic procedures were applied at all times during collection, receiving, transporting, storage, processing and analysis of samples. Both fixed and non smears were arranged in safe slide boxes and transported to KNH/UON cytology laboratory where all the samples were processed following the SOPS of KNH/UON cytology laboratory. (Appendix iii)

3.9.1 Staining method

The staining procedure was done at KNH/UON cytology laboratory .The fixed smears were stained using Pap and H& E staining methods while air dried slide was stained by giemsa method (10). All the standard operating procedures (SOPs) for the staining methods were followed to ensure the reliability and reproducibility of the results. (Appendix iii)

3.9.2 Cytological assessment and Reporting

All the samples were screened by the P1 and signed out by the supervising pathologists. The abnormal smears and 10% of the negative smears were signed out by in independent pathologist. Smears with discordant result were signed out by the two supervising pathologist in the department of human pathology using the teaching multi-head microscope. Presence or absence of cytological findings and the non-epithelial components were reported using The Bethesda Reporting System for cytology (TBS) 2001 (Appendix IV)

3.10 Variables

The independent variable in this study is presence of oral mucosal lesions.

The relationship between presence of oral neoplasm lesions and the following dependable (variables) were modelled: age, residence, gender, occupation, education level, marital status, duration (years) of chewing Tobacco amount chewed per day, length of time Tobacco bolus stayed in the mouth, frequency of chewing tobacco, alcohol use and duration of use, presence of oral cavity lesions. Oral and other cytological findings

3.11 Data Management

All participants were assigned a unique personal identification number (PIN). All data entered into the study database were delinked from the study subjects and only associated with a PIN in password protected files. Double entry system for the data was at all times maintained. All paper research records were kept in a locked filing cabinet where only the PI had access. The biological samples were identified using PIN and were stored in boxes with restricted access to only the supervising pathologists.

3.12 Statistical Analysis

Statistical analysis was done using STATA version 12 (stat Corp LP, Texas, USA) at a significance level of $P \leq 0.05$. The prevalence of oral mucosal cytological changes and the distribution of the demographic characteristic were calculated using chi-square and presented in terms of frequency and percentage.

3.13 Quality assurance

Standard operating procedures of KNH/UON were adhered to, those pertaining to labelling of slide, specimen collection, transportation, analysis and posting of results.

Sample collection was aseptically done. Fixed and dried smears were packed in slide boxes and transported to KNH/UON cytology laboratory for processing and analysis. All reagents were prepared in accordance with standard operating procedures (SOPs) used at KNH/UON cytology laboratory (Appendix III). Equipment operation was done according to manufacturer`s instructions. Screened smears were confirmed by the supervising pathologist before results were signed out to the participant`s records. All positive smears and every fifth normal smear were re-screened by an independent pathologist. Where there was discrepancy a tie-breaker opinion was sought from a different pathologist.

3.14 Ethical Considerations

Permission to carry out the research was obtained from the KNH/UON ERC/Narok County Medical Superintendent (APPENDIX VI)

The participants were carefully taken through a consent process that informed them about the study, benefits and risks involved and what was required of them.

Consenting eligible participants, with a dentist or dental assistant as a witness signed the consent form before taking part in the study. Any information obtained from the participants was kept strictly confidential and used solely for the purpose of achieving the study objectives.

Participants were informed of their screening results in accordance with the established clinical practice and referred for further management to Narok and Kilgoris level IV Dental Unit where need arose or was indicated by result.(Appendix II)

4.0 RESULTS

4.1 Demographic characteristic

A total of 100 patients who visited the dental clinics, were tobacco chewers and consented for the study were enrolled. (74 (74. %)) were males (26(,26%)were females who were residents of Narok county. About one half, of the 100 (50%) had secondary level education and (50%) had less than primary education.

4.1.1 Distribution of patients by Gender

The participants by gender were 74% male versus 26% female and all the female were stay home mothers .None were students at any learning institution. The unemployed or stay home mothers the majority of them never completed primary school education.

Patients' Gender	**Frequency(n)**	**Percentage(%)**
Male	74	74
Female	26	26
Total	**100**	**100**

Table 4: Distribution of Patients by Gender

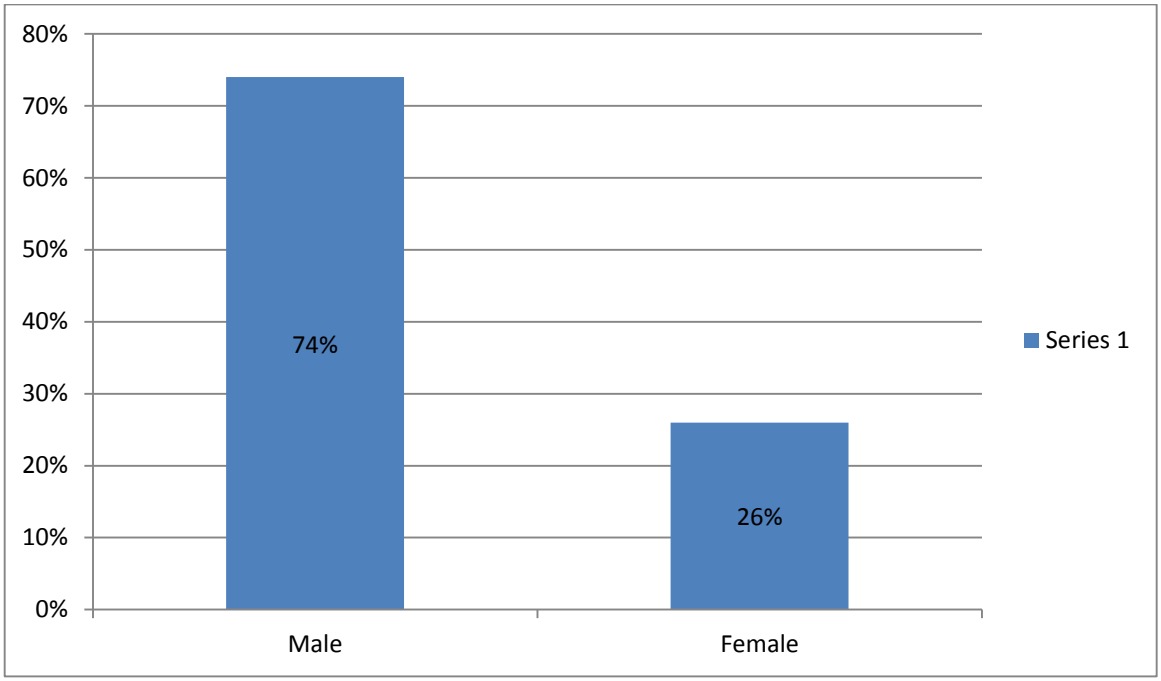

Figure 1 Distribution of patients by gender

4.2 Age distribution

Mean age of the100 participants was 38.73 years (range 26 – 90 years) and there were three peak age groups; those aged 34 to 45 years (40.2%, followed by those aged 45 to 54 years (29.7%) and those age 55 years and above (21.9%) (Figure1).

Age distribution	Frequency (n)	Percentage (%)
25-34	8	8
35-44	15	15
45-54	25	25
55-64	24	24
65-74	17	17
75-84	8	8
85+	3	3
Total	**100**	**100**

Table 5: Distribution of Patients by Age

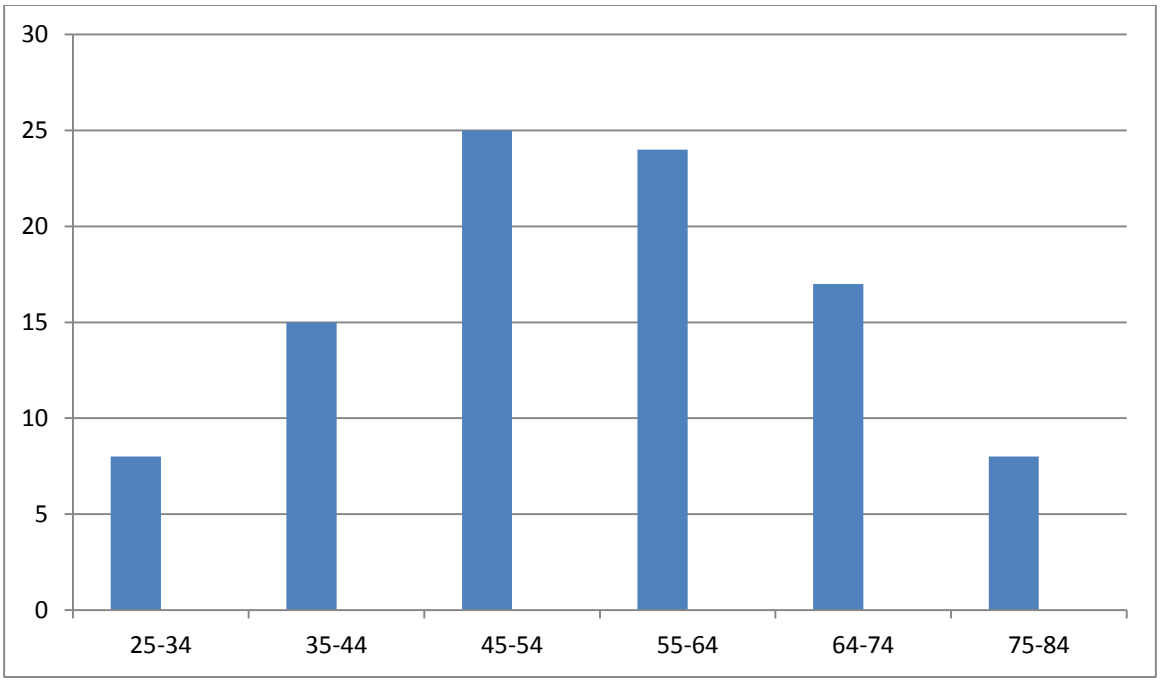

FIGURE 2 Distribution of patients by Age

4.3 Duration of tobacco chewing

There were two distinct periods of times in which the participants chewed could chew tobacco (table).majority (38%) had chewed tobacco for more than 30 years while (62%) had chewed tobacco for more than 10 years.(table 5)

Duration of tobacco Chewing in years	Zample size	Percentage%
6-15	12	12
16-25	22	22
26-35	28	28
36-45	22	22
46-55	9	9
56-65	5	5.0
66+	2	2.0
Total	**100**	**100**

Table 7: Distribution of Patients by Number of Years of Tobacco Use

Frequency of tobacco chewing

In terms of the frequency of tobacco chewing, majority (92%) chewed on a daily basis while a few 8% chewed about twice a week.

Chewing daily	Sample size	
	No	**%**
Yes	92	92%
No	8	8%

Table: 7 Frequency of tobacco chewing

Concerning the amount of tobacco chewed daily, majority 92(92%) chewed on a daily basis the tobacco bolus was kept in the mouth during chewing between 1-6 hours in about half of the participants 50 (50%) while 40 (40%) reported keeping their bolus for 6-12 hours. Only 10(10%) reported keeping Tobacco bolus in their mouth slightly over 12 hours or as long as they were awake and not having a meal . A few revealed of getting wet chewed tobacco from their family members that has already been chewed before when they were totally out of tobacco. These were family members like husband, wife, brother or parent. Health wise it's not proper especial this as it could be port of transmission of such diseases fungal, HIV, HSV and other communicable diseases.

There were two forms of tobacco available for chewing, the loose leaves and the powder forms.

Form of Tobacco	**Frequency (n)**	**Percentage(%)**
Loose leaves	90	90
Powder tobacco	10	10
Total	**100**	**100**

Table6: Form of tobacco used by patients.

4.6 Pattern of cytological findings on oral mucosa

Almost all the participants 100(95%) had negative (for intraepithelial lesion) oral mucosa cytological findings, twenty (20%) were negative but had bacterial and Candida infection, two (0.9%) were negative but with inflammation. Only five participants (5%) had atypical cytology changes. (Table5).

Characteristics	Sample size	
	No	%
Cytopathology finding		
Negative	95	95
Negative and bacteria	20	20
Negative and Candida	9	9
Atypia	5	5

Table 8: Pattern of cytologic finding of oral mucosa in tobacco chewers in narok county (n=100)

5.0 DISCUSSION

The aim of this study was to describe oral cytological changes among oral tobacco chewers presenting to two dental clinics in Narok County. Also to determine the prevalence of neoplastic lesions on oral mucosal cytology in oral tobacco chewers. One hundred (100) oral tobacco chewers for 6 years and above were studied. Majority (74%) of the participants in this study were male and this compares well with a similar study by Ahmed et al (16) where he found that Tobacco chewing is a predominantly (100%) male habit. However, the mean age was lower 38 years in this study compared to 47 years in the study by Ahmed et al (16); in Yemen the habit of tobacco chewing is declining in the younger generation but it still common in the older age group.

Tobacco use was most common among these patients as the majority were not in any formal employment. (80%); were involved in farming or doing business. This translates to having no formal rules guiding them at their places. 20% were stay home mothers who shared tobacco use with their husbands, co-wives and children.

No relationship was found between oral mucosa changes and duration of frequency of Tobacco use, this compares well with a study by Ahmed et al (16). Approximately half of the population in this study drunk alcohol either locally brewed or commercially sold, but no association was found between alcohol consumption compared to oral mucosal neoplastic lesions. Macigo et al (14) study found these risk factors not to be statistically significant.

The clinically observed lesions, included brown-black teeth, cracked gums and few ulcerated lips, this is consistent with a case report by Hassan (28), who concluded that the brown-black teeth is an example of dental pathology caused by complications of chewing tobacco.

Oral cytology using a cytobrush which was applied in this study was a simple and non-invasive technique that provided sufficient cells for analysis of non-neoplastic and neoplastic oral lesions.

This study found five cases with atypical squamous cells of undetermined significance (ASC-US). Shown on photomicrographs 1-2. The rest 95% were negative for oral mucosal neoplasia. This contrasts to Scuibba et al (48) study conducted in a study conducted in 2015 in India who found the prevalence of oral lesions among tobacco chewers to be 41%. They found Leukoplakia to be prevalent in the 41 -50 year olds. The population in Sujath's study (50) had a mean age of 47 years while the mean age in this study was 38 years. This study recruited older population same as Scuibba et al (48) suggesting that Scuibba et al (48) study participants might have had longer duration of chewing and also various forms and mixed with other chemical substances with tobacco hence more exposure and more time for the development of oral neoplastic lesions. The low prevalence of neoplastic lesions in this study does not suggest that tobacco chewing is a safe but underscores the fact that carcinogenesis is a process that is dependent on several factors including; time, type and amount of exposure to carcinogens, immune status of the individuals and other interventions (10,48). Time is an important factor in carcinogesis; an example is the development cervical cancer where it takes about 10-20 years before precursor lesions develop into cancer TBS (Appendix IV). This therefore could explain the reason for low prevalence in this general population in Narok County.

About (20%) of the patients had bacterial infection and (9%) had Candida. This may be attributed to poor oral hygiene which may have been as a result of chewing habits, lack of brushing the teeth as well as keeping the bolus in the mouth and sharing of chewed tobacco sometimes mixing it with other substances. Gorsky et al (49) in a study of oral white lesions

keratotic lesions caused by qat and tobacco chewing also found increased incidence of gingivitis among khat chewers but no neoplastic lesions. With increased prevalence of HIV/AIDS this could lead to increased risk of opportunistic infections in this population if one is HIV infected.

Several additional risk factors associated with oral lesions were evaluated in this study. They included alcohol use, and non-smoked tobacco. Despite the fact that approximately half of the participants (70%) consumed alcoholic substances, Chewing Tobacco in the short term does not seem to have deleterious effects but its long term use is more important in carcinogenesis and morphologic cellular changes (45). From a preventive perspective, the observed prevalence of oral neoplastic lesions (5%) underlines its public health significance in the population; funds should be directed towards screening of high risk groups and setting the stage for treatment.

5.1 CONCLUSION

1. Oral mucosal neoplastic is not common in persons with an average tobacco chewing period of five years in this fairly general population in Narok County.
2. Inflammatory lesions were the most common positive findings and this could be attributed to poor oral hygiene and party due to physical damage to the oral mucosa from oral Tobacco chewing
3. Almost half of the participants were exposed to alcohol and oral tobacco which are known long term risk factors for oral neoplasia.

5.2 RECOMMENDATIONS

1. A longitudinal study including an older age group who probably have a long time exposure to tobacco chewing who are in the villages or manyatta and follow-up of young people who are likely to be long term tobacco chewers should be carried out.
2. Promotion of oral hygiene among tobacco chewers is recommended so as to reduce oral inflammatory disease.
3. Health education should be given a priority by the county about alcohol and use of tobacco as long term known risk factors to neoplasm of the oral mucosa and other sites.

5.3 STUDY LIMITATIONS

The sampling technique used in this study precluded age stratification. This led to predominance of relatively middle aged and above participants and this would have affected the outcome of this study. The participant recruitment sites, which were the dental clinics, were visited mainly by middle aged people, who were able to commute the distance between their homes and the two hospitals, and therefore might have missed older generation of tobacco chewers who might have longer exposure to oral tobacco chewers who could not access the dental clinics due to their inability to walk to the dental clinics.

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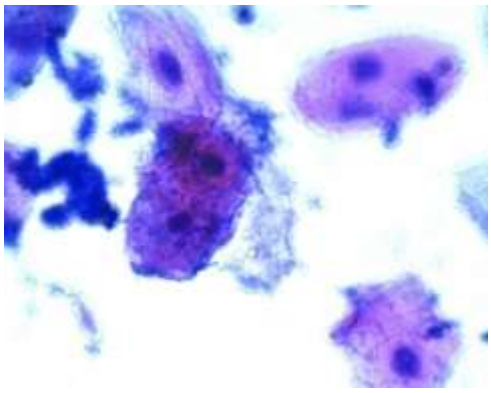
Photomicrographs

Photo micrograph 1Ascus



micrograph 2 Ascus

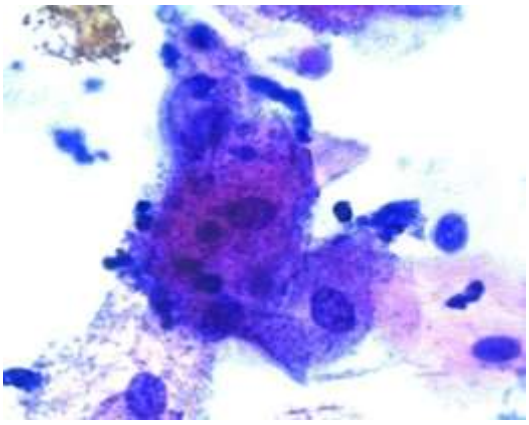


Photo micrograph 3 Normal cellular morphology

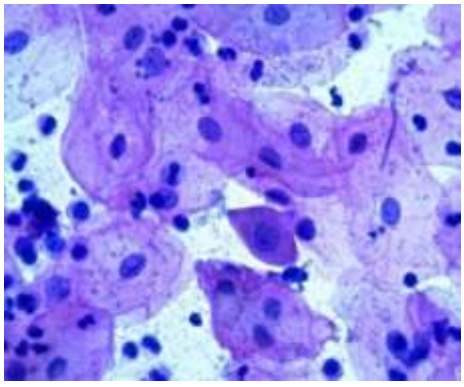
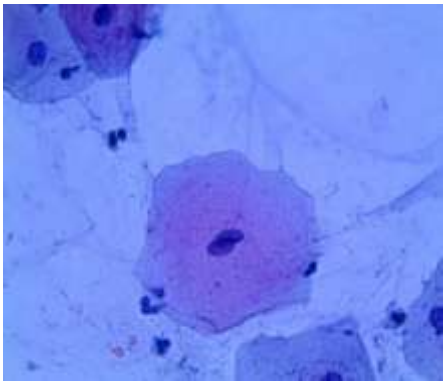
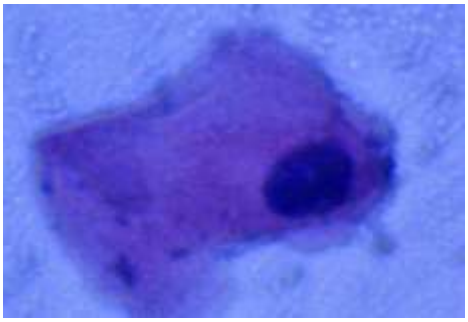


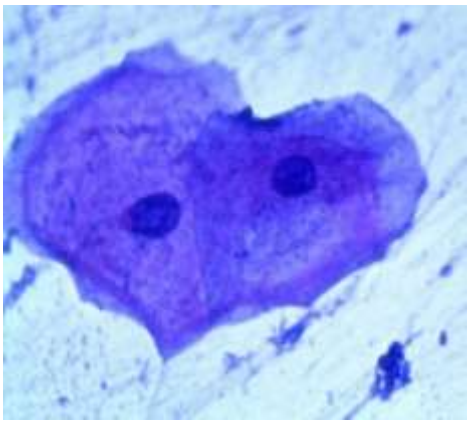
Photo micrograph 4 Normal cellular morphology



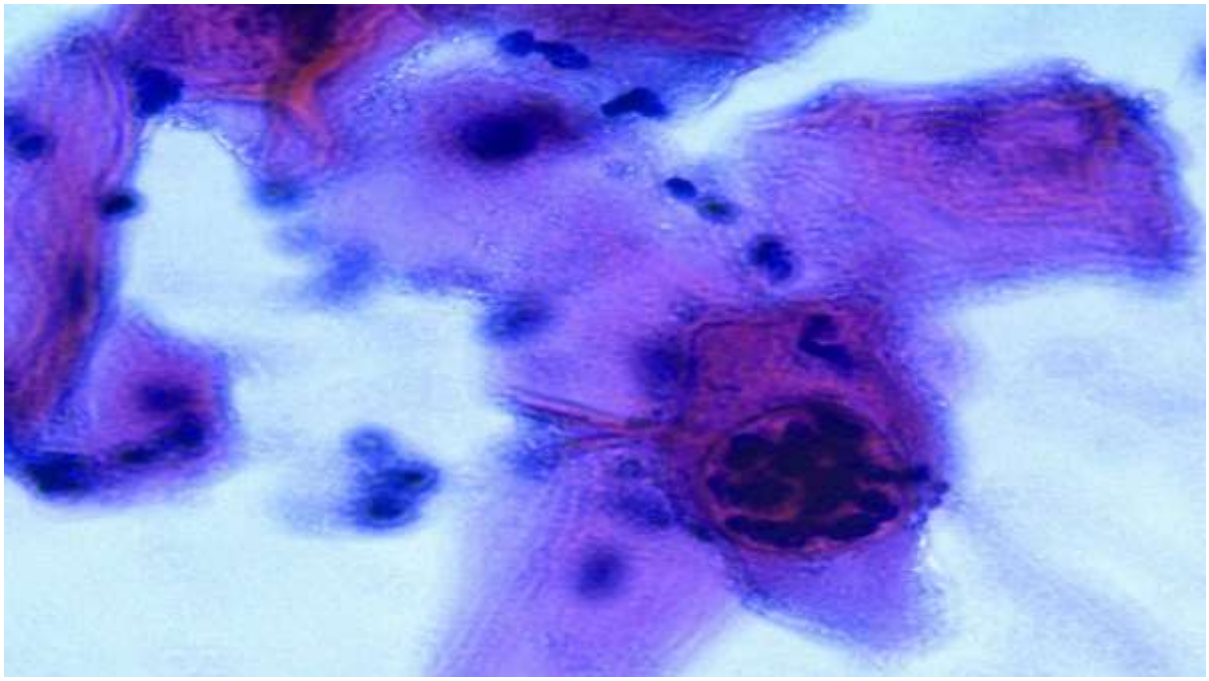
Photomicrograph 5 Normal Cellular Morphology

Photomicrograph 6 Two normal cells





Photomicrograph 7 Normal cell with Neutrophils filled Nucleus. X 40



NAROK COUNTY MAP

Map courtesy of narok county tourism development board.

APPENDIX I: INFORMED CONSENT AND CONSENT FORMS

Topic: oral mucosal cytological changes among tobacco chewers presenting to Narok County Kenya

Consent explanation: My names are Peter Mbaka, a student at the University of Nairobi department of Human Pathology. University of Nairobi P.O Box 20732 Nairobi, Kenya; Phone: 0722914850. I am conducting a study seeking to find out what is the effect of routine tobacco chewing on your mouth tissues. The information in this form will help you make an informed decision whether or not to participate in this study. Please read through carefully and feel free to ask any question about the study. I will read it out to those who are not able to read.

Description: Previous studies in other regions have shown that those people who routinely chew tobacco are likely to develop changes in their mouths which could lead to cancer.

Purpose: This study is interested in finding out what are the changes likely to cause cancer of the mouth among those who routinely chew tobacco

Benefits: You will benefit from free screening of your mouth for cancerous tissues. In case of any abnormal findings you will be counselled, informed in confidentiality and referred to where you will receive care and early management.

Risks: One potential risk of being in the study is the loss of privacy. However, we will do our best to make sure that the personal information gathered during this study is kept private.

Also, you might feel a little discomfort at the time we will be taking your mouth samples

Procedure: if you accept to participate in this study, we will ask you to attend the Narok/Kilgoris Level 4 Hospital dental unit where you will meet study consultant. You will undergo face to face interviews using language you are comfortable with to answer a few questions concerning your demographic parameters as well as collect mouth sample from you using special brush.

Voluntarism: participation in this research study will be voluntary without any coercion

Follow up: participants found with abnormal mouth finding will be contacted for review and referral to a management facility.

Subject`s rights: if you have read this form and have decided to participate in this project, please understand your participation is voluntary and you have the right to withdraw your consent or discontinue participating at any time without penalty. You have the right to refuse to answer particular questions. Your individual privacy will be maintained in all published and written data resulting from the study.

If you have questions about your rights as a study participant, or are dissatisfied at any time with any aspect of this study, you may contact – anonymously, if you wish – KNH/UON ERC (Chairperson of the Scientific Steering Committee, P.O Box 20732 Nairobi, Kenya; phone: 02-7263000 Ext 44102

I have read this form or had it read to me in a language that I understand. I have discussed the information with study staff. My questions have been answered. MY decision whether or not to take part in the study is voluntary. If I decide to join the study I may withdraw at any time.

By signing this form I do not give up any rights that I have as a research participant.

_____	_____	_____
Participant Name	participant Signature/Thumbprint	Date
_____	_____	
_____		
Study Staff Conducting	Study Staff Signature	Date

APPENDIX 1: CONSENT FORM:

I ……. after reading and being explained the study purpose and what it involves do hereby give informed consent to participate in the diagnostic study fully aware of the benefits and risks. I have not been pressurized to participate in this study in any way or baited by any incentive. I understand that participation in this study is completely voluntary and that I may withdraw from it at any time and without loss of any benefit or quality of management to which I am entitled. I am fully aware that the results of this study will be used for scientific purposes and may be published in journals.

Participant Signature: ……..Date……..

Doctor/Nurse Signature…….. Date ……..

Principal investigator Signature.....… Date ……..

APPENDIX II: QUESTIONNAIRE

The oral mucosal cytological changes among Tobacco chewers in Narok County Kenya

The participant will fill the questionnaire before oral smears are collected.

Section 1: Social demographic data

1. Study number ---------- Date (dd/mm/yyyy)-------/----------/----------
2. Sex: Male ☐ female ☐ Age ☐
3. Occupation: farmer ☐

   ☐ Tobacco seller

   Others (specify)

4. Are you a residence of Narok County? Yes ☐ No ☐

   I yes which sub county ----------

   If No, specify county of residence

5. Marital status: Single ☐

   Married ☐

   Divorced ☐

   Windowed ☐

6. What is your level of education?

   None ☐

   Primary ☐

   Secondary ☐

   Tertiary ☐

7. How long have you chewed tobacco? (Year)

   ≤ 5 ☐

   5-10 ☐

   ≥ 10 ☐

8. How many grammes of Tobacco do you chew in a day?

   ≤ 100 ☐

   100 – 3000 ☐

   ≥ 300 ☐

9. How often do you chew Tobacco?

   Daily ☐

   Once in a weak ☐

   Occasionally ☐

10. How long does the bolus stay in your mouth? (Hours)

    1 – 6 ☐

    6 – 12 ☐

    ≥ 12 ☐

11. Do you smoke? Yes ☐ No ☐

    If yes, specify: Tobacco (sniffed) ☐

    Smokeless Tobacco ☐

    Cigarettes ☐

    Do you mix your tobacco with salt? ☐

12. Do you drink alcohol? Yes ☐ No ☐

    If yes, specify**: a)** Bottled ☐ Traditional brew ☐

b) Quantity per day:

≤ 5 bottles ☐

6 – 10 bottles ☐

≥ 10 bottles ☐

c) Duration of use (yr):

≤ 5 ☐

5 – 10 ☐

≤ 10 ☐

13. Do you use/chew other drugs? Yes ☐ No ☐

If yes specify, Name and Mode of intake

14. Do you have a lesion in the oral cavity? Yes ☐ No. ☐

If yes, are you under any medication for the same? Yes ☐ No ☐

Specify

FOR LABORATORY USE ONLY. By (PI)

RESULTS:

SPECIMEN ADEQUACY:

Satisfactory ☐

Unsatisfactory ☐

Epithelial cells features

Negative ☐ Inflammatory ☐ ASCUS ☐

LSIL ☐ ASC-H ☐ HSIL ☐

SCC ☐ OTHER SPECIFY----------

APPENDIX III: LABORATORY METHODS

Papanicolaou Staining procedure

Principle of the stain

Hematoxylin stains the nuclei blue. The eosin azure solution being acidic stains the cytoplasm which is basic so that the eosin has affinity for the mature cells while light green has affinity for the young cells. Orange G also being an acidic dye has an affinity for the cytoplasm and stains keratin.

Staining technique

1. Slides with smears will be fixed in 95% ethanol for 15 minutes.
2. Hydrated in descending grades of alcohol, 80%, 70%, 50%, 10 dips in each
3. Rinsed in tap water, 10 dips.
4. Stained with Harris Hematoxylin stain for 4 minutes
5. Rinsed in tap water until excess stain is drained off.
6. Differentiated in three changes of 0.05% acid alcohol, 10 dips in each
7. Rinsed in running tap water
8. Blued in Scott`s tap water for 1 minute, and rinsed in tap water, 10 dips
9. Dehydrated in ascending grades of alcohol, 70%, 90%, 10 dips in each.
10. Stained with orange G-6 for 2 minutes
11. Dehydrated in three changes of 95% alcohol, 10 dips in each.
12. Stained in Eosin Azure EA-36 for 3 minutes.
13. Dehydrated in three changes of 95% alcohol, 10 dips in each.
14. Cleared in three changes of Xylene, 10 dips n each.
15. Mounted with DPX (Diestrene Plasticizer Xylene), and observed under microscope

Reagent preparation

Harris alum Hematoxylin to prepare 1 Litre

1. Hematoxylin 50gm
2. Absolute alcohol 100ml
3. Ammonium alum 100gm
4. Distilled water 1000ml
5. Mercuric oxide 3gm
6. Glacial acetic acid 40ml

Method of preparation

1. Dissolved Hematoxylin in absolute alcohol (solution 1).
2. Dissolve Ammonium alum in water (Solution 2).
3. Mix solution 1 and solution 2 and heat to boil.
4. Add mercuric oxide and cool rapidly.
5. Add glacial acetic acid.
6. Solution is ready for use as soon as it cools.
7. Filter before use

E.A. 36

To prepare 2 litres

1. Light green 1gm
2. Bismark brown 1 gm
3. Eosin yellow 5gm
4. Phosphotungstic acid 4gm
5. Distilled water 600ml

6. Absolute ethanol 1400ml
7. Glacial acetic acid 20ml

Method of preparation

1. Dissolve in order of light green then bismark brown then eosin yellow into water
2. Add Phosphotungstic acid slowly in stage while agitating
3. Add alcohol slowly while agitating
4. Add glacial acetic acid then agitate

- Ph of E.A 36 should be 4.5-5 to achieve maximum results.

O.G. 6

To prepare 4 litres

1. Orange green (O.G) 9.4gm
2. Phosphotungstic acid 0.6gm
3. Distilled water 1200ml
4. Absolute ethanol 2800ml

Preparation Method

1. Dissolve O.G in water then Phosphotungstic acid
2. Add absolute ethanol

0.05% acid alcohol

To prepare I litre of 0.05% acid alcohol

1. Distilled water 999.5ml
2. Conc.HCL 0.5ml

Scott's tap water

To prepare 1000ml

Sodium bicarbonate	3.5gm
Magnesium sulphate	2gm
Distilled water	1000ml
Thymol	1 tablet

Quality check

1. Stains will be stored in dark coloured, stoppered bottles
2. Fresh amounts of Haematoxylin will be added to replace stain loss due to evaporation.
3. O.G and E.A were replaced as soon as the cells appear without crisp staining colors
4. Water rinses will be done under running tap water.
5. Alcohol will be replaced on a rotating basis
6. Alcohol will be changed as soon as it becomes tinted with any of the cytoplasmic stains or becomes slightly milky due to presence of water.
7. An agitation of the slides by dipping will be done to remove excess dye.
8. Dipping will be done gently to avoid cell loss and the slide carrier was not hit the bottom of the staining dish.

APPENDIX I V: BETHSDA SYSTEM FOR RE PORTING CERVICAL CYTOLOGY

1. SPECIMEN ADEQUACY

- Squamous cellularity
- Unsatisfactory
- Satisfactory
- Transformation zone component
- Obscuring factors

NON-NEOPLASTIC FINDINGS

a) Organisms

- Fungal organisms morphologically consistent with *Candida* species
- Cellular changes consistent with Herpes simplex virus

b) Other non-neoplastic findings (optional to report)

- Reactive cellular changes associated with:
- Inflammation (includes typical repair)
- Radiation

c) Non-neoplastic findings , not specifically listed in 2001 Bethesda terminology

- Keratotic cellular changes
- Lymphomas
- Other

2. ATYPICAL SQUAMOUS CELLS

a) Of undetermined significance (ASC-US)

b) Cannot exclude HSIL (ASC-H)

3. EPITHELIAL ABNORMALITIES: SQUAMOUS

a) Low grade squamous intraepithelial lesion (LSIL)
   Encompassing: HPV/ mild dysplasia/ CIN 1
b) High grade squamous intraepithelial lesion (HSIL)
   Encompassing: moderate and severe dysplasia, CIS; CIN2 and CIN3
   With features suspicious for invasion (if invasion is suspected)
c) Squamous cell carcinoma
   Squamous epithelial abnormalities, not specifically listed in 2001 Bethesda terminology
   - Keratinizing lesions
   - Squamous intraepithelial lesions (SIL) – borderline cases
   - SIL with gland involvement

4. EPITHELIAL ABNOMALITEIS: GLANDULAR

A typical glandular cells, NOS or specify in comments

- Atypical Glandular cells, favour neoplastic
- Adenocarcinoma
- Not otherwise specified (NOS)

5. OTHER MALIGNANT NEOPLASMS

- Carcinomas
- Sarcomas
- Other tumours

ORAL CYTOLOGY

- Unsatisfactory
- NILM: negative for intraepithelial lesion or malignancy
- Epithelial cell abnormalities

APPENDIX V DATA COLLECTION AND REPORTING SHEET

1- Patient identification

Identification number Age ☐ X ☐ In patient (1) ☐ (2) ☐

2- Tobacco use history

a) Chewing yes----------No of years ----------
b) Snuff yes ----------No of years----------
c) Cigarettes yes ----------No of years----------
d) Other tobacco products ----------

2- Clinical history: Any lesion in the oral mucosa visible macroscopically

a) Location of the lesion …….
b) For how long has the lesion been there.....?
c) The number of specimen taken ……..
d) Clinical diagnosis…….

3- Specimen Adequacy :(1) Satisfactory ☐ (2) Unsatisfactory ☐

4- Microscopic description

5- Interpretation

Specimen adequacy:

(1) Satisfactory …….. (2) Unsatisfactory ……..

Negative for malignancy: …….Specify ……

Suspicious for malignancy; ……..Specify ……

Positive for malignancy: ……...Specify ……

Infections: ……..Specify ……

Signatures

Cytologist ----------

Pathologist (1) ----------

Pathologist (2) ----------

APPENDIX VI: ETHICAL AND COMPLIANCE FORMS.

Attached are the clearance letter from Uon/Knh Erc, and Narok county government clearance to conduct the research project with narok count.