THE UTILITY OF IMPRINT CYTOLOGY OF GASTROINTESTINAL ENDOSCOPIC TISSUE BIOPSIES AT KENYATTA NATIONAL HOSPITAL

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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 higher learning institution.

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DEDICATION

I dedicate this dissertation to my husband Jean De Dieu for his spiritual, social and financial support and encouragement which enabled me to succeed in my studies and also to my two sons Igor and Hygues who remained wise despite my absence at very tender age.

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ABBREVIATIONS

ADC**:** Adenocarcinoma

AIDS: Acquired Immune Deficient Syndrome

BE: Barrett's Esophagus

CRC: Colorectal Cancer

CT: Computed Tomography

DCBE: Double-Contrast Barium Enema

DNA: Deoxyribonucleic Acid

DPX: Diestrene Plasticizer Xylene

EA: Eosin Azure

FIT: Fecal Immunochemical Test

GC: Gastric Cancer

GERD: Gastro Esophageal Reflux Disease

GI: Gastrointestinal Tract

GIT: Gastrointestinal Tract

H&E: Hematoxylin and Eosin

HPV: Human Papilloma Virus

IARS: International Agency for Research on Cancer

IC: Imprint Cytology

KNH: Kenyatta National Hospital

MALT: Mucosa Associated Lymphoid Tissue

NPV: Negative Predictive Value

NSAID: Non- Steroidal Anti-Inflammatory Drugs

OG: Orange G

PAP: Papanicolaou

PET: Positron Emission Tomography

PI: Principle Investigator

PPV: Positive Predictive Value

QA: Quality Assurance

SCC: Squamous Cell Carcinoma

SOPs: Standard Operating Procedure

SPSS: Statistical Packages for Social Sciences

UON: University Of Nairobi

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Abstract

Background: Endoscopic histology is the gold standard for diagnosis of gastrointestinal pathology. Touch imprint cytology of endoscopic biopsies is rapid, inexpensive method for diagnosis of gastrointestinal pathology. Whereas there are less than eight published studies that examine the utility of imprint cytology of endoscopic biopsies as a tool for rapid diagnosis of gastrointestinal infections and malignancies, none are from Africa. This study examined the utility of imprint cytology of endoscopic biopsies for rapid diagnosis of inflammatory, pre-malignant and malignant gastrointestinal pathology.

Objectives: The main objective was to establish the utility of imprint cytology in the diagnosis of GIT lesions at Kenyatta National Hospital.

Materials and Method: This study was a cross sectional descriptive study and was carried out on 124 patients in Endoscopy Unit, Kenyatta National Hospital, within a period of 3 months**.** **E**ndoscopic biopsies were gently rolled on two microscopic slides to make imprint smears- prior to formalin fixation. Both slides were air dried and subsequently stained with Papanicolaou and Giemsa stains. Cytological features were described and displayed using photomicrographs. Diagnostic performance of imprint cytology was calculated and expressed in percentage.

Results: A total number of 124 participants were included in this study and Imprint cytology revealed that 37 (29.83%) were positive for malignancy whereas 34 (27.41%) were positive *H.pylori.* The overall accuracy of imprint cytology for malignancy and for *H.pylori* was excellent (94% and 90% respectively).

Conclusions: Imprint cytology is an easy and rapid procedure for detection of infectious, benign and malignant diseases of gastrointestinal tract. Imprint cytology has a high accuracy, sensitivity and specificity in diagnosis of GIT malignancy and for *H .pylori* infection.

Recommendations: Imprint cytology can be routinely performed alongside endoscopic biopsy for diagnosis of bacteriologic and helminthic infections in the digestive tract. Imprint cytology should be used to enable early planning for further management of the patient and to help to avoid a repeat of procedure in case of inadequate biopsy.

1.0. INTRODUCTION

Diagnosis of gastrointestinal diseases relies upon history, physical examination, endoscopy, radiology and laboratory investigations. In anatomic pathology, tissue diagnosis is the gold standard in diagnosis of disease and relies upon the identification of specific histological patterns, cells, cell products and etiological agents (1).

Cytology has emerged as a valuable diagnostic tool and an adjunct to histopathology. This relies upon the identification of cells, cellular patterns, cell products such as mucin and etiological agents.

Gastrointestinal cytology is performed on specimens obtained using the following techniques: brush cytology, crush preparation and endoscopic fine needle aspiration (1). These techniques have low yield, specimens are relatively difficult to process. Touch preparations of endoscopy specimens is a reliable source of cytological specimens.

Imprint cytology is a technique used for collecting cells by applying gentle pressure on surgical tissues onto a clean glass slide. Slides are then fixed and stained using alcohol based stains such as Papanicolaou or Romanowsky stains. Microscopy using screening objective (x 10), high power (x 40- x 100) can be performed by an experienced cytopathologists and diagnostic information documented (2). The main advantage of imprint cytology of endoscopic biopsy specimens is that it can be performed on the biopsy specimen without the requirement for an additional invasive procedure (3). Imprint cytology is quick, non-invasive, easy and relatively inexpensive (3). When used with histology, imprint cytology is useful for diagnosis of infectious diseases such as *Helicobacter pylori*, identification of gastric or esophageal intestinal metaplasia, pre-malignant and malignant disease (1).

Imprint cytology is currently not performed in the endoscopy clinic in Kenyatta National Hospital. Within referral hospitals as well as tertiary health care institutions, imprint cytology can be used for rapid diagnosis of infections such as *Helicobacter pylori*, screening for metaplasia, pre-malignant and malignant disease. This study evaluated the diagnostic utility of imprint cytology on gastrointestinal endoscopic biopsies at an endoscopy unit of a referral hospital in Kenya. This information would be useful for effective histological evaluation by aiding the selection of special stains, immunohistochemistry or molecular techniques.

2.0. LITERATURE REVIEW

2.1. Normal histology and cytology of gastrointestinal tract

2.1.1. The Esophagus

The adult esophagus is 18-25 cm long and 2-3 cm in diameter , which is composed of striated skeletal muscle in the upper part, smooth muscle in the lower part, and a mixture of the middle (4). The mucosa consists of stratified non keratinized squamous type. The basal layer may contain melanocytes and neuroendocrine cells. The submucosa is made up of loose connective tissue that in the distal portion contains mucous gland. The muscularis propria and the submucosa which contains mucous glands distributed through the esophagus (5).

Esophageal cytology specimens mainly consist of superficial and intermediate squamous cells in large flat sheets, in small clusters, in pearls, and as solitary cells. Parabasal cells 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 esophagus. Contaminants like ciliated columnar respiratory cells, alveolar macrophages, oral cavity microbes and food may be present (1).

Imprint cytology of esophageal biopsies can detect *Candida* species, inflammatory lesions, Barrett's Esophagus by identifying benign columnar metaplasia with Goblet cells; squamous cell carcinoma by identifying pleomorphic squamous cells in clusters with orangeophilic cytoplasm as well as adenocarcinoma by clusters of malignant columnar cells (6).

2.1.2. The stomach

The stomach is divided into four anatomic zones: the cardia, fundus, body and antrum. The antral foveolae are lined by mucin-secreting cells that form shallow foveolae. The antral foveolae are lined by mucin- secreting cells and endocrine cells, such as G cells, that release gastrin to stimulate luminal acid secretions by parietal cells within the gastric fundus and body. The foveolae of the body and fundus also contain Chief cells that produce and secrete pepsin (7).

Gastric cytology specimens are represented by columnar cells, occurring singly or forming cohesive fragments of cells with opaque or clear cytoplasm. The columnar configuration of the component cells is seen at the edge of such clusters whereas the center of the cluster shows `honey comb` pattern. The mucus-producing antral columnar cells display an abundant, clear

cytoplasm a flattened surface, tapered basal surface. In mucus-producing columnar cells, the nuclei may be located at the basal surface and may resemble the Goblet cells (8).

The neutral mucin can be found in the surface epithelia of the stomach. Several staining technics are used to demonstrate the two types: Alcian blue is used alone to demonstrate acid mucins and combined with PAS staining procedure to demonstrate both acid and neutral mucins (9). Romanowsky stains can also be helpful for identifying components in a tissue such as mucin. Mucin stain purple, which is helpful in identifying them (10).

The use of imprint cytology can detect *Helicobacter pylori* gastritis on a smear showing benign columnar cells with tiny curved or spiral shaped *H. pylori* organisms in the background. *Helicobacter pylori* stain dark blue with Giemsa stain. Malignant tumors like adenocarcinomas can be detected by identification of tumor cells in clusters and acinar pattern with individual cells being columnar with mucin filled cytoplasm, vesicular nuclei and prominent nucleoli. Signet-ring cells are also present and predominant in signet-ring adenocarcinoma. Lymphoma also can be detected by the presence of monomorphic dyscohesive lymphoid cells with high N/C ratio (6).

2.1.3. The small intestine

The small intestine is divided into 3 portions: duodenum, jejunum, and ileum. The small bowel is composed of three layers: mucosa, muscularis propria, and serosa (11) . The mucosa of the small intestine is principally composed of absorptive enterocytes, mucous producing goblet cells, neuroendocrine and Paneth cells (8). In a normal small intestine, goblet cells contain neutral and sialomucins. The sialomucins are found more to the level of villus top. Both these types of mucin can be stained by PAS and Alcian blue (9) .

Cytology specimens mainly consist of cells arranged in a honey comb pattern with the nucleus centrally located within the cytoplasm. The cytoplasm is finely vacuolated but generally opaque. Goblet cells may be present singly or in clusters among the columnar cells (8).

Imprint cytology can detect adenocarcinoma and lymphoma according to the morphological features of cells. Infections, for example *Giardia lambria* can be detected by their morphological characteristics. Carcinoid and stromal tumors may be detected although there is no data showing their detection on imprint cytology of endoscopic biopsies.

2.1.4. The large intestine

The large intestine is 1-1.5m. Anatomical regions consist of the cecum, ascending(right) colon, transverse colon, descending (left) colon, sigmoid colon and rectum (11). The colorectal mucosa is normally composed of an epithelium that forms straight non branching glands. The epithelium is composed of absorptive cells and is rich in goblet cells (8). In a normal colon, goblet cells produce neutral mucin and sulfated acid mucin. They can be stained by PAS stain, Alcian blue, a combination of Aldehyde-Fuschin and Alcian blue as well as the combination of Alcian blue and PAS (12).

Cytology specimens mainly consist of relatively large cells, monolayered sheets with distinct edges. The sheets are cohesive and single cells are sparse. The cells are uniform and orderly forming a honey comb arrangement when seen *en face* and palisaded when seen from side. Normally, inflammatory cells are seen in background and possibly, mucus and undigested food as well as debris (8).

Imprint cytology can detect malignant tumors found in the large intestine such as adenocarcinoma, lymphoma, stromal tumors, carcinoid tumors; as well as metastatic tumors. Benign tumors can also be detected (13), (14). Inflammatory and infectious diseases can be detected on imprint smear of endoscopic biopsies. Debris and undigested food are also expected to be seen.

2.2. Diseases of GIT

2.2.1. Infections

The human, gastrointestinal tract can be infected by a wide range of microbial pathogens that can affect human hosts. These infections are common in countries regions with poor hygiene and sanitation. Infections of GIT can be caused by viruses, bacteria, parasites and fungi (1). Some of these infections are common in immunocompromised patients; however, they may be diagnosed in immunocompetent patients.

Fungal infections may affect any part of gastrointestinal, but the esophagus is the most affected. *Candida albicans* is the most common. Other fungi such as *Aspergillus* species, *Histoplasma* species, *Pneumocystis Jiroveci* and *Cryptococcus neoformans* are also found in gastrointestinal

tract (1), (15). Viral infections which may be found in gastrointestinal tract are *Herpes* virus which is common in esophagus and anorectum; *Human Papillomavirus* which is common in esophagus and *Cytomegalovirus* which is mostly found at the level of stomach and intestine (1). Parasitic infections are common in small and large intestines. These include protozoa like *Entamoeba histolytica* and *Giardia lamblia* which are the most common; coccidial infections and helminthic infections (1). Bacterial infections also are found in gastrointestinal tract and are commonly found in small intestine. These include Enterobactericeae and most of them are gram-negative rod-shaped pathogens like *Escherichia coli, Shigella, Klebsiella, Salmonella, Enterobacter, Camphylobacter* species etc. *Mycobacterium tuberculosis* is found at any part of GIT. *H. pylori* is also found in the stomach and is associated with gastritis and other ulcer diseases (1)

On gross examination, depending on the species of infectious pathogen, the mucosa may appear non-specific, normal, ulcerated, nodular, necrotic and also `flask-shaped` lesions in case of *E. histolytica* infection (1), (8). Microorganisms are identified according to their morphological criteria. Viral infections are identified by their cytopathic effects (8). Although microorganisms may be identified on H&E or Papanicolaou stains, special stains are the best diagnostic aids. These include GMS, PAS and Giemsa which stains fungi, parasites and bacteria such as *H. pylori*; Ziehl Neelsen stain *Mycobacterium tuberculosis.* Trypomastigotes of *T. cruzi* has been reported on imprint smear stained by Giemsa by Marcero et al(16)Viruses may be identified using immunohistochemistry (15).

Gastrointestinal infections can be detected on imprint cytology as it has been shown by studies (17), (6). Imprint cytology may help in establishing a quick diagnosis of these infectious diseases and patient management especially for premalignant infections such as *H. pylori* .The literature data also showed that eradication of *H. pylori* reduced the gastric carcinoma and gastric MALT-omas in 70% to 80% of patients (17).

2.2.2. Other non-neoplastic and neoplastic GIT disorders

2.2.2.1. Non-neoplastic disorders

Non-neoplastic disorders in gastrointestinal tract are common. These include congenital malformations, metaplastic lesions, autoimmune and other inflammatory diseases. The persistence of these diseases may lead to malabsorption, ulcers, fistulae and malignancy (8).

Barrett's esophagus was described in 1950 by Barret; it consist of replacement of distal esophageal squamous epithelium by columnar epithelium of gastric or intestinal type. The presence of Goblet cells is a characteristic feature (18). Barrett's esophagus is thought to result from chronic gastro-esophageal reflux and more than 90% of esophageal adenocarcinoma arise from BE (18) . The intestinal type epithelium contains mucus producing Goblet cells which may be stained by Alcian blue stains. Both gastric and intestinal type epithelium are PAS and mucicarmine positive (8). Barrett's esophagus has a prevalence of 1-7%. Its prevalence in GERD and adenocarcinoma range from 4 -7% and 0.4- 1.9% respectively.

Gastritis may be acute or chronic (18). It has been shown that lithium may be occasionally associated with gastritis (18). Gastric ulcer is seen in all ages but is more frequently seen in adults. Its complications include gastric hemorrhage and perforation of gastric wall (18). Acute gastritis refers to inflammatory damage of gastric mucosa. Among the causative agents of acute gastritis include drugs like NSAID and aspirin, infections, stress, irradiation, allergies etc.(1), (19). Intestinal metaplasia refers to replacement of normal gastric epithelium by cells like those of mucus-producing epithelium lining the small intestine. This occurs in the distal portion of the stomach (1). Intestinal metaplasia is considered as a risk factor of gastric cancer because the majority of gastric cancer arise in this environment of intestinal metaplasia (8). Intestinal metaplasia grade III is considered to develop in dysplastic lesion (1). Alcian blue and PAS stains demonstrate the type and the magnitude of intestinal metaplasia (1). The normal gastric neutral mucins stain magenta with PAS stain while in intestinal metaplasia, acid mucins stain blue or pulple with Alcian blue. Sialomucins seen in type I intestinal metaplasia stain blue and sulfomucins seen in type III intestinal metaplasia stain brown. Type II intestinal metaplasia show a mixture of gastric and intestinal mucins (20). Intestinal metaplasia present as a component of atrophic gastritis. Auto-immune gastritis is defined as chronic inflammation involving the corpus mucosa whereby a patient has high serum autoantibodies against parietal cells and intrinsic

factor antibodies in 55% to 60% cases. Many of these patients develop pernicious anemia which is associated with intestinal-type gastric cancer. Patient with autoimmune gastritis have a high chance of developing hyperplastic and adenomatous polyp, carcinomas and endocrine tumors (1), (21), (22).

Numerous inflammatory abnormalities may involve the small intestine. Some may be congenital such as Merckel's diverticulum, others are due to ischemic disorders, malabsorption disorders, autoimmune diseases, bacterial over growth, inflammatory bowel disease (Crohn's disease)etc. (1). Crohn's disease is a chronic inflammatory condition that can affect any part of GIT. One of its characteristics is the presence of non- caseating granuloma in 50% to 60% of cases. The duodenum and the terminal ileum are the most involved in small intestine. According to studies, Crohn's disease is being diagnosed more often in teenagers and children. Women and smokers are also more affected (1).

Two principal forms of inflammatory bowel disease in large intestine are ulcerative colitis and Crohn's disease. These diseases may cause serious complications including colon cancer (18) . When chronic, the disease is characterized by formation of confluent mucosal ulcers limited to various segments of the colon. Polypoid masses or nodules may occur to the edges of the ulcer. Patients with ulcerative colitis are prone to the development of colonic adenocarcinoma (18).Ulcerative colitis involve both rectum and colon, only 1% of colorectal adenocarcinoma arise from a colitic colon (1). The cytological features show cells forming cohesive sheets showing nuclear enlargement and prominent nucleoli (18).

Crohn's colitis may involve any part of GIT. It has been shown that the involvement of the colon is at 10% to 20%. The cytological features of Crohn's colitis are the presence of leucocytes, as well as features of repair. Glandular cells are singly dispersed or in loosely cohesive clusters but always in columnar shape with pale nuclei and prominent nucleoli. Non-necrotizing granuloma is also present. Studies showed that the chance of developing dysplasia and adenocarcinomas are similar for both ulcerative colitis and Crohn's colitis (1).

Barrett's Esophagus has been identified on imprint cytology by Vijayanarasimha et al, 2014 though its sensitivity for detection of this lesion is not known. There is no data on the identification of these other non-neoplastic disorders on imprint cytology. Imprint cytology can

help in early diagnosis of BE as well as other potentially malignant non-neoplastic disorders. The early management of BE may lead to reduction of the number of esophageal carcinoma.

2.2.2.2. Neoplastic disorders

2.2.2.2.1. Benign neoplasm

Benign neoplasms in gastrointestinal tract are common and include polyps, papilloma, leiomyoma, lipoma, adenoma and hamartomas. Gastrointestinal stromal tumours (GIST) are frequently benign. These cause disease by mass effect. Although excision may be curative, targeted therapy may be required GIST.

The squamous papilloma in the esophagus has been described in 1982 by Syrjanen et al and HPV infection has been found in association with this kind of polyp in 78% (1). Approximately 25% of small intestine benign neoplasms are adenomas and most of them occur in duodenum papilla. It has been shown that these lesions have pre- malignant potential (23). Dysplastic adenomas are graded as low-grade where there is no significant architectural changes and high-grade where severe nuclear changes and complex architectural abnormalities are characteristics (1). High-grade dysplastic adenomas and larger than 4cm adenomas are the one at high risk for malignancy (1).

It has been shown that polyps cannot be identified in brush smears. Their identification in imprint smears is not known (18). Imprint cytology technique can help in screening of GIT malignancies arising from these benign neoplasms, like adenomas which are high risk for malignancy.

2.2.2.2.2. Premalignant lesions in GIT

Esophageal premalignant lesions are classified as mild, moderate and severe dysplasia. Low-grade squamous intraepithelial lesions of the esophagus are characterized by well-differentiated superficial and intermediate squamous cells with marked nuclear enlargement and hyperchromasia. High-grade squamous intraepithelial lesions comprise parabasal type cells. The cells are characterized by enlarged hyperchromatic nucleus, increased nucleus cytoplasmic ratio and clustering of cells (18). Atypical glandular cells, low grade or mild dysplasia is described as slight atypia of the columnar epithelial cells. Adenocarcinoma in situ 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. Studies showed that the prevalence rate of adenocarcinoma, high-grade dysplasia and low-grade dysplasia in BE are 6.7%, 3% and 7. 3% respectively (24).

Precursor lesions of gastric carcinoma include carcinoma in situ (early superficial carcinoma) and dysplasia. Dysplasia of gastric epithelium is defined as where there occur epithelial abnormalities that are clearly on the border of cancerous changed in the form of atrophic gastritis with good preservation of glandular pattern, but cells with nuclear abnormalities within glands (18). According to the four potential international systems for the classification of dysplasia and early cancer in the stomach, low-grade dysplasia is characterized by hyperchromatic elongated cells with crowding whereas high-grade has more severe cytologic atypia and loss of polarity (1). The early stages of intestinal type of gastric carcinoma are characterized by glandular cells with hyperplastic nuclei often with prominent nucleoli. For the gastric type carcinoma, there is accumulation of small, often signet-ring type of cancer cells within the epithelium (18).

Precursor lesions may be identified cytologically. Histologically and cytologically, it is difficult to clearly separate adenocarcinoma in situ from adenocarcinoma (18). Imprint cytology would show moderately enlarged epithelial cells with hyperchromatic or clear nuclei and the presence of distinct enlarged nucleoli.

2.2.2.2.3. Malignant neoplasms

Squamous cell carcinoma is as many as 90% to 95% of esophageal cancer (8). Half occur in the distal esophagus and a third occurs in the middle esophagus. Squamous cell carcinoma represent 0.04% - 0.09% of gastric carcinoma. In small and large intestine, the primary squamous cell carcinoma is rare. Metastasis of squamous cell carcinoma to the small intestine is commonly from cervix and lung (1).

The study done by Rabson Kachala showed that SCC is common in Sub Saharan Africa (25). Esophageal adenocarcinoma is more common in United States (7). SCC is more common in males of African descent (7). It is endemic disorder in northern Iran, in parts of China, among the Chinese in Singapore, among Africans in southern Africa, and among men in Brittany (18). The study done by Dawsey et al, 2013 at Tenwek Hospital in Kenya, showed 95% cases of

esophageal squamous cell carcinoma are under 30 years of age (26). Most of SCC grows as polypoid masses, ulcerative cancers and as diffusely infiltrative. The prognosis is poor, with less than 5% five years' survival, because these tumors are usually detected late. SCC of the esophagus varies from well differentiated to poorly differentiated, keratinizing or non-keratinizing (8).

According to studies, imprint cytology has a high sensitivity and specificity for diagnosing squamous cell carcinomas in gastrointestinal tract (6), (27). With imprint cytology, squamous cell carcinoma can be reported easily and in a limited time. A study done in Japan on 1345 asymptomatic individuals, who were screened by endoscopy, showed that 3% were dysplastic lesions. But the association between dysplasia and squamous cell carcinoma is still not known (28).

Adenocarcinomas are seen in 10% of patient with Barrett's esophagus (8), (29). Adenocarcinomas represent 95% of gastric cancers (1). Gastric carcinoma is subdivided into intestinal-type which retain glandular structure and which is more localized; and diffuse type which has no glandular structure and this is more spread out (1). The morphologic types include well differentiated adenocarcinoma, composed of large, mucus-producing cells. The diffuse type of gastric carcinoma is less common and is derived from glandular crypts. This type of gastric carcinoma is composed of signet-ring cells but also contain a mixture of poorly differentiated, pleomorphic cells with scant to abundant cytoplasm. This is not associated with intestinal metaplasia and its prognosis is poor. Signet-ring cells are recognized by their usually large size and large cytoplasmic vacuoles pushing the nucleus to the periphery (1), (18).

Primary epithelial malignancies of the small bowel are unusual; most of them arise in the duodenum. A small percentage of tumors arise at the level of ileum and jejunum. There is a male preponderance and higher incidences are observed in males than females and more in persons of African Descent as compared to Caucasian. Some diseases like Familial adenomatous polyposis, Peutz-Jeghers syndrome, long-standing Crohn's disease are linked to small intestine malignancy (1).

Adenocarcinomas of the colorectum usually arise in adenomas. Colorectal adenocarcinomas are usually moderately to well differentiated (8). Most colonic carcinomas are diagnosed by colonoscopic biopsies (18).

The cells obtained from well-differentiated adenocarcinomas of the colon are large, often columnar or cuboidal in configuration, with large irregular hyperchromatic nuclei, prominent nucleoli, occurring singly and in clusters. Mitotic figures are usually abundant (18). In signet-ring cell carcinoma, more than 50% of neoplastic cells have signet-ring morphology. Signet-ring cell carcinomas account about 0.5% to 1.0% of all colorectal carcinomas. The neoplastic cells show mucin vacuoles that pushes the nucleus to the periphery of the cell cytoplasm. Colorectal signet ring cells are associated with abundant extracellular mucin (1).

Adenocarcinomas may be identified on imprint cytology. Atypical columnar cells in clusters with irregular hyperchromatic nuclei and prominent nucleoli are identified. Signet-ring cell adenocarcinoma show signet-ring cells on imprint smear (6) (27).

Gastric lymphomas account for about 5% of gastric malignant tumors. The mucosa-associated lymphoid tissue (MALT) lymphoma is the most common (8).The tumors are curable if diagnosed early. The diffuse large B cell lymphoma is the most frequent form of gastric lymphoma. Marginal zone lymphoma has an association with *H. pylori* and a high cure rate. Their cytological diagnosis is exceedingly difficult in gastric lavage material (18) (1). The gastric wall may sometimes be involved by Hodgkin's disease from adjacent lymph node. The primary gastric Hodgkin's disease is rare. Raskin et al (1958) and Rubin (1974) reported seeing Reed-Sternberg cells in gastric cytological material (18).

Lymphomas account for 0.5% of colonic malignancies. The cecum is the most involved followed by the rectum (1). The diffuse large B-cell lymphoma is the most common. Marginal zone B-cell lymphoma, mantle cell lymphoma, follicular lymphoma, Burkitt lymphoma and peripheral T-cell lymphoma are also found in the large intestine (1). Burkitt's lymphoma is associated with immunodeficiency.

Lymphomas may be identified on imprint cytology by identifying monomorphic population of lymphoid cell with nuclear abnormalities(6). They are CD20, CD3, CD45 and CD10 positive on immunostaining (1). Although in some studies, few cases of lymphoma were diagnosed as false

negative on imprint cytology, rare tumors like anaplastic large cell lymphomas were diagnosed on imprint smear by Ranjan et al 2012 (13)

Neuroendocrine tumors account less than 1%, and account for 1% to 2% of all gastrointestinal tumors. Although neuroendocrine malignancies are rare, it has been shown that they behave aggressive in the colon and rectum. At the level of the colon and rectum, neuroendocrine tumors are divided as low-grade atypia and malignancy; and high-grade atypia and malignancy. Neoplastic neuroendocrine cells have neurosecretory granules in their cytoplasm and neurosecretory markers that can be detected with immunohistochemistry using NSE and CD56 markers. The high grade neuroendocrine tumors may be divided into small cells. The cells are fusiform, morphologically characterized by scanty cytoplasm, finely granular chromatin and small or absent nucleoli. They are positive for chromogranin, synaptophysin, NSE, and CD56 markers. For the high grade, cells are morphologically round or polygonal with abundant cytoplasm, coarse chromatin and prominent nucleoli (30). Neuroendocrine tumors have been identified on ultrasound-guided FNA smears. The role of imprint cytology in diagnosis of these tumors on gastrointestinal endoscopic biopsies is not known.

Imprint cytology, according to studies, is a highly sensitive and specific technique for identification of gastrointestinal malignant neoplasms. Its simplicity may help for making an immediate diagnosis on these malignant tumors. Cytology is also a useful adjunct for histological diagnosis of gastrointestinal malignancies.

2.3. Cytological sampling of the GIT

Instruments adapted to the inspection of the esophagus, stomach, duodenum and colon are available and principles of collection of material are the same (8). These instruments include endoscopy, cytology sampling methods and histology.

Endoscopic biopsy has been used routinely to diagnose GIT pathology, but there was controversies regarding the role of cytology. Studies showed that imprint cytology has been considered as an invaluable adjunct which has been used where there is difficulty in obtaining adequate tissue(31). Contrary to the study done by Debongnie et al, 1989, that showed *Candida albicans*, *Campylobacter* and *Giardia lamblia* microorganisms were identified in imprint smears in upper GIT. In 55 patients, 26 cases were positive with *Candida*, 11 cases were positive with

Camphylobacter and 9 cases were positive with *Giardia* (32). Cytological examination during endoscopic biopsy is a rapid, useful and reliable adjunct to mucosal biopsy for diagnosis of GI tract lesions. However, during the last few years , use of GI cytology has declined due to preference for tissue biopsy (33).

Direct sampling cytology can be performed on accessible lesions. The direct brushing is the most useful for visible lesions and infections like *Candida* species but it still has limitations like inability to distinguish between dysplasia, carcinoma in situ and invasive carcinoma (34). The patient can also swallow abrasive balloon to collect cells from lesions of the upper GI tract (8). A study done by Hossain et al, 2008 showed that crush cytology had a sensitivity of 89% to detect *H. pylori* infection (35) .

When the cytology method is added to biopsy, this gives a correct diagnosis from 80% - 85% to 90% - 100% (8). Cytology may yield a positive result when tissue is falsely negative (8) and play a big role in sampling stenotic or small lesions in patients at risk for significant bleeding, and in patients with negative biopsies in which cancer is still suspected.

Although exfoliative cytology is useful in diagnosing lesions that involve tissue surface, neoplasms such as lymphoma and leiomyosarcoma are found in submucosa and muscularis propria (8). These may be sampled using endoscopic fine needle aspiration and imprint cytology methods. Endoscopic fine needle aspiration may be positive when brush cytology and tissue biopsy are negative, thus increasing diagnostic sensitivity (8) . The study done by Dhakhwa et al 2012 showed that the sensitivity of imprint cytology to identify malignancy is 91. 6% (31). The study done by Vijayanarasimha et al, 2014, showed that the sensitivity of IC for neoplastic lesions in GIT were 94.3%, 88.2% and 100% for esophageal lesions, gastric lesions and duodenal lesions, respectively; this study showed also that imprint smear has a sensitivity of 80% for H. pylori infection (6). The study showed that the sensitivity of this method in diagnosis of H. pylori was 100% (36). The study done by Mysorekar et al showed that the overall accuracy of imprint cytology in diagnosis of GIT malignancy was 100%; 96.7%; 95. 8%; and 95.8% for the diagnosis of the esophagus, stomach, duodenum and colorectum respectively (37).

The utility of imprint cytology has been shown in comparative study of imprint cytology and frozen sections done by Sukumar et al, where the imprint cytology was found to be comparable

with frozen section in diagnostic accuracy and sensitivity. This made them to conclude that imprint cytology can be used as an alternative to frozen section in hospitals where frozen section facilities are not available (38).

The study done by Ahmareen Khalid showed that the sensitivity of both imprint cytology and frozen section were comparable and showed that touch imprint provide better cellular details and few artifacts (39). It is unclear whether imprint cytology should be used instead of frozen section for rapid diagnosis of gastrointestinal pathology. This would be of great interest specialist facilities in low resource settings.

2. 4. Imprint cytology of the GIT and its utility in tertiary and remote health care facilities in Africa

The use of imprint cytological preparations in the diagnosis of GIT pathology has the potential for major cost savings. A previous study showed that touch preparation cytology slides were highly cheaper to prepare than histology slides of formalin- fixed tissue (40). Glass slides and relevant stains are the basic tools needed for touch preparation slides, while various processing equipment are additional requirements for histologic samples (40). The significantly lower cost for cytological assessment can be a very useful advantage in the provision of health care, particularly in tertiary and remote health care facilities in Africa.

The results of the studies showed that IC is an important diagnostic technique with significant diagnostic accuracy(37),(38),(6). It is easy to perform in limited time and even at centers with low medical facilities while considering in African set-up, it can be performed at the level of district hospitals, where many surgeries are being conducted. If a cytologist is available in the hospital, it can be reported in a limited time easily. The lack of artifact imposed by Frozen sections and decreased cost has made imprint cytology to be the most common method of analysis in intraoperative diagnosis of tumor (13). In another study, it was concluded that touch smear cytology may improve upon pathological diagnosis of malignancies when used in conjunction with biopsy (41).

In conclusion, more studies are required to explore the potential of imprint cytology in diagnosis of various lesions in GIT as well as in other tissues, especially using a large number of

participants. The role of imprint cytology in tumor typing is not also well known, further researches are recommended.

3.0. JUSTIFICATION

Imprint cytology has a high accuracy between 91.6% and 100%. When used in conjunction with biopsy, this gives a correct diagnosis from 80% - 85% to 90% - 100%. All these studies examined the utility of imprint cytology in diagnosis of esophageal, gastric and duodenal malignancy. One study examined the role of imprint cytology in the evaluation of both upper and lower gastrointestinal malignancy. No studies have examined the role of imprint cytology in the evaluation of benign lesions such as infections (other than H. pylori) and benign neoplasm. No studies have been carried out in Africa on touch imprint cytology in diagnosis of gastrointestinal tract endoscopic biopsies.

The significantly lower cost for cytological assessment can be a very useful advantage in the provision of health care, particularly in primary and remote health care facilities where complicated surgeries are performed. Imprint cytology is easy to perform in a limited time and even at centers with basic medical facilities considering many African settings, it can be performed at the level of district (level 3) hospitals where many surgeries are being conducted.

Implementation science projects that establish the utility of imprint cytology in endoscopic biopsies would focus upon rapid diagnosis and accuracy. Such projects would establish the role of imprint cytology for improving efficiency of histology through early selection of special stains or techniques and increase index of suspicion, resulting in reduction in observer variability hence quality through improved precision and accuracy.

As currently conceptualized, a cross-section study was performed at Kenyatta National Hospital where imprint cytology is obtained from gastrointestinal endoscopy. Specimen would be examined using Romanowsky and alcohol based stains. These findings were compared to those of biopsy material, to determine the accuracy and utility of touch imprint biopsy in endoscopic specimens. The benefit of this study is that imprint cytology is an easy technique that can be done in a resource contained area. The outcomes can guide in early management of the patients.

4.0. MATERIAL AND METHODS

4.1. Research Question

What is the utility of imprint cytology in evaluation of endoscopic tissue biopsies at Kenyatta National Hospital?

4.2. Objectives

4.2.1. Main objective

The objective of the study is to establish the utility of imprint cytology in the diagnosis of GIT pathology at Kenyatta National Hospital.

4.2.2. Specific objectives

1. To describe cytological features of esophageal, gastric and intestinal imprint cytology of endoscopic biopsies.

2. To establish sensitivity, specificity, negative predictive value, positive predictive value and the overall accuracy of imprint cytology of endoscopic biopsies for diagnosis of gastrointestinal diseases.

4.3. Study design

The study was a cross sectional descriptive study.

4.4. Study site

Endoscopy unit, Kenyatta National Hospital, Nairobi, Kenya. Endoscopy unit is located at clinic 23. It open every day from Monday to Friday and serves an average number of 13 patients per day. Endoscopies are performed for upper and lower gastrointestinal tract. Diagnostic and therapeutic endoscopies are performed. Endoscopic biopsies are taken by consultant gastroenterologists assisted by nurses. Endoscopy biopsies are normally fixed in formalin and taken to the histology laboratory for processing and reporting.

4.5. Study population

The study population comprised patients referred for endoscopy at Kenyatta National Hospital, Endoscopic Unit. Imprint cytology samples were collected until the required sample size was achieved.

4.6. Selection criteria

4.6.1. Inclusion criteria

Patients in whom endoscopy of esophagus, stomach, duodenum, and colon was indicated.

4.6.2. Exclusion criteria

Patients in whom endoscopic biopsies were indicated but not performed during the procedure.

4.7. Sample size determination

The number of samples for the study population was calculated using prevalence of gastrointestinal malignancy of 8.8% obtained in the study done in Lusaka-Zambia from 2132 upper gastrointestinal tract endoscopic records examined in the year between 1999-2005(42).

The sample size was calculated using the Fisher's formula:

Sample size n = $\mathbf{[DEFF \times Np\ (1-p)]/\ [(d^2/Z^2_{1-\alpha/2} x\ (N-1) + p\ (1-p)]}$

n=124

n= sample size
N is an estimate of patient's size served by Kenyatta National Hospital per month that corresponds to 120,816.
P is the known prevalence
Z is the normal standard deviate that correspond to 95% confidence interval
d is margin of error degree of precision set at +/- 5%
DEFF is the design effect equal to 1

4.8. Sampling method

Convenience sampling method was used. Endoscopy biopsies were taken from all patients referred to Endoscopy clinic and who had GIT lesions. Imprint cytology samples were collected until the required sample size was achieved.

4.9. Specimen collection procedures

Cytological slides were given identification numbers before sample collection. From biopsies taken by the physician, a minimum of 2 imprint smears were prepared by the researcher (PI) and the assisting nurse in theatre, from fresh biopsy by rolling the tissue on glass slides using needle by applying gentle pressure; both smears were air-dried; one smear was rehydrated in 0.9% normal saline for 3 minutes, fixed in 95% ethanol and stained with Papanicolaou stain and the second slide was stained with Giemsa stain. Tissue biopsies were fixed in 10% formalin and processed in the usual manner for histological diagnosis. Samples were processed from the University of Nairobi's Anatomic Pathology core laboratory. Tissue biopsies were sent to Kenyatta National Hospital, Anatomic Pathology laboratory. The nurse was adequately trained for her to be familiar with the procedure in order to be able to assist if the PI stepped out of the theatre for any reason. However, the PI was available to prepare most of the imprint smears from the endoscopic biopsies.

4.10. Histopathology and Cytopathology evaluation of specimen

Imprint cytology were screened by the PI and reviewed together with the pathologists. Discrepant findings were evaluated by a third pathologist. Histology sections were reported by staff pathologists blinded to the findings of imprint cytology.

Cytology results were classified for adequacy as unsatisfactory or satisfactory; and interpretation as positive, suspicious and negative for malignancy. Cytology slides with few cells, poorly preserved, degenerated cells, obscuring inflammation, blood or necrosis were classified as unsatisfactory. Cytology slides with unequivocally malignant cells were classified as positive for malignancy. Cytology slides with atypical cells, suspicious but not confirmatory for malignancy were classified as suspicious for malignancy. Cytology slides with unequivocally negative or atypical cells consistent with inflammatory or reparative process were considered negative.

Pathogenic organisms were diagnosed on their morphologic appearance and were classified as negative or positive. Where adequate criteria for a specific cytologic diagnosis e.g. adenocarcinoma, squamous cell carcinoma, signet-ring type adenocarcinoma, small cell carcinoma, are present, then these were reported.

On histology, lesions were categorized as negative for any pathology, inflammatory lesion, and dysplasia and positive for malignancy.

4.11. Data management

Data was collected and stored in hard cover register, Microsoft excel as well as SPSS software. Data was collected from hard cover register and kept in lockable cabinet where only the researcher would access thus confidentiality was maintained. Information was stored in soft copy and protected from access by unauthorized persons by password which was changed periodically. All records were identified by study identification number. Cytological features were described and displayed using photomicrographs. Kappa statistics test was used to calculate the degree of agreement between 2 tests. A 2x2 contingency table was used to determine sensitivity, specificity, NPV, PPV and the overall accuracy of imprint cytology compared to histology. The results were presented in tables and charts. This data will be disseminated through seminars, conferences and publications in peer reviewed journals upon completion.

4.12. Quality Assurance

Imprint smears were prepared on a clean slide. All reagents were prepared in accordance with standard operation procedure (SOPs) and with the manufacturer's instructions. The fixation was made immediately using a recommended fixative and the fixation time was respected. All stains and reagents were kept covered, they were filtered after each use and they were replaced after every week. Cross-contamination from one slide to another was avoided. All the smears were examined by the principal investigator and the study pathologists. All positive and 10% of negatives smears, randomly selected, were re-examined by an independent pathologist. All discrepancies were confirmed by a third pathologist as tie breaker.

4.13. Ethical consideration

Before commencement of the study ethical clearance was obtained from KNH/UON Ethics and Research Committee; and permission to conduct research in the unit was sought from the manager in charge of Endoscopic clinic/Kenyatta National Hospital. Written informed consent was obtained from all the participants in the study. The physician took consent for the study at the time of taking consent for the endoscopy procedure. All tissue biopsy samples were carefully used to make imprints smears to avoid risks such as crush artifacts that can be caused by repeat of procedure. Patient privacy and confidentiality were strictly observed. All results of imprint cytology were communicated to the attending physician. The study not involve any extra procedure to obtain a separate sample other than the endoscopy already planned, therefore no added risk or harm from the study was foreseen. All data collected in hard copy was kept in a lockable cabinet where the researcher only can access to maintain confidentiality. Information stored in soft copies was protected from access from unauthorized persons by password which was being changed periodically. All records were identified by study identification number. All data collected (soft and hard) will be kept for a minimum period of 5 years as per The North American Free Trade Agreement (NAFTA) regulation for protection of clinical data.

5.0. RESULTS

5.1 Demographic characteristics of the study population

The present study was carried out on 124 participants, with age between 20 and 87 years. The overall mean age was 55.22 ± 15.24 . The majority of study participants were female 67/124 (54.03%) and male were 57/124 (45.96%). **(Figure 1)**

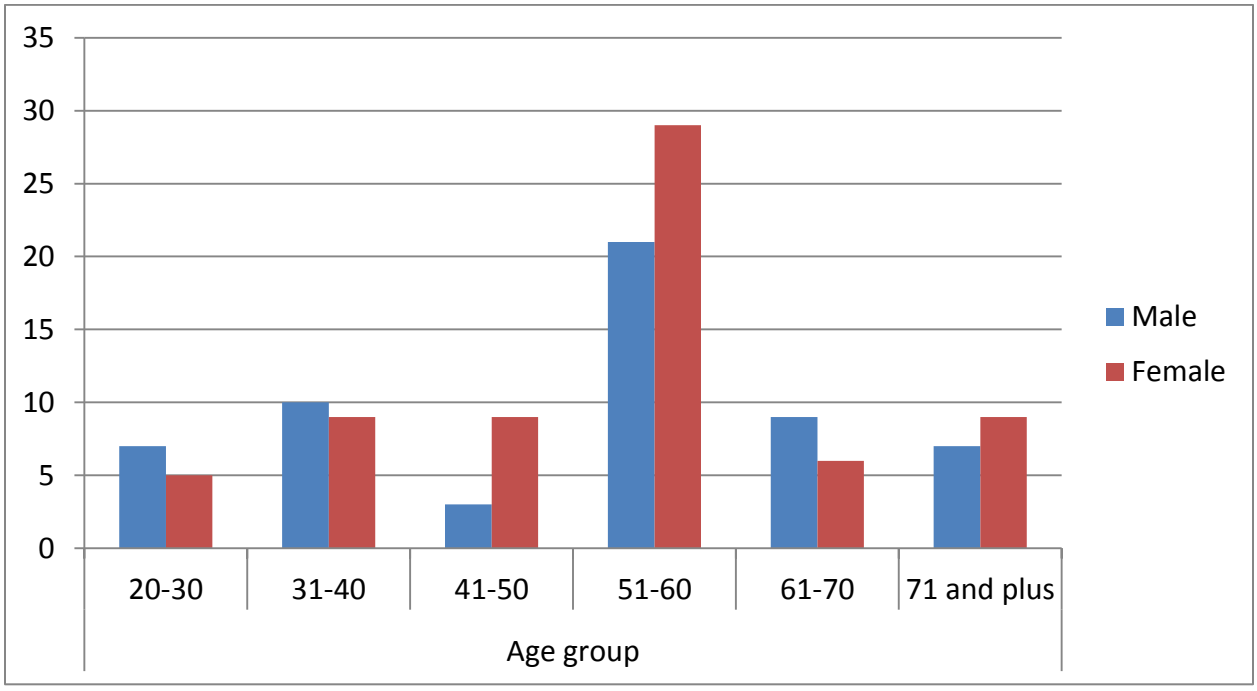

Figure1: Age and sex distribution

5.2 Cytological features of oesophageal, gastric and intestinal imprint cytology of endoscopic biopsies.

The description of imprint cytology of the biopsies obtained from the esophagus, stomach and intestine was based on three major variables namely: Cytomorphology, *H.pylori* presence and other infections seen. The results revealed that 37 (29.83%) were positive for malignancy, of those 16 cases were from age group of 51-60. The same age group was also most affected by *H.pylori* whereby 15 cases (27.42%) of all 34 positive cases were in this group **(Figure2).**

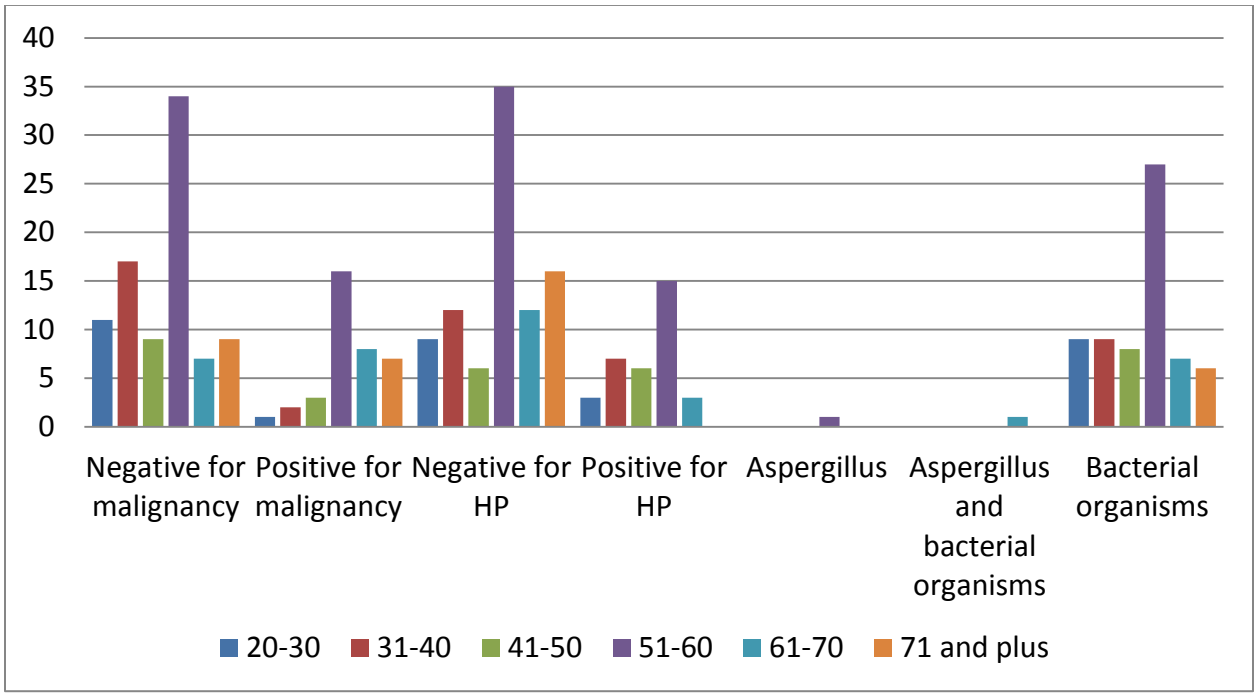

Figure2. Cytological features according to age group

5.3. Cytomorphology of imprint cytology findings

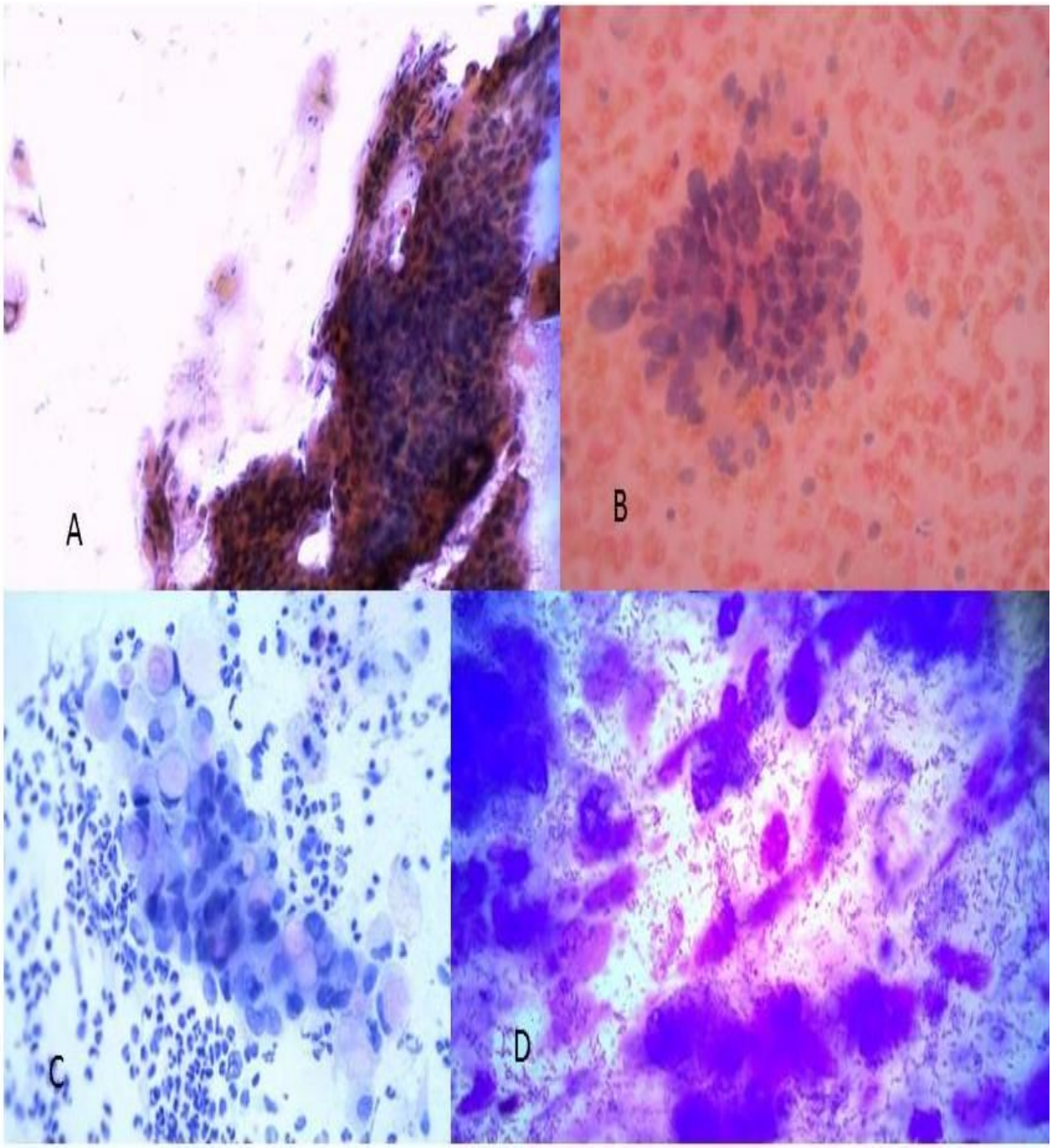

Figure 3. Photomicrographs of: A) SCC: Sheet of pleomorphic squamous cells with orangeophilia in cytoplasm([Pap]x20) B) ADC: Cluster of tumour cells with feathering and gland opening([Pap]x40) C) Signet-ring cell ADC: Showing signet-ring cells([Pap]x40) D) H.pylori organisms in the background by their spiral shape([Giemsa]x100).

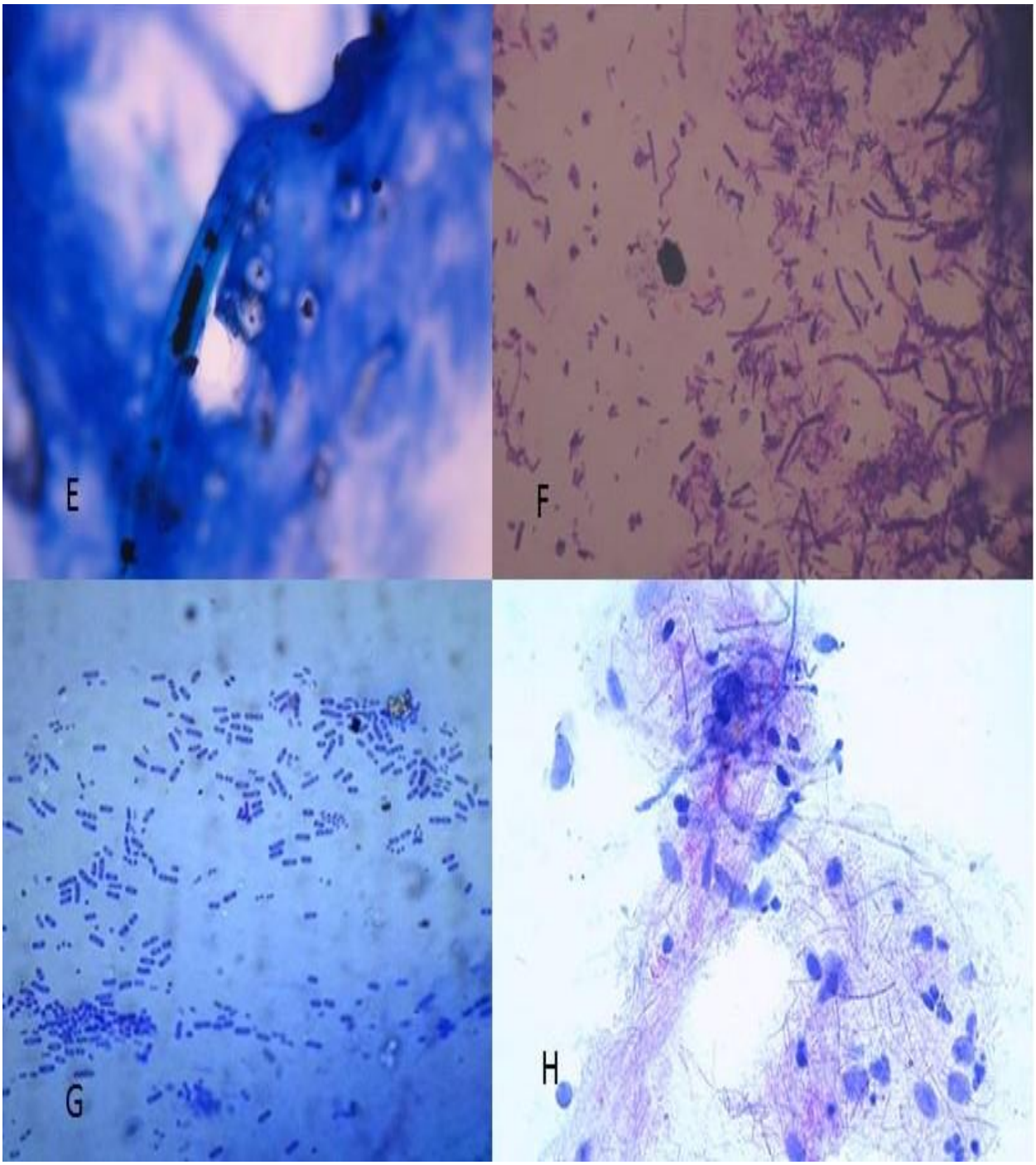

Figure4. Photomicrographs of: E) Helminthic hookworm: Elongated parasite with internal particles ([Giemsa] x20) F) Fungi morphologically consistent with Aspergillus: Wide Fungi with hyphae tending to have an acute angle ([Giemsa] x100) G) Bacterial organisms ([Giemsa] x100) H) Fungi morphologically consistent with candida ([Pap] x40).

5.4. Gender and distribution of cytological features

According to Gender, 16/37 cases from male were positive for malignancy, and 21/37 cases from female were positive for malignancy. There was no statistical difference with regard to positivity and negativity for malignancy. (**table 1).**

Table1. Cytological features according to gender (n=124)

		Gender		Total (%)
Variables	**Categories**	**Male**	**Female**	
Cytology	Negative for malignancy	41	46	87 (70.16)
	Positive for malignancy	16	21	37 (29.83)
H.pylori	Negative for HP	42	48	90 (72.58)
	Positive for HP	15	19	34 (27.41)
Other infections	Aspergillus	1	0	1 (1.37)
	Aspergillus and bacterial organisms	1	0	1 (1.37)
	Bacterial organisms	29	37	66 (90.41)
	Candida species	1	2	3 (4.11)
	Helminth larva and bacterial organisms	0	2	2 (2.74)

5.5. Cytological features according to anatomical sites

The findings from this study show different frequency of lesions according to anatomical sites. Out of a total of 37 cases (29.83%) positive for malignancy, 25 cases were from the oesophagus whereas 12 cases were from the stomach. This shows that malignancy was more common in oesophagus than the stomach, the difference was statistically significant (P.*value* =0.00).

However, the stomach was the most common site for bacterial infections with 59/66 cases (83.3%) **(Table2).**

Table2. Cytological features vs anatomical sites (n=124)

	Anatomicalsites				Frequency (%)	
Disease categories	**Esophagus**	**Stomach**	**Duodenum**	**Colon**		***P.value***
Negative for malignancy	6	72	8	1	87 (70.16)	0.001
Positive for malignancy	25	12	0	0	37 (29.84)	
Negative for HP	31	52	6	1	90 (72.58)	0.001
Positive for HP	0	32	2	0	34 (27.42)	
Aspergillus	0	1	0	0	1 (0.81)	0.001
Aspergillus and bacterial organisms	0	1	0	0	1 (0.81)	
Bacterial organisms	4	59	3	0	66 (53.23)	
Candida species	3	0	0	0	3 (2.42)	
Helminth larva and bacterial organisms	1	1	0	0	2 (1.61)	

5.6 The diagnostic performance of imprint cytology of endoscopic biopsies for diagnosis of gastrointestinal diseases.

The diagnostic performance of imprint cytology was compared against 105 histology reports that were available, histology was considered as the gold standard. With regard to the presence or the absence of malignancy, the level of agreement between the two methods was fair with Kappa *value* = 0.75 **(table 3**).

Table3. Cross-tabulation of Cytology and Histology findings (n=105)

	Histology		Total	Kappa Value
Cytology	Negative for Malignancy	Positive for Malignancy		
Negative for malignancy	73	3	76	0.75
Positive for malignancy	3	26	29	
Total	76	29	105	

Helicobacter pylori was positive in both cytology and histology in 23/105 cases and 5 cases were positive on cytology while negative on Histology, the level of agreement was good with Kappa *value*=0.74 **(table 4).**

Table4. *H. pylori* (HP) findings on Cytology and Histology (n=105)

	Histology		Total	Kappa *value*
Cytology	Negative for HP	Positive for HP		
Negative for HP	71	6	77	0.74
Positive for HP	5	23	28	
Total	76	29	105	

The sensitivity of imprint cytology was 90% and the specificity was 96% for the detection of malignancy. However, for *H. Pylori* detection the sensitivity was 82% whereas the specificity was 92%. The overall accuracy of imprint cytology was 94% for detecting malignancy and 90% for *H. Pylori* detection **(table 5).**

Table5. Diagnostic performance of imprint cytology (n=105)

Performance of cytology	Malignancy	*H. Pylori*
Sensitivity	0.90	0.82
Specificity	0.96	0.92
Negative predictive value	0.95	0.93
Positive predictive value	0.90	0.79
The level of agreement	0.75	0.74
The overall accuracy	0.94	0.90

According to anatomical sites, cytology was more sensitive for lesions in the stomach (91%) the oesophagus (89%). On the other hand, cytology had a high specificity for lesions in the oesophagus, duodenum and colon at 100%. The sensitivity and the positive predictive value of cytology in the duodenum and colon were not applicable because all the cases were negative on both cytology and histology **(table 6).**

Table 6. The performance of cytology according to anatomical sites

Performance of cytology	Esophagus (n=24)	Stomach (n=73)	Duodenum (n=7)	Colon (n=1)
Sensitivity	89%	91%	N/A	N/A
Specificity	100%	95%	100%	100%
Negative predictive value	8%	98%	100%	100%
Positive predictive value	55%	77%	N/A	N/A
The overall accuracy	92%	95%	100%	100%

6.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

6.1 Discussion

The diagnosis of gastrointestinal diseases relies upon history, physical examination, endoscopy, radiology and laboratory features. Endoscopy has been a great tool for detection of gastrointestinal lesion (31) and histology is the gold standard. Imprint cytology has been an invaluable adjunct to histology and it has been used where there is difficulty in obtaining adequate tissue (31). The overall accuracy from this study was also very high. There was a good agreement (Kappa value of 0.75 and 0.74) between IC and histology findings.

The present study was carried out on 124 patients with a mean age of 55.22 ± 15.24 which is similar to Muhammad et al, 2013 who carried out a study on 120 patients with a mean age 57 ±17.99 (36); the majority were female at 54.1%, but in the study by Muhammad et al, the majority were males (36). The majority in this study were female 67 (54%); this is due to the fact that the majority of patients attending KNH are females(43) probably because they are more assertive in health seeking than men. Cytological features of oesophageal, gastric and intestinal imprint cytology of endoscopic biopsies revealed that 29.83% were positive for malignancy and the findings from this study showed a different distribution according to anatomical sites. Out of a total of 37 cases (29.84%) of positive for malignancy 20.2% cases were from the esophagus whereas 9.6% cases were from the stomach. In the study done by Vijayanarasimha et al, 2014 on 110 patients, 34 esophageal lesion, 17 gastric lesions and 5 duodenal lesions were neoplastic(6). This might be due to that the majority of these cases were from the age group of between 51-87 years which is a high risk age group for GIT malignancy(44).

The diagnostic performance of imprint cytology was compared with histology and the sensitivity, specificity and accuracy were 90%, 96% and 94% respectively. This means that the IC is an excellent diagnostic tool for detecting malignancy. The study by Dhakhwa et al in 2012 showed a comparable sensitivity of 91.6% and specificity of 100% (31). The reasons these figure were higher was because of some cases that were reported negative for malignancy on cytology and positive on histology. For instance one case was reported as Kaposi Sarcoma on histology, but showed sheets of benign epithelial cells mixed with inflammation on imprint cytology. Another case from the oesophagus was diagnosed as tumor on endoscopy and reported as oesophageal carcinoma on imprint cytology, but was non- diagnostic on histology because of

lack of oesophageal mucosa. Reactive atypia may be misled with malignancy especially in the presence of inflammation. However, a study by Dhakhwa etal 2012 showed that cytology may diagnose malignancy on cases which were initially negative on histology. This was proven by a repeat of biopsy. In such cases, there must be unequivocally malignant cells in touch smear. Small clusters of malignant cells may also be missed when a conclusive tissue pattern is lacking on histology probably due to poor sampling (31). These and other similar few cases contributed to the slight differences in performance compared with the above mentioned study. In cases with positive cytology and negative histology, it was however recommended that a repeat biopsy should be done to confirm the diagnosis or correlate with clinical and endoscopy diagnosis. The findings from this study highlight the use of imprint cytology in the diagnosis of gastrointestinal disease.

With regard to the performance of imprint cytology in the detection of *Helicobacter pylori*, the sensitivity of 82%, specificity of 92% and the overall accuracy of 90% were recorded. In approximately 10% of cases *H .pylori* were identified on imprint cytology in some cases but not on histology. The findings from this study were comparable to other findings that showed a sensitivity that range from 78.4% to 100%, specificity from 83.3% to 100% and an accuracy that ranges from 90% to 100% (6)(17)(31). This means that IC is a good diagnostic tool for detecting *H. pylori*. The false negatives were probably due to low density of bacteria rods or the presence of numerous other bacterial organisms in the background(45). It is likely that IC could be a better tool for diagnosis of *H. pylori*.

For duodenum and colon lesions, all cases were reported negative for malignancy. Other studies recorded adenocarcinoma and lymphoproliferative disorders from the duodenum(6). This could be due to fewer cases in our study compared to other studies mentioned. Although IC showed an excellent performance for detecting malignancy and *H. pylori* showed by this study, and the ability of this technique to show benign lesions, parasitic, fungal and bacterial infections, IC was unable to diagnose some benign lesions such as polyp, atrophy, edema, and foveolae hyperplasia which were diagnosed by histology. This is due to the nature of these lesions; it has been shown that lesions such as hyperplastic polyp cannot be recognized using cytology(18).

The main limitation of this study is that observed microorganisms were not further identified using special stains such as Gram (bacteria) GMS (fungi) and PAS (parasites). However, use of a

Romanowsky stain in conjunction with PAP in this study resulted in identification of cocci/bacilli (bacteria) and helminthic larvae. Romanowsky stains have many advantages, as morphological diagnosis of infectious pathology was possible in this study and should be applied in routine IC of GI biopsies(15).

5.2 Limitations of the study

The colonoscopy was not being performed at the time of sample collection; hence only one sample was obtained. The fact that all histological reports were not available for comparison with all cytological results at the time of data analysis is another limitation that we think might have influenced the findings in one way or another. Some tissues biopsies were reported from private laboratories and therefore could not be traced for review. Fungal, bacteria organisms and helminths seen on imprint cytology were not specifically identified as special stains and cultures were outside the scope of this study.

5.3 Conclusion

Imprint cytology is an easy and rapid procedure for detection of infectious, benign and malignant diseases of gastrointestinal tract. Imprint cytology has a high accuracy, sensitivity and specificity in diagnosis of GIT malignancy and *H. pylori* infection.

5.4. Recommendations

Imprint cytology can be routinely performed alongside histology of the endoscopic biopsy for diagnosis of bacteriologic and helminthic lesions in the digestive tract. Imprint cytology should be used as a rapid diagnostic tool to enable early planning for further management of the patient and to help to avoid a repeat of procedure in case of inadequate biopsy.

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Appendices

Appendix I

Papanicolaou Staining Procedure, Progressive Method

Principle of the stain

Hematoxylin stains the nuclei blue by dye-like 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 stain keratin.

1. Smears are fixed in 95% ethanol
2. They are hydrated through ethanol grade 80%, 70%, 50%
3. Smear are rinsed in distilled water (6-8 dips until glossy look disappears)
4. Smears are stained with Harris hematoxylin (undiluted) for 6min
5. They are rinsed in tap water
6. Smears are differentiated in 0,05% acid water 10 dips
7. They are rinsed in tap water and blued in running tap water 10 dips
8. They are rinsed in 95% ethanol
9. Smears are stained with O.G-6 for 2 minutes
10. They are rinsed in 95% ethanol 10 dips
11. They are stained in EA50 for 3 minutes
12. They are rinsed in 95% ethanol 10 dips
13. They are dehydrated in changes of absolute ethanol 10 dips each
14. They are cleared in 3 changes of xylene 10 dips each
15. They are mounted in D.P.X

Appendix II

Data collection sheet

1- Patient identification

Identification number Age ☐ Sex ☐ In patient (1) ☐ Outpatient (2) ☐

2-Clinical history:

a) Location of the lesion. ……..

b) For how long has the lesion been there? **.....**

c) The number of biopsies taken…….

d) Clinical diagnosis……

e) Endoscopic diagnosis……..

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

4-Microscopic description

5-Interpretation

-Specimen adequacy –Satisfactory (1)…….Unsatisfactory (2) ……

- Negative for malignancy : (3)……Specify……

- Suspicious for malignancy : (4)…….Specify…….

- Positive for malignancy : (5)…….Specify…….

- Infections : (6)……... Specify……

Signatures

Cytologist Pathologist

Appendix IIIa

Informed consent explanation and consent form

Introduction

My name is Nadia KALINGANIRE, a Master's of Science (clinical cytology) post graduate student in 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 voluntary.

Research Title

The utility of imprint cytology in evaluation of gastrointestinal endoscopic tissue specimens at Kenyatta National Hospital.

Purpose of Study

The study aims to evaluate the utility of a technique called imprint cytology in the diagnosis of diseases of the digestive system (esophageal, gastric and intestinal (GIT) diseases) at Kenyatta National Hospital. I wish to evaluate the utility of this new laboratory technique which can be used for the early management of these diseases. This technique is simple, inexpensive and can improve upon other pathological diagnostic techniques when used together.

Benefits and risks

Imprint cytology can be used for rapid diagnosis of infections such as *Helicobacter pylori*, screening for many diseases among which some can lead to cancer and cancer disease as well. The results obtained from this study will help policy makers in making informed decisions best suited in treatment and management of infectious, pre-cancer and cancer. You will benefit from obtaining both endoscopy and biopsy as well as imprint cytology results. The sample collection will not require another procedure. It will be obtained from the endoscopic biopsies already taken, therefore no added risk or harm.

Procedure

Once you have accepted to participate and you are eligible for the study, the tissue biopsy that will be taken during endoscopy procedure as explained to you by the surgeon or physician before the procedure will be used to make samples for this study as described below.

1- Endoscopic tissue will be retrieved from the forceps by a fine needle
2- Imprint smear will be made by rolling the tissue on glass slides using a needle by applying gentle pressure. The smears on the slides will be stained for interpretation by myself together with the pathologist/supervisors. A report will then be issued appropriately and sent to your file.

Confidentiality

Participation in this study is voluntary and it is part of your medical evaluation.

Names will not be required for the study and you will be identified by study numbers.

You will get your results in the usual manner during your next visit. The results will be discussed with you by your doctor.

Withdrawal from study

In case you do not wish to continue participating in the study you are free to withdraw at any time without loss of any benefit or quality of management to which you are entitled in this hospital.

Contact Information

If you have any question regarding the study please contact me Nadia KALINGANIRE University of Nairobi P.O BOX 19676-00202 Nairobi on Mobile number 0704520769 or my supervisor, Prof. L.W. Muchiri, Dr. E.Walong and Dr E.Kamau; P.O BOX 19676-00202 Nairobi Tel: 726300 Ext. 43774. And if you have any ethical issue please contact Prof. M. L. CHINDIA, the Secretary, KNH/UON Ethical Research Committee, Tel: 726300-9 Ext 44102.

CONSENT FORM

I……..after reading and being explained the purpose of the study and what it entails

purpose do hereby give informed consent to participate in the diagnostic study fully aware of the benefits .

I am aware that I can withdraw from this study without loss of any benefits or quality of clinical services and care to which I am entitled in this hospital.

Participants Signature/Thumb print ……Date…….

Doctor/Nurse

(Witness)……..Date…….

Principal investigator

(Witness)……Date…….

Appendix III b:

FOMU YA IDHINI KICHWA CHA UTAFITI

MAELEZO KWA UFUPI

Jina langu ni Nadia KALINGANIRE mwanafunzi wa chuo kikuu cha Nairobi idara ya Human Pathology. Ningependa kukuelezea kuhusu utafiti ambao nafanya; kwa lengo la kutoa taarifa muhimu ambazo zinaweza kukusaidia kufanya maamuzi iwapo utashiriki kwa hiari yako au la.

KICHWA CHA UTAFITI

Utumishi wa cytologia ya kuguza katika tathmini ya saratani utumbo kutumia nyama zilizopasuliwa kupitia njia ya endoscopy katika hospitali kuu ya Kenyatta.

MADHUMUNI YA UTAFITI

Utafiti unalenga kutathmini matumizi ya mbinu ya cytologia ya kuguza katika uchunguzi wa saratani za koromeo la umeo na utumbo katika hospitali kuu ya Kenyatta. Ningependa kufanya tathmini ya matumizi mbinu hii mpya ya maabara ambayo inaweza kutumika kwa ajili ya uchunguzi wa mapema wa saratani na magonjwa mengine.

Mbinu hii ni rahisi, nafuu na inaweza boresha mbinu nyingine za pathologia kwa uchunguzi wakati zikitumika pamoja.

FAIDA NA HADHARI

Mbinu hii ya kuguza cytologia inaweza kutumika kwa ajili ya utambuzi wa saratani na magonjwa mengine kama vile Helicobacter pylori mapema na uchunguzi wa magonjwa mengi kati ya ambayo baadhi inaweza kusababisha saratani. Matokeo kutoka utafiti huu itasaidia watunga sera katika kufanya maamuzi sahihi bora inayofaa katika matibabu na usimamizi wa kuambukiza, kabla ya saratani na saratani. Wewe utafaidika kutokana na kupata.

UTARATIBU

Ukikubalika kushiriki kwa utafiti huu nyama zinazotokana na upasuaji kwa njia ya endoscopy zitachukuliwa ili zitatumike kuchukua sampuli kwa ajili ya utafiti huu kama ilivyoelezwa hapo chini.

1- Nyama za upasuaji kwa njia ya Endoscopy zitashikwa kutumia makasi forceps na sindano laini
2- Nyama hizi zitaguzishwa juu ya kioo kitakachotumiwa kwa uchunguzi kwa kusindilia kidogo.

TARATIBU WA KUSHIRIKI

Watakao shiriki katika uchunguzi huu itakua kwa njia ha hiari bila kushuritishwa. Kutoshiriki hutapoteza kwa njia yeyote haki yako kuhundumiwa unavyostahili.

Majibu ya uchunguzi huu utapata kwa njia ya kawaida wakati wa kufuata kiliniki yako ya kawaida.

IDHINI YA MSHIRIKI

Watakao shiriki katika utafiti huu itakuwa kwa hiari bila kushurutisha. Una uhuru wa kutoshiriniki, kutojibu swali lolote kwenye dodoso au kukatiza kipindi cha maswali iwapo hautaridhika na jambo lolote. Pia waweza kutamatisha ushirika wako kwenye utafiti huu bila kupoteza haki yako ya kutibiwa katika hospitali hii.

ANWANI

MUCHUNGUZI, Nadia KALINGANIRE Chuo Kikuu Chaa Nairobi SLP 19676-00202 Nairobi Numbari ya simu 0704520769 Wasimamizi Prof. L.W. Muchiri; Dr. E.Walong na Dr E. Kamau; S.L.P 19676-00202, Nairobi Nambari ya Simu 726300 Ext. 4377, Na kama una suala lolote kuhusu maadili tafathali wasiliana na Prof. M.L.CHINDIA Maadali ya utafiti ya KNH/UONERC SLP 20732-0200 Nairobi Kenya. Nambari ya simu 726300-9 Ext. 44102.

IDHINI YA MSHIRIKI

Kama utashiriki tafadhali tia sahihi yako kwenye pengo lilioachwa hapa chini

Mimi----------nimesoma na nimeelewa nia

ya uchunguzi huu, utaratibu utaotumika kuchukua kipimo, faida na madhara yanayohusika

na uchunguzi huu. Nimekubali kushiriki kwa hiari bila kushurutishwa.

Sahihi ya mushirika----------Tarehe ----------

Sahihi ya shahindi----------Tarehe……...

Appendix IV:

Sample collection procedure- Flow chart.