CYTOLOGICAL FINDINGS OF THE ESOPHAGUS USING SPONGE CYTOLOGY ON PATIENTS REFERRED FOR ESOPHAGEAL ENDOSCOPY AT KNH

By
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A Dissertation Submitted in Partial Fulfillment of the Award of Master of Science Degree in Clinical Cytology at the University of Nairobi.

2013
DECLARATION

I hereby declare that this dissertation is my original work and has not, to the best of my knowledge, been submitted to any other institution of higher learning.

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DEDICATION

I dedicate this dissertation to my late grandfather Stephen Gichobi and cousin Elias Karimi who prior to their demise were diagnosed with esophageal cancer.
ACKNOWLEDGEMENT

I thank God for His strength and grace that has brought me this far.

I wish to extend my sincere gratitude to all those who have contributed directly and indirectly to the success of this project.

Special gratitude to my supervisors Dr. Muchiri L. and Prof. Lule G. for their tireless efforts in guiding me to put this work together.

Special appreciation to KNH endoscopy unit staff for their outstretched assistance.

Great appreciation goes to Actimed Company Switzerland for providing the cytosponge.

Sincere gratitude to Mr. John Kairu and Mr. Willis Ochuk for their ever ready help.

My heartfelt appreciation to my family for encouragement and support.
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<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AGC</td>
<td>Atypical Glandular Cells</td>
</tr>
<tr>
<td>ASC-H</td>
<td>Atypical Squamous Cells High Grade Squamous Intraepithelial Lesion cannot be excluded</td>
</tr>
<tr>
<td>ASC-US</td>
<td>Atypical Squamous Cells of Undetermined Significance</td>
</tr>
<tr>
<td>BE</td>
<td>Barrettts Esophagus</td>
</tr>
<tr>
<td>CIN</td>
<td>Cervical Intraepithelial Neoplasia</td>
</tr>
<tr>
<td>CIS</td>
<td>Carcinoma In-Situ</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DPX</td>
<td>Diestrene Plasticizer Xylene</td>
</tr>
<tr>
<td>EC</td>
<td>Esophageal Cancer</td>
</tr>
<tr>
<td>ESC</td>
<td>Esophageal Sponge Cytology</td>
</tr>
<tr>
<td>E.A</td>
<td>Eosin Azure</td>
</tr>
<tr>
<td>FNA</td>
<td>Fine Needle Aspiration</td>
</tr>
<tr>
<td>GERD</td>
<td>Gastroesophageal Reflux Disease</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HPV</td>
<td>Human Papilloma Virus</td>
</tr>
<tr>
<td>HSIL</td>
<td>High Grade Squamous Intraepithelial Lesion</td>
</tr>
<tr>
<td>ID</td>
<td>Identity Number</td>
</tr>
<tr>
<td>IP</td>
<td>Inpatient</td>
</tr>
<tr>
<td>KEMRI</td>
<td>Kenya Medical Research Institute</td>
</tr>
<tr>
<td>KNH</td>
<td>Kenyatta National Hospital</td>
</tr>
<tr>
<td>LSIL</td>
<td>Low Grade Squamous Cell Intraepithelial Lesion</td>
</tr>
<tr>
<td>NILM</td>
<td>Negative for Intraepithelial lesion or Malignancy</td>
</tr>
<tr>
<td>No.</td>
<td>Number</td>
</tr>
<tr>
<td>NOS</td>
<td>Not Otherwise Specified</td>
</tr>
<tr>
<td>O.G</td>
<td>Orange G</td>
</tr>
<tr>
<td>OP</td>
<td>Out Patient</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolution Per Minute</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
</tr>
<tr>
<td>---------</td>
<td>-----------</td>
</tr>
<tr>
<td>SOPs</td>
<td>Standard Operation Procedures</td>
</tr>
<tr>
<td>Spp</td>
<td>Species</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Packages for Social Sciences</td>
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<tr>
<td>TBS</td>
<td>The Bethesda System</td>
</tr>
<tr>
<td>UON</td>
<td>University Of Nairobi</td>
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ABSTRACT

**Background:** Esophageal cancer is the 9th most common cancer in the world, and the 5th most common cancer in developing countries. In Kenya, it is the most common cancer in men and the third in women; with the highest mortality rate of 10.2%. Esophageal cancer has poor prognosis because most of the patients present in advanced stages of the disease when current treatment modalities are not very effective. There are known precancerous lesions which can be diagnosed through exfoliative cytology to improve the esophageal cancer survival rates and at the same time reduce its mortality. Sponge cytology, balloon cytology and sponge-mesh are screening methods which have been extensively studied and are now being assimilated into clinical practice. Studies have shown sponge cytology to be readily accepted by patients compared to the other methods. Hence it was the method of choice for this study.

**Objective:** To describe cytological findings of the esophagus using sponge cytology on patients referred for endoscopy at KNH.

**Design:** A cross-sectional descriptive study.

**Setting:** Kenyatta National Hospital endoscopy unit.

**Study Population:** Both men and women who were referred for esophageal endoscopy.

**Method:** A calculated sample size of sixty (60) patients was recruited and a structured questionnaire used to collect socio-demographic data and risk factors for esophageal disease. Smears made from specimen collected using a cytosponge® (Oesotest, Actimed Switzerland) were fixed then stained with Papanicolaou stain. The smears were reported using The Bethesda Reporting System 2001.
Results: All the 60 participants were blacks. The female to male ratio in this study was 2:1 (68.33% & 31.67% respectively). The peak age group was 41-50 yrs (25.0%) and with mean age of 43.77 yrs (SD - 14.623). Majority of patients hailed from Central and Nairobi provinces. Of the clinical information elicited, persistent heartburn (59%) was most common complaint with dysphagia (8%) being among the least. Cytological findings were; (86.6%) NILM, intestinal metaplasia (10%), HSIL (1.7%) and SCC (1.7%). Among the 52 NILM patients, 9.6% were reported as inflammatory smears and 17.3% had candidiasis. Cytological findings compared well with endoscopic and biopsy findings with Kappa value of 0.588 (measure of agreement). This study reported few cases of esophageal cancer and therefore could not deduce a significant association between esophageal cancer and the associated risk factors.

Conclusions: Majority of the patients in this study had non-neoplastic lesions and only a few with malignant lesions. Therefore, sponge cytology examination of the esophagus can be employed as a primary test whenever there is any suspicion of an esophageal lesion especially in clinical set-ups where endoscopy facilities and medical professionals are not available. Risk factors for esophageal cancer could not be assessed in this study hence, larger studies may be helpful in assessing the risk factors associated with esophageal cancer.

Recommendation: Sponge cytology is a simple and inexpensive technique which can be used as a triage test for patients with clinically indicated esophageal lesions.
1.0 INTRODUCTION

Esophageal cancer is the 9th most common cancer in the world, and the 5th most common cancer in developing countries (1). Esophageal cancer has incident rates of 3.8%, 3.9% and 8.4% in the world, Africa and Kenya respectively. Its mortality rates are, 5.4%, 4.9% and 10.2% in the world, Africa and Kenya respectively (2). The overall five-year survival rate is approximately 15%, with most patients dying within the first year of diagnosis. Cancer is the third commonest cause of death in Kenya after infectious diseases and cardiovascular diseases (3). Esophageal cancer has the highest mortality rate of all the cancers in Kenya (2).

The prognosis of esophageal cancer is poor, because most patients present with advanced disease. By the time the first symptoms start manifesting, the cancer has already advanced and the current treatment modalities, including radiation, chemotherapy, and surgery, are not very effective (4). According to the Nairobi Cancer Registry based in KEMRI, about 80% of reported cases of cancer in Kenya are diagnosed at an advanced stage, when very little can be achieved in terms of curative treatment (3). Thus there is dire need for early diagnosis through screening programmes in high risk groups.

Globally, esophageal cancer hot-spots areas include northern Iran, Kazakhstan, South Africa, and northern China, where annual incidence can exceed 200 per 100 000 per year (1). Though Kenya is not among the hot spot areas, certain regions of central and western Kenya report esophageal cancer as the first or second most common cancer. According to a study done at Tenwek Hospital (Bomet District), among all the malignancies diagnosed, esophageal cancer was the most common, accounting for 19% of the total malignancies diagnosed (5). Blacks are 4.5 times more likely to develop this cancer than are whites. Esophageal cancers are more common in men than women. In whites, the male to female ratio is 3:1 while in blacks it is
Among Kenyan men, esophageal cancer is the most common cancer while in women it is third most common following breast and cervical cancers (2).

There are two main histological types of esophageal cancers: squamous cell carcinomas and adenocarcinomas. Squamous cell carcinoma (SCC) is the most common accounting for over 90% of esophageal cancers. The esophageal adenocarcinoma is less common than SCC but its incident rate has increased though the underlying cause has not been established (6).

Various risk factors for esophageal cancer have been identified, but those identified in this study are use of tobacco and alcohol. Preventive measures, such as mass education on harm of tobacco and alcohol use and importance of early diagnosis (screening) could help fight this almost universally fatal cancer which has no effective treatment.

Several screening and diagnostic techniques have been used though endoscopy and subsequent biopsy are the commonly used methods. Cytology based screening methods such as balloons, sponge and sponge-mesh, which are cheaper, non-invasive, easy to perform and readily acceptable by patients, have been used. The results of abrasive cytological diagnostic methods in this and other studies carried out elsewhere have been impressive. In China, the 5-year survival rate of 85% to 90% and the 10-year survival rate of 55.6% were recorded subsequent to the esophageal balloon cytology screening (4). Sponge cytology has shown to be readily acceptable by patients over the other cytological methods with sensitivity of 81-90% and specificity of 92-99% (7, 8). Sponge cytology was well tolerated by patients. Due to its minimal discomfort to the patient and the relative simplicity of collecting and interpreting esophageal cells, sponge cytology is a suitable procedure for screening patients.
2.0 LITERATURE REVIEW

2.1 Normal Histology and Cytology of the Esophagus

The adult human esophagus is an 18 - 25 cm long and 2 - 3 cm in diameter muscular tube, which is composed of striated muscle in the upper part, smooth muscle in the lower part, and a mixture of the two in the middle (9).

The esophagus is normally lined by non-keratinizing squamous cell though the distal most part (1-2cm) is lined by simple columnar cells epithelium which is mucin or non-mucin producing (6). Right beneath the epithelium is the lamina propria made of loose connective tissues within which are glandular structures that predominantly produce neutral mucin. The glandular structures in the submucosal lining of the esophagus produce predominantly acidic mucin (6).

The cytology specimen mainly consists of superficial and intermediate squamous cells in large flat sheets, in small clusters, in concentric arrangements ("pearls"), and as solitary cells. Parabasal cell are rare and are assumed to be due to vigorous sampling, inflammation and presence of an ulcer. Glandular cells may also be present presumably from the distal most esophagus. Ciliated columnar respiratory cell, alveolar macrophages, oral cavity microbes and food remains may be present as contaminants (7, 8)

2.2 Diseases of the esophagus

2.2.1 Non-neoplastic esophageal disorders
a) **Infections**

Esophageal infections are common, but not exclusively, in immunocompromised individuals (11). Among the infection of the esophagus, Candida is the most common cause of esophagitis (9, 10). Cytological techniques are superior to biopsies in the diagnosis of Candida esophagitis (10). Other infections include; Bacterial, Aspergillus, Herpes Simplex Virus, Cytomegalovirus and Human Papilloma Virus infections.

b) **Gastroesophageal reflux disease (GERD)**

This refers to regurgitation of gastric and often duodenal contents, through a variably incompetent lower esophageal sphincter, to the mouth. The squamous epithelium comes into contact with irritating substances such as hydrochloric acid, pepsin and bile components leading to reflux esophagitis (6).

c) **Barrett’s esophagus**

Barrett’s Esophagus (BE) refers to condition in which the normal stratified squamous epithelium of the distal esophagus is replaced by columnar epithelium which can be of cardiac, fundic, or intestinal type, but the increased risk of adenocarcinoma is associated with intestinal-type epithelium. Therefore, BE is defined by the presence of intestinal-type epithelium, characterized by goblet cells (11). Barrett's esophagus is thought to result from chronic gastroesophageal reflux. The native squamous epithelium is replaced by a chemically resistant metaplastic gastrointestinal glandular epithelium that is better able to withstand the action of gastric digestive juices (12). More than 90% of esophageal adenocarcinomas arise from BE (11).
c) **Chronic and acute esophagitis**

The most common cause of esophagitis is GERD (6). Esophagitis may also be caused by trauma, reaction to swallowed corrosive liquids such as alcohol, smoking, lye and hot drinks, cardiospasm of long standing, hiatus hernia, Crohn's disease, sarcoid, radiation/chemotherapy, infectious agents, uremia, and other sorts of injury (8, 10).

### 2.2.2 Benign esophageal neoplasm

They include; squamous papillomas, leiomyomas, lipomas, granular cell tumors, hemangiomas and lymphangiomas. They are particularly the soft tissue tumors that occur in the submucosa and usually covered by an intact mucosa and thus are successfully diagnosed by endoscopic fine needle aspiration biopsy rather than exfoliative cytology (9, 10).

### 2.2.3 Esophageal cancers precursor lesions

Following histologic and cytologic criteria, precursor lesions can be divided in-to dysplasia and carcinoma in situ. Dysplasia can further be divided into mild, moderate and severe dysplasia. Just as in the uterine cervix, the lesions may be divided into high grade and low grade precursor lesions.

Low-grade squamous intraepithelial lesions of the esophagus which encompasses mild or moderate dysplasia are characterized by well-differentiated superficial and intermediate squamous cells with marked nuclear enlargement and hyperchromasia. In some patients, koilocytes may be observed.
High grade squamous intraepithelial lesions which includes high grade dysplasia and carcinoma in situ, comprises parabasal type of cells. The cells are characterized by enlarged hyperchromatic nucleus, increased nucleus cytoplasmic ratio and clustering of cells.

Atypical glandular cells, low grade or mild dysplasia, is described as slight atypia of the columnar epithelial cells. Adenocarcinoma in situ, high grade dysplasia consists of nuclear enlargement and hyperchromasia in the columnar epithelial cells, occasionally with branching or distortion of the affected glands and a marked increase in abnormal mitoses. The lesions are very similar to precancerous abnormalities and carcinoma in situ of the gastric epithelium. It is difficult either histologically or cytologically to clearly separate adenocarcinoma in situ from adenocarcinoma (10).

2.2.4 Malignant esophageal neoplasms

a) Squamous cell carcinoma

This is the most common esophageal cancer worldwide accounting for over 90% of all the esophageal cancers. However, this is different in United States where adenocarcinoma is the leading esophageal carcinoma (13). It is a quasi-endemic disorder in northeastern Iran, in parts of China, among the Chinese in Singapore, among Africans in southern Africa, and among men in Brittany (10). Squamous cell carcinomas are more common in blacks worldwide (13). This carcinoma occurs preferentially in areas where the esophagus narrows, that is, near the thyroid cartilage, the bifurcation of the trachea, and at the level of the diaphragm (10). Prolonged mucosal exposure to potential carcinogens such as those contained in tobacco and alcoholic beverages is associated with the majority of SCC in Europe and the United States (13). Recent studies done in China did not reveal any risk factor for SCC except, perhaps, diet. However,
Auerback et al, in 1965, demonstrated a high frequency of squamous carcinoma in situ of the esophagus among smokers (10). Recently, it has been suggested that HPV may also have a role in some individuals (6).

Squamous cell carcinoma of the esophagus varies from well differentiated, highly keratinized types to poorly differentiated squamous cancer and, rarely, small-cell (oat cell) type of carcinoma (8, 10).

b) **Adenocarcinoma**

Adenocarcinoma is usually found in the distal esophagus, near the gastric cardia, though it can develop at any level of the esophagus. Unlike SCC, adenocarcinoma is more common in white men than the blacks, three times more often in whites than blacks (7, 10). Men are affected much more frequently than women. In whites, the male-to-female ratio is 7:1, in blacks the difference is even greater (6). Adenocarcinomas seem to arise from dysplastic mucosa in the setting of Barrett esophagus (13).

c) **Small cell carcinoma**

Primary small cell carcinomas of the esophagus are rare but when present they usually arise at the mid or distal third (12).

2.3 **Epidemiology of esophageal carcinoma**

An estimated 482,300 new esophageal cancer cases and 406,800 deaths occurred in 2008 worldwide (14). The World Health Organization (WHO) 2009 report indicated that cancer accounted for 7.9 million deaths which are 13% of all deaths worldwide (3). According to the esophageal cancer statistics released by World Cancer Fund in December 2010, Mongolia has the highest incidence rate in the world with 18.7 cases per 100,000 people (15).
Studies on esophageal cytology have been done since 1950’s. Several esophageal cytology sampling devices have been invented and improved since then. Sponge cytology was first introduced in Japan in 1977. Similar sponges have been used in China, Switzerland and South Africa in different studies. A study was done to compare the three exfoliative esophageal cytology sampler and all the three samplers; balloon, sponge and sponge-mesh, obtained satisfactory yield of both squamous and glandular cells. However, sponge was preferred by participants (7).

Incidence rate of esophageal adenocarcinoma is increasing in the United States. A study was done by Tsang et al. to determine the reliability of balloon cytology in detecting esophageal carcinoma in US veteran where 87 people participated. The results from this study showed balloon cytology to have a sensitivity of 91% and specificity of 94% in esophageal carcinomas (4). The principle drawback of EBC is the discomfort experienced by the subjects during intubation and retrieval of the balloon (7). In a study carried out in United Kingdom between 2008 and 2009 on acceptability and accuracy of non-endoscopic screening test for Barrett’s esophagus in primary care, sponge cytology was well tolerated by 99% of the 504 participants and had sensitivity and specificity of 73.3% compared with gastroscopy (16).

China is one of the esophageal cancer hot spots. Generalized cytology screening is being performed where more than 500,000 people have been subjected to esophageal balloon cytology (EBC) screening in regions of China, in which the prevalence of esophageal cancer ranges from 80 to 800/100,000 people and where esophageal cancer accounts for 22% of all cancer deaths (17). The results of the massive screenings, performed in 1970-1972 and 1974-1975 in Linxian County in China, were impressive where 70% to 80% of detected esophageal carcinomas were at very early stages; carcinoma in situ or limited to submucosa (4). The 5-year survival rate of 85%
to 90% and the 10-year survival rate of 55.6% were recorded subsequent to the EBC screening (4). This shows a positive impact of exfoliative cytology. Results from screening of 81,187 asymptomatic people over the age of 30 in the high-risk Henan province in China indicated that 880 esophageal cancers were diagnosed (a huge prevalence rate of 1%), out of which 649 (73.7%) were in early stages treatable by surgery (10).

In sub-Saharan Africa, esophageal cancers are on the increase with uneven geographical distribution where Eastern and Southern Africa is its epicenters (18). The highest incidence rates reported from Southern Africa are those for the south of Transkei and Soweto in South Africa (19). Cancer of the esophagus is the commonest cancer in South African black males (19). Venter did a research on early detection of esophageal cancer in Transkei using the sponge. Of 152 asymptomatic volunteers, 23% showed normal cytology while the rest showed various degrees of abnormal cell growth (19). A pilot study done in rural Ciskeians in 1992, sponge cytology showed to have 90% sensitivity and 99.9% specificity (20). In a study done between 1999 and 2003 on esophageal cancer in Transkei using sponge cytology, 847 asymptomatic individuals participated among which 717 were women and 130 men. Normal smears or only inflammatory changes accounted for 82%, suspected malignancy 2.3%, severe dysplasia 2.3%, moderate dysplasia 4.7% and mild dysplasia 8.7% (21). Berry et al, 1981 and Jaskiewicz et al., 1987 also researched on EBC cancer screening (10).

In Uganda, at Mulago hospital, a study was done on factors associated with carcinoma of the esophagus. The study enrolled 219 people where the endoscopic diagnosis showed that 20% had esophageal cancer, 4% esophagitis, 5% candida esophagitis and <1% had esophageal ulcers(22).

In Kenya, a research study was done on esophageal cancer in North Rift Valley of Western Kenya at Moi Teaching and Referral Hospital from 1994 to 2004. Out of the all the neoplasm
reported during the study period, esophageal cancer was leading accounting for 468 (13.8%) patients. Esophageal squamous cell carcinoma accounted for more than 90% and the male to female ratio was 1.5:1 (1). In the year 1989 to 1998, White et al studied on esophageal cancer; a common malignancy in young people of Bomet District, Kenya. The results showed that esophageal cancer was the most common with 274 (19%) of 1459 malignances diagnosed (5). Twenty one percent (21%) of 274 were 30 years and below. In 1978, Gatei and colleagues reported that incidence among the Kipsigis and related peoples of the Rift Valley was 0.2 per 100 000 per year, and the overall incidence for the country was 0.67 per 100 000 per year (1).

2.4 Risk Factors for esophageal carcinomas

There are a number of risk factors associated with esophageal carcinomas. They include:

- Using tobacco
- Excessive alcohol use
- Barrett esophagus
- Race
- Age
- Gender
- Being malnourished (lacking nutrients and/or calories)
- Infection with human papillomavirus (HPV)
- Tylosis
- Achalasia
- Having swallowed lye
- Drinking very hot liquids on a regular basis
- Gastroesophageal reflux disease (GERD)
- History of using drugs that relax the lower esophageal sphincter
- Being overweight (probably related to higher frequency of GERD)

2.4.1 Tobacco

Smoking causes acid reflux and also damages cell DNA of the esophagus. Tobacco has more than sixty nine known carcinogens. Some of these carcinogens includes Arsenic, Benzene, Beryllium (a toxic metal), 1,3–Butadiene (a hazardous gas), Cadmium (a toxic metal), Chromium (a metallic element), Ethylene oxide, Nickel (a metallic element), Polonium-210 (a radioactive chemical element), Vinyl chloride, Formaldehyde, Benzo[α]pyrene, Toluene (23). Smoking and/or chewing tobacco increases the risk of both esophageal squamous cell carcinoma and adenocarcinoma (24).

2.4.2 Alcohol

Although there is no evidence that alcohol itself is a carcinogen, alcohol may act as a co-carcinogen by enhancing the carcinogenic effects of other chemicals like tobacco. Persons who take alcohol and tobacco are at higher risk of developing esophageal carcinomas. Excessive drinking of alcohol increases the risk of squamous cell carcinoma (24).

2.4.3 Diet

Diet, which is high in fat, low in protein and low in carbohydrate, has shown to increase the risk of esophageal cancer. Use of nitrosamine, a food additive sometimes used in Chinese food,
may increase the risk of esophageal cancer (25). Vitamins A, C and E, and folate, acts as antioxidant together with other substances in fresh foods, may help to prevent damage to the lining of the esophagus that can lead to cancer. Higher levels of selenium in the blood were shown to reduce the risk of esophageal cancer by almost 50% in a recent study. Selenium is found in all fresh fruit and vegetables, meat and eggs (24). Very hot drinks may damage the lining of the esophagus and increase the risk of esophageal cancer. Some studies have reported up to 3 times the risk in people who regularly drink hot drinks when they are burning hot (24). Epidemiologic studies have identified a strong link between the consumption of a fungus *Fusarium verticillioides* on contaminated maize and the incidence of esophageal squamous cell carcinoma (26).

### 2.4.4 Barrett’s esophagus

People with long term GERD may develop Barrett’s esophagus due to long term irritation of esophagus lining by the acid. BE is associated with a very high risk of esophageal adenocarcinoma. People with BE are up to 125 times more likely to develop adenocarcinoma of the esophagus than the average person (24).

### 2.4.5 Age, Gender and Race

The risk of developing esophageal cancer increases with age. The highest risk of esophageal cancer is in age group 70 to 80. Men have higher risk of developing esophageal cancer compared to women. The African American race has a three-fold increased risk of developing esophageal cancer compared to the Caucasians (25).
2.4.6 Other medical conditions

Tylosis is associated with very high risk of esophageal squamous cell esophageal carcinoma. It is a rare inherited skin condition characterized by too thick skin on palms of the hands and soles of the feet (24).

Achalasia refers to incomplete relaxation of the lower esophageal sphincter in response to swallowing. People with achalasia have 10 to 11 times higher risk of both the squamous cell and adenocarcinima of esophageal than people without achalasia (12, 20)

Plummer - Vinson Syndrome is associated with anaemia as a result of iron deficiency. This condition has been associated with esophageal SCC. Patients also develop small, thin growths of tissue which block part of their food pipe, making swallowing difficult (24).

2.5 Signs and Symptoms associated with esophageal cancer

Difficulty in swallowing (dysphagia) is one of the most common symptoms associated with esophageal cancer. As the tumor grows there may be pain in swallowing (odynophagia) which is normally associated with late stages of the disease. Some patients present with chest pain which they describe as a feeling of pressure or burning in the chest. These symptoms are more often caused by problems other than cancer, such as heartburn and pain associated with difficulties in swallowing. About half of the patients lose weight as a result of swallowing problems which keep them from eating enough to maintain their weight, decreased appetite and an increase in metabolism from the cancer. Esophageal bleeding is also noted in a number of patients. The blood passes through the digestive tract and causes the stool to turn black. Other sign associated
with esophageal cancer include; hoarseness of voice, constant cough, hiccups, bone pain and pneumonia due to the blocking mass (27).

2.6 Diagnosis of esophageal lesions

2.6.1 Endoscopy

If esophageal cancer is suspected, endoscopy is often the first choice to determine the nature of the patient’s problem. The physician is able to see the esophagus lining and can examine for the presence of cancer. If any suspicious sites are detected, a biopsy is taken and sent to the laboratory for histological diagnosis (25).

2.6.2 Barium swallow

Barium is swallowed to coat the walls of the esophagus and then X-rays of the esophagus are taken. Presence of tumor causes the barium to coat that area of the esophagus unevenly. Even small, early cancers can often be seen using this test. Barium studies are much less invasive and causes little discomfort compared to endoscopy, hence this test is often the first test used if an esophageal cancer is suspected. This technique will not result in confirmation of diagnosis of esophageal cancer since it is not possible to do a biopsy. Also it only shows the shape of the inner lining of the esophagus, thus cannot be used to determine how far a cancer may have spread outside of the esophagus (21, 23).

2.6.3 Computed tomography (CT) scan

Unlike a regular x-ray, a CT scan creates detailed images of the soft tissues and organs in the body. It is very useful test to determine the extent of spread of esophageal cancer once the
Diagnosis is made but not for initial diagnosis. These scans can also show the nearby organs and lymph nodes as well as distant areas of cancer spread. The CT scan can help to determine whether surgery is a good treatment option (21, 23).

2.6.4 Biopsy

This involves the removal of tissues, often done during endoscopy, so they can be viewed under a microscope by a pathologist to check for signs of cancer. Biopsy samples can be taken from several different areas in the lining of the lower part of the esophagus to detect early Barrett esophagus in patients who have risk factors for Barrett esophagus (28).

2.6.5 Cytology sampling

Cytology sample collection methods have shown a number of advantages over biopsy. Cytology sampling can cover a wider area than biopsy especially for pre-invasive neoplastic lesions and high grade dysplasia in which no obvious lesion is seen but poorly defined areas of irregularities. A properly prepared cytological smear can give well preserved isolated lymphoid cells unlike distorted and squeezed biopsies. Cytology sampling is less invasive and can be very useful than biopsies to patients with clotting disorders or vascular tumors. Cytology preparations have short turn-around time which is of great importance when quick decision on patient management is needed (11).

There are various exfoliative cytology methods of sample collection. Esophagus washing involves instilling large volumes of fluid usually saline through the mouth and then maneuvering the patient into various positions so that cell from the entire mucosal can be obtained. This method is no longer being used to diagnose gastrointestinal neoplasm (6). Direct brushing of visible esophageal lesions by use of fiberscope is the commonly used cytology sample collection
method (11). Salvage cytology though not a commonly use method; have been used to retrieve material present on the external surface of the biopsy forceps dislodged during withdrawal of the forceps while taking biopsy. The channel is flashed with saline or fixative (6). Endoscopic fine needle aspiration (FNA) is also used especially for lesions confined to lamina propria or the submucosa and muscularis (11). Cytology esophageal balloons with an abrasive surface have also been used as esophagus sampling method. Gelatin coated sponge has also been used. Studies have shown that it is well tolerated by patients (10). Patients swallow product of their choice and gelatin is waited for 5 to 10 minutes to dissolve before the sponge is withdrawn. Balloon sampler has been shown to be more sensitive than the sponge sampler for detecting esophageal squamous carcinoma (29).

Generalized screening to detect early esophageal carcinoma, using both sponge and balloon cytology has been used only in China, Iran, and South Africa, where rates of disease are sufficiently high to render screening cost-effective (4). It is not known how exfoliative sponge method would compare with contemporary endoscopy, which because of cost and limited availability could not be used on a very large scale for purposes of esophageal cancer detection (10).
2.7 Justification

Esophageal cancer is the 9th most common cancer in the world, and the 5th most common cancer in developing countries. Esophageal cancer has known pre-cancerous lesions which can be detected early through cytological diagnosis. With HIV epidemic there is an increase in cancer incidence, resurgence of endemic infections and a wide range of opportunistic infections. Esophageal cancer has high incidence and poor survival rate and therefore, there is great need for screening for precursor lesions that identify individuals at high risk of developing invasive esophageal carcinoma.

There is no standard screening method for esophageal cancers though endoscopy has been most commonly used. Although endoscopy has high sensitivity and specificity of more than 90%, due to its high cost, it is not readily acceptable by patients. Endoscopists and pathologists in the country are few and mainly based in City of Nairobi, making it difficult for patients in the rural areas to access their services.

Studies have shown esophageal sponge cytology as a sensitive (sensitivity of 81 – 90% and specificity of 92 – 99.9%), cheaper, less invasive and readily acceptable by patients as an esophageal cancer screening method. It has also shown to be sensitive in the detection of esophageal infection especially in patients with acquired immunodeficiency disease. This sponge sampling procedure can be effectively performed by non physician medical professionals quickly and with little discomfort to patients; thus it can be used as screening method especially in areas where endoscopy services are not available. No studies have been carried out in Kenya on esophageal sponge cytology.
2.8 Research Questions

What are the cytological findings of the esophagus using sponge cytology on patients referred for esophageal endoscopy at KNH?

What are the risk factors associated with esophageal carcinoma in this population?

2.9 Objectives

2.9.1 General objective

To describe the cytological findings of the esophagus using sponge cytology in patients referred for esophageal endoscopy at KNH.

2.9.2 Specific Objectives

a) Primary

1. To obtain specimen from the esophagus for cytological evaluation using a sponge in patients referred for esophageal endoscopy at KNH.

2. To describe the pattern of esophageal cytological findings using sponge cytology in patients referred for esophageal endoscopy.

b) Secondary

3. To compare sponge cytological findings with endoscopy findings and biopsy results.

4. To identify possible risk factors associated with esophageal diseases.
3.0 MATERIALS AND METHODS

Study type: A descriptive cross-sectional study

Study site: Kenyatta National Hospital (KNH) endoscopy unit. KNH is located in Nairobi County which has a population of approximately 3,138,369. It has total bed capacity of 1800. Endoscopy unit is identified as clinic number 24 and serves an average of 75 patients per week which is about 1,900 patients per year. A patient who is referred for endoscopy is informed on the preparations needed before the procedure is done then booked for endoscopy procedure on a later date. During the procedure a biopsy for histological evaluation may be taken when necessary.

Study Duration: From March to May 2013

Population: Adult patients referred for esophageal endoscopy

3.1 Selection criteria

3.1.1 Inclusion criteria

1. Adult male and female 18 years and above referred for esophageal endoscopy at KNH and were eligible to undergo endoscopy.
2. Individuals who gave informed consent.

3.1.2 Exclusion criteria

1. Individuals with known esophageal carcinoma and any other known primary malignancy.
2. Patients with history of liver cirrhosis and/or esophageal varices.
3.2 Sample size determination

The sample size was calculated using Fisher’s formula. Esophageal cancer prevalence of 11.7% used in this study was obtained from a study done in Transkei, South Africa where 847 patients were examined during 1999-2003, of which 10 cases were diagnosed as suspected malignancy, 13 – severe dysplasia, 30 - moderate, and 56 - mild dysplasia (21).

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

Where,

n is the minimum sample size

Z is the normal standard deviate that corresponds to 95% confidence interval

P is the known prevalence

d is the margin of error degree of precision set at ±5%

\[ n = \frac{1.96^2 \times 0.117(1-0.117)}{0.05^2} = 158.75 \]

\[ n = 159 \]

However, for this study, due to time and financial constraints a sample size of sixty (60) was used. This is a descriptive study and a minimum sample size of thirty (30) is sufficient (30).

3.3 Sampling method

Non probability convenient sampling method was used. Both males and females of 18 years and above who met inclusion criteria were recruited into the study until the desired sample size was achieved. There was no randomization.
3.4 Data collection

Individuals, who were eligible for this study and met the inclusion criteria and gave informed consent, were requested to provide information in the questionnaire. Socio-demographic information and clinical history was extracted from filled questionnaires.

3.5 Specimen collection Procedure

Study subjects were requested to fast overnight as is usual for endoscopy, before the procedure was done. Before examination, dental prostheses were removed and the mouth rinsed with water. With the patient sitting upright, the researcher put the encapsulated sponge (Oesotest, Actimed Switzerland) attached to a string into the back of the throat and the patient was requested to swallow using 1-2 sips of water. Once in the stomach, within 5-10 minutes gelatin dissolved allowing the sponge to expand before it was withdrawn by the attached string. The withdrawn sponge was rolled on a labeled glass slide and a conventional smear. Sponge was then rinsed in normal saline, centrifuged at 1500 rpm for 5 minutes and a smear made from the cell button (31). The smears were fixed immediately in 95% alcohol for at least 15 minutes. Papanicolaou stain was used to stain the smears.

3.6 Cytological Evaluation and Interpretation

There is a remarkable similarity between the cytological presentation of carcinoma in situ and also related lesions of the esophagus and those of the uterine cervix (10). Therefore, the cellular adequacy criteria as well as the interpretation were similar to The Bethesda System (TBS) 2001. Adequate smear for evaluation were conventional smear with well preserved and
visualized squamous cells covering more than 30% of the slide surface. Adequate smear but limited had well preserved and visualized squamous cells covering 10-30% of the slide surface. Inadequate smear had less than 10% of the slide surface covered by squamous. Adequate glandular cells had at least two well preserved and well visualized clusters each with at least five glandular cells (7). Recommended interpretations of squamous cell included; negative for intraepithelial lesion or malignancy (NILM) or epithelial cell abnormality, atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells cannot exclude HSIL (ASC-H), low grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), and squamous cell carcinoma. Interpretations for glandular cells included; atypical glandular cells (AGC), atypical gland cells favour neoplastic, adenocarcinoma in situ (AIS) and adenocarcinoma.

3.7 Data management

Data collected was stored in hard cover register, Microsoft excel spread sheets as well as SPSS software. Data collected from the questionnaires and in hard cover register was kept in lockable cabinets where only the researcher has access thus maintained confidentiality. Information stored in soft copies was protected from access from unauthorized persons by a password. All records were identified by study ID numbers.

All data was analyzed using SPSS version 20. Mean and median were used for continuous variables while proportions used for categorical variables. Chi-square was used to determine association between cytological findings and clinical summaries and risk factor. Kappa test, McNemar and Cochran test were used to compare cytological, endoscopic and histological
findings where appropriate. All statistical tests were performed at 5% level of significance (95% confidence levels). Results were presented in tables and graphs.

3.8 Quality assurance

All reagents were prepared in accordance with the standard operation procedures (SOPs) and as per the manufacturer’s instructions. All the smears were reviewed and signed out by the principal investigator together with consultant pathologist. All positive and 10% of the negatives smears, randomly picked, were re-examined by an independent pathologist.

3.9 Ethical considerations

Before commencement of the study permission was sought and obtained from the UON/KNH ethical review committee. Informed consent was obtained from all potential participants. Care was taken to minimize discomfort for the patient and those who were unable to swallow the capsule because of severe dysphagia were excluded. All results of sponge cytology screening were communicated to the attending physician. Risks involved in this study were minimal which included slight discomfort (gagging) when withdrawing the sponge. Care was not denied to those who declined to participate. All the information obtained in the study remained confident
4.0 RESULTS

4.1 Socio-demographic factors

A total of sixty (60) patients recruited into the study met the inclusion criteria and had satisfactory smears for evaluation. Females were the majority group, 68.33%, and 31.67% were males giving a female: male ratio of 2.2:1 (Table 1). All the 60 participants were blacks.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20</td>
<td>0</td>
<td>1</td>
<td>1 (1.7%)</td>
</tr>
<tr>
<td>21-30</td>
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<td>7</td>
<td>11 (18.3%)</td>
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<td>5</td>
<td>8 (13.3%)</td>
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<tr>
<td>&gt; 70</td>
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<td>0</td>
<td>2 (3.3%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>19 (31.7%)</strong></td>
<td><strong>41 (68.3%)</strong></td>
<td><strong>60 (100.0%)</strong></td>
</tr>
</tbody>
</table>

Table 1 Age group verses gender cross tabulation

4.1.1 Age distribution

The peak age groups was between 41 to 50 years accounting for 25.0%. (Table 1 and Figure 1). The age range was 57 year (18 to 75 years), mean age of 43.77 years (standard deviation of 14.623.), a median age of 42.0 years and mode age of 42years.
4.1.2 Residence

The highest number of participants accounting for 63.33% resided in Nairobi, 25.0% Central, and the least number (1.67%) resided in Rift Valley province (Figure 2). In this study, there was no association between area of residence and development of esophageal cancer (p value 0.102).
4.2 Cytological Findings

Out of all the participants, 52 (86.7%) were negative for intraepithelial lesion or malignancy. Among the 52 NILM patients, 5 (9.6%) were reported to have inflammatory smears and 9 (17.3%) had candidiasis. Intestinal metaplasia was reported in 6 (10%) of all the patients, 1 (1.7%) HSIL and 1 (1.7%) SCC (Table 2).

<table>
<thead>
<tr>
<th>Findings</th>
<th>Frequency</th>
<th>Percent</th>
<th>Cumulative Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>NILM</td>
<td>52</td>
<td>86.6</td>
<td>86.6</td>
</tr>
<tr>
<td>Intestinal metaplasia</td>
<td>6</td>
<td>10.0</td>
<td>96.6</td>
</tr>
<tr>
<td>HSIL</td>
<td>1</td>
<td>1.7</td>
<td>98.3</td>
</tr>
<tr>
<td>SCC</td>
<td>1</td>
<td>1.7</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>100.0</td>
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</tbody>
</table>

Table 2 Pattern of cytological findings
4.2.1 Cytomorphology of various cytological findings

Figure 3 Photomicrographs of; a) Normal smear, b) an inflammatory smear, c) Reactive smear, d) Candida species, e) Intestinal metaplasia, f) HSIL and g) SCC
4.2.2 Pattern of cytological findings by gender and age.

Female participants outnumbered their male counterparts in cytological findings classified as NILM (2:1), inflammation (5:1), Candida species (2:1) and intestinal metaplasia (5:1). However both HSIL and SCC were found in men (Figure 4). All the cytological findings were fairly distributed among all age groups apart from HSIL and SCC findings which were found in the 6th and 7th decades of life (Table 3). Cytological findings were found to have statistically insignificant association with both age and gender (p value 0.447& 0.194 respectively).

Figure 4 Pattern of cytological findings by gender
Table 3 Pattern of cytological findings by age group

4.2.3 Cytological findings by clinical summaries

There was statistically significant association between cytological findings and patients who presented with persistent heartburn and pain in swallowing (p value 0.001 and 0.017 respectively) (Table 4).

Table 4 Cross tabulation of clinical summaries by cytological findings
4.3 Endoscopic diagnosis

Out of all the patients who underwent endoscopic examination of the esophagus and the findings were as follows: normal esophagus 40 (66.67%), GERD 11(18.33%), Candidiasis 7(11.67%) and tumor 2(3.33%). The distribution of endoscopic results among age groups was statistically insignificant ($\chi^2 - 27.19$, df - 18, p value - 0.75). Patients found to have esophageal tumor in this study happened to be all men in their 6th and 7th decade of life (Table 5 & Figure 5).

<table>
<thead>
<tr>
<th>Endoscopy results</th>
<th>Normal</th>
<th>GERD</th>
<th>Candidiasis</th>
<th>Tumor</th>
<th>Total</th>
<th>$\chi^2$</th>
<th>df</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group</strong></td>
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<tr>
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<td>0</td>
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<tr>
<td>21-30</td>
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<td>0</td>
<td>0</td>
<td>11</td>
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<tr>
<td>31-40</td>
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<td>3</td>
<td>2</td>
<td>0</td>
<td>14</td>
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<tr>
<td>41-50</td>
<td>12</td>
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<td>1</td>
<td>0</td>
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<tr>
<td>51-60</td>
<td>6</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>9</td>
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<tr>
<td>61-70</td>
<td>4</td>
<td>1</td>
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<td>8</td>
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<td></td>
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<tr>
<td>&gt;70</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Total</strong></td>
<td>40(66.67%)</td>
<td>11(18.33%)</td>
<td>7(11.67%)</td>
<td>2(3.33%)</td>
<td>60(100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Male</td>
<td>13(32.5%)</td>
<td>3(27.27%)</td>
<td>1(14.29%)</td>
<td>2(100%)</td>
<td>19</td>
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<tr>
<td>Female</td>
<td>27(67.5%)</td>
<td>8(72.73%)</td>
<td>6(85.71%)</td>
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<td>41</td>
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<tr>
<td><strong>Total</strong></td>
<td>40</td>
<td>11</td>
<td>7</td>
<td>2</td>
<td>60</td>
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</table>

$\chi^2$-Chi square; df - Degrees of freedom; P value - Level of significance

Table 5 Cross tabulation of endoscopic findings by age group and gender.
Figure 5 Endoscopic results by gender

4.4 Comparison between cytological findings and endoscopic findings

There was good agreement between endoscopic and cytological findings (sponge cytology) of the esophagus with kappa value of 0.588 and p value of 0.001.

Table 6 Cross tabulation Endoscopic results verses Cytological Findings Cross tabulation
4.5 **Comparison between cytological findings and histological findings**

Of all the patients who were evaluated by cytological method, 2(3.33%) biopsies were taken for histological diagnosis. On cytology, one was reported as HSIL while the other as SCC while on histology (gold standard) they were both reported as SCC. Small sample size (n=2) could have resulted to poor measure of agreement although there was statistically significant association (Kappa value - 0.338, p value - 0.001) between cytological findings and histological findings.

4.6 **Analysis of risk factors for esophageal cancer**

The number of esophageal cancer in this study was small therefore statistical inferences on risk factors (tobacco, alcohol, food additives, very hot beverages among others) associated with esophageal cancer could not be made due to reduced power of association test.

4.7 **Clinical Summary**

Clinical summary as indicated in the questionnaire was categorized as: difficulties in swallowing, pain in swallowing, persistent heartburn, previous esophageal diagnosis, family history of esophageal cancer and use of drugs to relax the esophagus. Majority of the patients accounting for 59.1% presented with persistent heartburn while patients with family history of esophageal cancer accounted for only 3.3% of the total participants. None of the patients diagnosed with esophageal cancer reported to have had family history of esophageal cancer. Participants who experienced pain in swallowing were 8% of the total (**Figure 6**). None of the patients reported to have used drugs to relax the esophagus.
4.7.1 Previous esophageal diagnosis

Ninety percent 54/60 (90%) of the total patients had no previous esophageal diagnosis and were being examined for the first time. Patients on follow up having been diagnosed with GERD accounted for 5/60 (8.3%) and only one patient (1.7%) had been diagnosed with BE who came for re-examination after treatment (Figure 7).

Figure 7 Previous Esophageal diagnoses
5.0 DISCUSSION

This study aimed to describe the cytological findings of the esophagus using sponge cytology in patients referred for esophageal endoscopy at KNH. One of the most importance indications for referral for endoscopy is clinical suspicion of esophageal cancer.

Esophageal cancer remains a global challenge due to its high mortality rate as a result of late diagnosis of which prognosis is to the grave (2). Studies on use of cytological methods for early diagnosis have reported promising findings as esophageal precancerous lesions have been diagnosed. Most of these studies have been conducted in China, Iran and South Africa regions (hot- spots for esophageal cancers) but no documented studies have been carried out in East Africa and Kenya in particular. According to Globocan report 2010, esophageal cancer in Kenya has been ranked as the leading cancer in men and the third in female after cervical and breast cancer.

5.1 Socio-demographic factors

All the participants were blacks. Female to male ratio in this study was 2.2:1 unlike in other studies where the number of males surpassed that of females (7, 29,31). Majority (25%) of the participants were in their 4th and 5th decade of life with the mean age being 43.77 years. The highest risk of esophageal cancer is in 70-80 years with men having a higher risk than women hence this could contribute to few (3.33%) patients found to have esophageal cancer in this study. A study done by Dawsey S. et al. recruited patients between 50- 60 years (high risk group) and the number of esophageal cancers were high(32%) (29). Patients diagnosed (histologically)
with SCC were both males in their 6\textsuperscript{th} and 7\textsuperscript{th} decade documented in literature where males are at higher risk of developing esophageal cancer than women (10-12; 25).

Majority of patients hailed from Central and Nairobi provinces. The possible reason for this is that KNH is in close proximity to patients from central and Nairobi provinces and therefore easily accessible on logistical grounds.

5.2 Cytological findings

All the participants (100%) swallowed the encapsulated sponge successfully and had smears with satisfactory material for evaluation. The cytosponge technique was well tolerated by patients and also had good cellular yield similar to other studies done elsewhere (7; 29; 33).

The minimal discomfort to the patient and the relative simplicity of collecting and interpreting esophageal cells make this procedure suitable for screening patients. However, patients with moderate to severe dysphagia could not swallow the encapsulated sponge hence were excluded from the study. Therefore, sponge cytology is not suitable for this group of patients and other interventions should be used.

Negative smears for intraepithelial lesion or malignancy, including those cases comprised of only inflammation as well as infections,- were the most common (86.6%), intestinal metaplasia was reported in 10% of all the patients, 1.7% HSIL and 1.7% SCC. These results compared well with Stepien’s study of 2009 in South Africa (21). However, other studies done in China and South Africa reported higher numbers of intraepithelial abnormalities (29; 32; 34; 35). This could be accounted by the fact that these studies used very large sample size (625- 12,877) and also these regions are considered hot spots for esophageal cancer. In addition, patients with
severe progressive dysphagia, the ones likely to have malignancies, were also excluded from this study as they were unable to swallow the sponge capsule.

Of all the participants, 10% had smears showing intestinal metaplasia which suggested GERD and/or BE which is a precursor lesion for adenocarcinoma of the esophagus. UK study by Fitzgerald et al. used cytosponge to obtain specimen for biomarker screening for BE (36). A cohort study by Kadri et al looked at acceptability and accuracy of a non-endoscopic (sponge cytology) screening test for BE in primary care found sponge cytology to have a sensitivity of 73.3% (95% confidence interval 44.9% to 92.2%) (16). Rader et al, studied on Cytological Screening for BE and concluded that sponge cytology is a sensitive, inexpensive, and minimally invasive approach to evaluating patients with GERD and BE (37).

Just as it is documented in other studies done elsewhere, Candida species (15% of all the participants) was the most common infection (9; 10). However, in this study the figure is higher compared to a study in Uganda where diagnosis of Candida species was done endoscopically (22). This can be explained by fact that cytology has better results in picking esophageal infections compared to endoscopy (6; 11; 38).

5.3 **Comparison between cytological, endoscopic and biopsy findings.**

There was good agreement (Kappa value of 0.588) between endoscopic and cytological findings just as reported in other studies (39). Endoscopy is a very sensitive technique for identifying clinically significant esophageal lesions in referral centre. In most settings however, it will be more appropriate as a secondary test to confirm and localize lesions identified by a cheaper, less invasive primary procedure, in this case sponge cytology and as a triage for endoscopic biopsy.
Of all the patients who were evaluated cytologically, only 3% had biopsies taken for histological diagnosis. Histology confirmed that the two cases to be significant lesions – squamous cell carcinoma, as picked up by cytology. Although cytology reported one as HSIL and the other as outright SCC, the intervention after screening was the same – surgical management. Therefore screening with sponge cytology correctly identified those cases. The numbers were however, too few for statistical analysis.

5.4 Risk factors associated with esophageal cancer.

One the secondary objective of this study was to identify possible risk factors associated with esophageal diseases. However, due to small sample size and inability to achieve this objective using a descriptive cross section study design, risk factors associated with esophageal cancer could not be evaluated. Nevertheless, within the boundaries of those limitations – some findings were noted- contributory or not.

Although intake of boiling hot beverages have been associated with esophageal cancer according to a study by Dawsey S. et al. at Tenwek hospital Kenya, this study could not deduce association between esophageal cancer and intake of boiling hot beverages. Reports from studies done in China have documented that food additives containing nitrosamine increased the risk of developing esophageal cancer (35). However, this study reported few cases of esophageal cancer and therefore could not deduce a significant association between food additives and increased risk of esophageal cancer.

Tobacco and alcohol use has been associated with increased risk of esophageal cancer. Patients diagnosed with esophageal SCC had history of smoking tobacco and alcohol use. But the small sample size could not be used to make statistically significant inference. Therefore,
larger series may confirm the apparent risk in case of tobacco, and further explore the role of alcohol.

5.5 Clinical summary

Clinical summaries of participant indicated persistent heartburn (59.1%) as the most common presenting symptom while dysphagia (8%) among others was among the least and therefore indication for endoscopic examination in this study group.

There was association between cytological findings and patients who presented with persistent heartburn and pain in swallowing (p value 0.001 and 0.017 respectively). However, the clinical indications are non-specific for particular esophageal lesions hence need for further evaluation examinations. Sponge cytology could be of importance in filtering patients with findings that would require endoscopic examination with subsequent biopsy if warranted.
5.8 Conclusions

1. Esophageal sponge obtains satisfactory specimen for cytological evaluation.

2. Majority of the patients in this study had non-neoplastic lesions and only a few with malignant lesions which were correctly identified by sponge cytology when compared to the gold standard – tissue biopsy.

3. Combined use of cytology, endoscopy and biopsy correctly identified potentially curable cancer.

4. Risk factors associated with esophageal diseases could not be assessed in this study due to the small number of cancer cases reported.

5.9 Recommendations

1. Sponge cytology is a simple and inexpensive technique which can be used as a triage test for patients with clinical esophageal symptoms.

2. Sponge cytology may be employed as a primary test whenever there is any suspicion of an esophageal lesion especially in clinical settings where endoscopic facilities and medical professionals are not available.

3. Sponge cytology can be useful in follow up of patients with BE and GERD rather than commonly used endoscopy which is expensive and unavailable in most of the clinical setting except in tertiary and private health facilities.

4. Clinical signs and symptoms are non-specific for a particular esophageal lesion hence there is need for further evaluation among which sponge cytology could be of importance
in filtering patients with findings that would require endoscopic examination with subsequent biopsy if warranted.

6.0 Study Limitations

1. Sponge cytology was a new procedure in the KNH setting. Therefore skills limitations might have compromised the quality of the specimen collection.

2. Those who were unable to swallow the sponge capsule because of severe dysphagia were excluded and therefore probably resulted in the fewer numbers of neoplastic disease picked up by sponge cytology.

3. Risk factors associated with esophageal cancer could not be assessed in this study due to the small number of cancer cases reported.
References


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Appendices

Appendix i: Client consent explanation form

My name is Ruth Waithira Muriithi from the Department of Human Pathology at the University of Nairobi. I would like to introduce to you a research study that I am conducting, with the aim of giving you relevant information that may help you make an informed decision on whether or not you are willing to participate voluntarily.

This form entails information that might be of help in making an informed decision to either participate or decline. Read it carefully and be there questions or areas you need clarification be free to ask. If you are unable to read, I will read it out aloud to you in a language that you will understand.

Research Title
Cytological Findings of the Esophagus using Sponge Cytology on Patients Referred for Esophageal Endoscopy at KNH

Introduction and Purpose of the Study

Esophageal cancer is the most common cancer in men and third common in women in Kenya. Early detection of esophageal cancer improves treatment and management outcome. Sponge cytology is one of the methods which can detect signs of development of this cancer early enough.

The primary objective of this study is to describe the findings of the esophagus using a sponge on patients referred to esophageal endoscopy at KNH. The findings will be interpreted and reported by a pathologist where the results will be compared with those you will get from endoscopy and may be biopsy results. The outcome will determine whether this sponge method is a useful to test for esophageal cancer.

Benefits of the study

- The results will be confidentially given to the doctors looking after you or placed in your file, will be passed to you and you will benefit from obtaining both endoscopy and biopsy as well as sponge cytology results.
Early detection of signs of development of esophageal cancer would be beneficial because they have good prognosis. This study will give you an opportunity to know early whether you have any signs or not.

In case of any signs of early development of a disease is detected, your doctor will be confidentially informed and you will be advised on the recommended treatment and management.

**Risks and discomfort**

There will be minimal discomfort during swallowing and withdrawal the sponge. There is potential risk of emotional stress due to anxiety of the awaited outcome but you will be assured and advised accordingly.

**Procedure**

Once you have accepted to participate and you are eligible for the study, you will fill the provided questionnaire and the below described procedure will be carried.

1. You can have dinner as usual, but do not have any breakfast in the day of the procedure.
2. While seated in an upright position, a sponge inside a capsule will be passed at the back of the mouth and you shall be requested to swallow it using water.
3. The sponge will then be allowed in the stomach for five to ten minutes and then pulled up.
4. The sponge will be rolled on a clean labeled glass slide to transfer the material obtained make a smear for laboratory analysis.
5. The smear will be processed in the usual manner and then analyzed.

**Voluntarism**

Participants in this research study will volunteer without any coercion. You may decline to participate or to answer any question in the questionnaire that you are not comfortable with or even terminate the interview at will without any consequence. You may also withdraw from the study at any time you wish should you change your mind about participating without loss of health care benefits to which you are entitled in this hospital.
• Do you have any question concerning the above explained information?

Yes ☐ No ☐

• Are you willing to participate in this research study?

Yes ☐ No ☐

If yes, kindly sign here below.

I .................................................. declare that this study described above has been explained to me and/or I have read it and understood. I volunteer to participate in this study.

Participant Signature/ thumbprint ..................................Date ........................................

Doctor/ Nurse ..........................................................Date ........................................

Principal Investigator/ Research Assistant ......................Date ........................................

Contacts
Should you have any concerns about how this study is being conducted you may get in touch with either with my supervisor or the Secretary of the ERC that gave approval to this study on the telephone number provided below. You also get in touch with me for any queries that you have at any time.

Researcher contacts
Ruth Waithira Muriithi
Mobile number: +254 723 359 603

UON contacts
Department of Human Pathology,
Tel. +254-2-7263000 Ext 43769,
+254-2-2725102

KNH/Ethical Research Committee Secretary,
02-726300 Ext 44102,
P. O. Box 20732, Nairobi Kenya.
Appendix ii : Fomu ya maelezo ya idhini

Jina langu ni Ruth Waithira Muriithi kutoka idara ya Human pathology katika chuo kikuu cha Nairobi. Lengo la ujumbe huu ni kukuleleza kwa kina kuhusu utafiti ninao fanya kwa nia ya kukusaidia kukata shauri kwa hiari kushiriki au kutoshiriki katika utafiti huu bila kushurutishwa.

Fomu hii ina ujumbe utakao kusaidia kufanya uamuzi wa busara. Isome fomu hii kwa makini. Una uhuru wa kuuliza swali lolote kuhusu utafiti huu; faida zake au madhara ambayo yaweza kukuweza kukukumba iwapo utashiriki. Iwapo huwezi kusoma, nitakusomea kwa sauti ili uweze kuelewa.

Kichwa cha Utafiti

Matokeo ya uchunguzi wa umoi kwa kutumia kifaa cha kisaitolojia , katika wagonjwa walioelekezwa kufanyiwa uchunguzi wa umio kwa njia ya ‘Endoscopy’ katika hospitali kuu ya Kenyatta.

Maelezo kwa ufupi na nia ya utafiti huu

Saratani ya umio ina ongoza kati ya saratani zinginezo kati ya wanaume na nambari tatu kati ya saratani zinginezo kwa wanawake humu inchini Kenyatta. Kuna dalili za saratani ya umio ambazo za julikana na zawezekufanyiwa uchunguzi wa umio katika hospitali kuu ya Kenyatta. Ugunduzi wa mapema wa saratani ya umio unamanufaa kwani huboresha matokeo ya matibabu.

Lengo kuu la utafiti huu ni kuelezea matokeo ya uchunguzi wa umio kwa kutumia kifaa cha kisaitolojia katika wagonjwa walioelekezwa kufanyiwa uchunguzi wa umio kwa njia ya ‘Endoscopy’ katika hospitali kuu ya Kenyatta. Matokea yatasomwa na daktari aliyehitimu na yatalinganishwa na matokeo ya ‘Endoscopy’ na yale ya ‘Biopsy’. Matokoe haya yatatadihihirisha ubora wa kifaa hiki kama njia mojawapo ya uchunguzi wa saratani ya umio.

Faida za utafiti huu

- Stakabadhi za matokeo yako zitashughulikiwa kwa njia ya siri; hakuna yeyeto asiye ruhusiwa atakaye zisoma. Majibu yatawekwa kwa faili yakani yatakufikia kupitia daktari wanaokutibu. Utanufaika kwa kupata matokeo zaidi ya moja; ya kifaa cha kisaitolojia, ya ‘Endoscopy’ pia ya ‘Biopsy’. 
Ugunduzi wa mapema wa dalili za saratani ya umio unamanufaa kwa kuwa unaboresha matokea ya matibabu. Utafiti huu unakupa nafasi ya kuchunguzwa iwapo una dalili za saratani ya umio au la.

Iwapo utapatikana na dalili za saratani ya umio, daktari wako atapewa matokeo yako kwa siri na utapewa mawaidha kuhusu matibabu yatakayo kufaa.

**Madhara ya utafiti**

Waweza kuhisi usumbufu katika hali ya kumeza na kutolewa kwa kifaa hiki. Waweza kupatwa na wasi wapi unaposubiri matokeo la utapewa hakikisho na ushauri ufaa.

**Utaratibu wa kushiriki**

Pindi utakapo kata shauri kushiriki bila kushiriki na kutolewa kwa kifaa hiki. Utapewa mawaidha kuhusu matibabu yatakayo kufaa na utapewa mawaidha kuhusu matibabu yatakayo kufaa. Utaratibu utakaofuatwa ndio huu.

1. Kula chakula chako cha jioni kama kawaida lakini usile kiamsha kinywa siku ya uchunguzi.
2. Kama umeketi, chombo maalum (kifaa cha kisaitolojia) kitapitishwa kwa mdomo kisha utaelekezwa kukimeza.
4. Kipimo hiki kitachunguzwa kwenye mahabara ili kubaini dalili za saratani.

**Idhini ya Mshiriki**

Watakao shiriki katika utafiti huu itakuwa kwa hiari bila kushirutishwa. Una uhuru wa kutoshiriki, kutojibu swali lolote kwenye dodoso au kukuja swali lolote. Pia waweza kutotarishwa ushirika wako kwenye utafiti huu bila kupoteza haki ya kutiwa katika hospitali hii.

- Una swali lolote kuhusu maelezo uliyopewa?
  
  Ndio [ ] La [ ]

- Utashiriki kwenye utafiti huu?
  
  Ndio [ ] La [ ]

  Kama utashiriki, tafadhali tia sahihi yako kwenye pengo lililoachwa hapa chini.
Mimi ..............................................................nimeshauriwa kamili kuhusu utafiti huu na
nimeamua bila kushurutishwa na yeyote kushiriki.

Sahihi ya mshiriki ....................................................Tarehe ...............................................

Daktari / Muuguzi ..................................................Tarehe ........................... .........................

Mchunguzi ..........................................................Tarehe ........................... .........................

Anwani

Ukiwa na maswali yoyote kuhusu utafiti huu, wasiliana na katibu Chuo Kikuu cha Nairobi idara
ya ‘Human pathology’ au katibu kamati ya maadili ya utafiti ya Hospitali kuu ya Kenyatta/ Chuo
kikuu cha Nairobi kupitia anwani ulizopewa.

Chuo Kikuu cha Nairobi

Idara ya Human Pathology

Nambari ya Simu +254-2-7263000 - 43769
+254-2-2725102

Kamati ya Maadili ya Utafiti ya Hospitali Kuu ya Kenyatta

Nambari ya Simu 02-726300 - 44102

S. L. P 20732,

Nairobi, Kenya.
Appendix iii: Questionnaire

Cytological Findings of the Esophagus using Sponge Cytology on Patients Referred for Esophageal Endoscopy at KNH

All the participants will be required to fill the questionnaire before specimen collection. Kindly tick (√) one of the choices given.

Section A: Socio-demographic information

Study No. ................................................................. Date .................................................................
OP/IP No................................................................. Phone No............................................................

1. Age ....................... Gender: Male ☐ Female ☐
2. Race: Black ☐ White ☐ Asian ☐ Colored ☐
3. Residence (Specify Province) ................................
4. Occupation ...........................................................
5. Preferred beverage temperature.
   Cold ☐ Hot ☐ Boiling Hot ☐
6. Take food with spices.
   Rarely ☐ Regularly ☐ Always ☐
7. Tobacco use. Yes ☐ No ☐
   If yes indicate: a) Duration of use (yr) b) Quantity per day;
   ≤5 ☐ ≤5 sticks ☐
   6 – 10 ☐ 6-10 sticks ☐
   ≥10 ☐ ≥10 sticks ☐
8. Alcohol use Yes ☐ No ☐
   If yes indicate: a) Duration of use (yr) b) Quantity per day
   ≤5 ☐ ≤5 bottles ☐
   6 – 10 ☐ 6 – 10 bottles ☐
   ≥10 ☐ ≥10 bottles ☐

Section B: Clinical History
9. Persistent heartburn
   Yes ☐ No ☐

10. Difficulties in swallowing
    Yes ☐ No ☐

11. Pain in swallowing
    Yes ☐ No ☐

12. Have had previous esophagus diagnosis of either;
    Barrett’s Esophagus ☐ GERD ☐ Others.................................

13. Have used drugs to relax the esophagus
    Yes ☐ No ☐

14. Family history of esophagus cancer
    Yes ☐ No ☐

Section C: For Investigator ONLY

1. Specimen Adequacy: Satisfactory ☐ Unsatisfactory ☐

2. Epithelial cell features:
   Inflammatory ☐
   Infection ☐ Specify .........................................................
   BE ☐ NILM ☐ LSIL ☐ HSIL ☐
   ASCUS ☐ ASC-H ☐ SCC ☐ AGC ☐
   Adenocarcinoma ☐

3. Endoscopy results: Normal ☐ Suspicious ☐

4. Histological results: Normal ☐ Mild dysplasia ☐
   Moderate dysplasia ☐ Severe dysplasia ☐
   Carcinoma in Situ ☐ SCC ☐
   BE ☐ Adenocarcinoma ☐
Appendix iv: Papanicolaou Stain

Principle of the stain
Haematoxylin stains the nuclei blue by dye lake formation. 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. The smear is fixed in 95% ethanol.
2. Hydrate smears by passing them through ethanol grades of 80%, 70% and then 50%.
3. Rinse smears in distilled water 10 dips.
4. Stain in Harris haematoxylin for 3 minutes.
5. Rinse in tap water.
6. Differentiate in 0.05% acid water 10 dips 
7. Rinse in tap water and blue in Scott’s tap water 10 dips
8. Rinse in 95% ethanol 10 dips
9. Stain in O.G 6 for 1½minutes
10. Rinse in 95% ethanol 10dips
11. Stain in E.A.50 for 3 minutes
12. Rinse in 95% ethanol 10 dips
13. Dehydrate in changes of absolute ethanol 10 dips each
14. Clear in 3 changes of xylene 10 dips each
15. Mount in D.P.X
Appendix v: Bethesda System 2001

**Specimen Type:** Indicate conventional smear (Pap smear) vs. liquid-based vs. other

**Specimen Adequacy**

- Satisfactory for evaluation (describe presence or absence of endocervical/transformation zone component and any other quality indicators, e.g., partially obscuring blood, inflammation, etc)
- Unsatisfactory for evaluation (specify reason)
- Specimen rejected/not processed (specify reason)
- Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify reason)

**General Categorization (Optional)**

- Negative for Intraepithelial Lesion or Malignancy
- Epithelial Cell Abnormality: See Interpretation/Result (specify ‘squamous’ or ‘glandular’ as appropriate)
- Other: See Interpretation/Result (e.g. endometrial cells in a woman > 40 years of age)

**Automated Review**

If case examined by automated device, specify device and result.

**Ancillary Testing**

Provide a brief description of the test methods and report the result so that it is easily understood by the clinician.

**Interpretation/Result**

**Negative for Intraepithelial Lesion or Malignancy** (when there is no cellular evidence of neoplasia, state this in the General Categorization above and/or in the Interpretation/Result section of the report, whether or not there are organisms or other non-neoplastic findings)

**Organisms:**

- Trichomonas vaginalis
- Fungal organisms morphologically consistent with Candida spp
- Shift in flora suggestive of bacterial vaginosis
- Bacteria morphologically consistent with Actinomyces spp
- Cellular changes consistent with Herpes simplex virus

**Other**

- Endometrial cells (in a woman > 40 years of age)

  (Specify if ‘negative for squamous intraepithelial lesion’)

**Epithelial Cell Abnormalities**

**Squamous Cell**

- Atypical squamous cells
  - of undetermined significance (ASC-US)
  - cannot exclude HSIL (ASC-H)
- Low grade squamous intraepithelial lesion (LSIL)
  - encompassing: HPV/mild dysplasia/CIN 1
- High grade squamous intraepithelial lesion (HSIL)
  - encompassing: moderate and severe dysplasia, CIS/CIN 2 and CIN 3
  - with features suspicious for invasion (if invasion is suspected)
- Squamous cell carcinoma

**Glandular Cell**

- Atypical
  - endocervical cells (NOS or specify in comments)
  - endometrial cells (NOS or specify in comments)
  - glandular cells (NOS or specify in comments)
- Atypical
  - endocervical cells, favor neoplastic
  - glandular cells, favor neoplastic
- Endocervical adenocarcinoma in situ
- Adenocarcinoma
  - endocervical
  - endometrial
  - extrauterine
  - not otherwise specified (NOS)