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SPUTUM CYTOLOGICAL FINDINGS COMPARISON IN HIV POSITIVE AND HIV NEGATIVE PATIENTS WITH PRODUCTIVE COUGH AT KENYATTA NATIONAL HOSPITAL

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# SPUTUM CYTOLOGICAL FINDINGS COMPARISON IN HIV POSITIVE AND HIV NEGATIVE PATIENTS WITH PRODUCTIVE COUGH AT THE KENYATTA NATIONAL HOSPITAL

**BY**

**ALICE BITENGO NYAGAKA**

**H56/38544/2020**

**DEPARTMENT OF HUMAN PATHOLOGY**

**FACULTY OF HEALTH SCIENCES**

**UNIVERSITY OF NAIROBI**

**A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN CLINICAL CYTOLOGY AT THE UNIVERSITY OF NAIROBI**

**MAY 2023.**

## DECLARATION

I hereby declare that this dissertation is my original work and has not been presented to any other university for any research for the award of a Degree or any other award in any other institution to the best of my knowledge.

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23.11.2023

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## DEDICATION

I dedicate this research proposal to my parents Mr and Mrs. David Nyagaka and all Msc clinical cytology students, UON.

## ACKNOWLEDGEMENT

My acknowledgement to my supervisors Prof. Muchiri and Dr. Ndung'u, who put in a lot of effort to help me through the entire process. They really helped me to decide on the best topic, proposal development, data analysis and final project write-up. Also grateful to my classmates who helped a lot in our brainstorming groups so that we could settle on the best areas to carry out our research on. Appreciation to Festus the Biostatistician, TUK research progress forum. I really could not have gone this far without their help.

## ABBREVIATIONS

AFB – Acid fast bacilli

AIDS – Acquired immune deficiency syndrome

ARDS – Acute respiratory distress syndrome

ASCUS – Atypical squamous cells of undetermined significance

BAL – Bronchoalveolar lavage

CBD – Central business district

CCC – Comprehensive care clinic

$CD_4$ – Clusters of differentiation

CDC – Centre for Disease Control

COVID-19 – Corona virus disease 2019

FNA – Fine needle aspiration

HAART – Highly active antiretroviral therapy

H & E – Haematoxylin and Eosin stain

HIV – Human immunodeficiency virus

HIV/AIDS – Human immunodeficiency virus/ Acquired immune deficiency syndrome

KNH – Kenyatta National Hospital

KS – Kaposi Sarcoma

MOH – Ministry of health

MTB – *Mycobacterium tuberculosis*

NCI – National Cancer Institute

NHL – Non-Hodgkin’s lymphoma

OIs – Opportunistic infections

Pap – Papanicolaou stain

PAS – Periodic acid Schiff

PCP – *Pneumocystis pneumonia*

PI – Principal investigator

PPEs – Personal protective equipment

TB - Tuberculosis

UNAIDS – United Nations Programme for HIV/AIDS

VCT – Voluntary counselling and testing

WHO – World Health Organization

ZN – Ziehl Neelsen

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## ABSTRACT

HIV patients are vulnerable and are therefore predisposed to opportunistic cancers and opportunistic infections (OIs).

With respect to cancers in HIV patients, lung cancer takes the lead as a non-AIDS defining cancer that is the most common cause of cancer deaths in HIV-infected patients. In terms of OIs, *Mycobacterium tuberculosis*, *Aspergillus fumigatus*, *Pneumocystis jirovecii*, *Pneumococcal pneumonia* are some of the most common bacterial and fungal microorganisms responsible for the secondary respiratory infections associated with HIV infection. Sputum examination, a minimally invasive, inexpensive and a simple method to obtain specimen can effectively be used for the rapid screening of respiratory disease.

**Broad objective:** To describe cytomorphological changes and opportunistic infection patterns in HIV positive patients being compared with HIV negative patients with a productive cough in KNH Chest Clinic and CCC.

**Study design and site**: A cross-sectional study conducted at the KNH CCC and Chest Clinic.

**Study population**: HIV positive and HIV negative patients with productive cough. The estimated sample size was 106 patients: 53 HIV +ve and 53 HIV -ve sampling both males and females with productive cough.

**Methodology**: Participants were recruited as they visited the clinics by convenient sampling. A consent form and questionnaire was administered as part of the recruitment protocol. Socio-demographic data, Clinical data and respiratory symptoms were recorded. Sputum was then collected, processed, and stained using various stains before microscopic examination.

**Results**: Four of the 106 participants in this study had atypical squamous cells of undetermined significance (ASCUS) (3.7%). There were no obvert cases of lung cancer detected. For ZN staining, about 8.5% of patients sampled tested positive for AFB, 6.6% tested positive for Candida Spp. on PAS staining. For Gram stain, 19.8% of the patients had only Gram positive cocci in clusters, 18.9% had only Gram positive cocci in chains and 7.5% of the patients had Gram positive diplococci.

**Conclusion**: Of the positive findings in this study, AFB and inflammatory changes were the commonest findings at 8.5% each and this concurs with previous studies. Of the 4 ASCUS findings, only one was an HIV positive patient. Lung cancers were therefore uncommon in this study population of HIV positive and HIV negative patients with productive cough.

**Recommendation**: Sputum cytology should continue to be the standard of care for patients with productive cough, both HIV positive and HIV negative. Careful evaluation of sputum for epithelial lesions, although rare, is recommended. However, routine screening for intraepithelial lesions is not recommended.

# 1.0 INTRODUCTION

## 1.1 Background Information

HIV is an important global public health issue with 36.3 million lives claimed so far. Although potential cure for HIV infection has been documented, it has not been widely available beyond research. However, access to operational HIV prevention, diagnosis, HAART and care, plus prevention and management of opportunistic infections, it is a manageable chronic health condition now (WHO, 2021).

HIV targets the immune system and impairs the body's ability to fight off many infections as well as destroying the body's tumour surveillance capability thereby setting the stage for the development of several types of cancer that people with healthy immune systems can resist. Depending on the individual, AIDS, the most advanced stage of HIV infection, might take several years to manifest. The development of severe, long-term clinical symptoms, certain malignancies, or specific infections are considered to be indicators of AIDS (WHO, 2021).

In the year 2020, approximately 37.7 million people lived with HIV, 25.4 million of them in the African region. It is estimated that between 480,000–1 million people died from HIV-related causes and approximately 1–2 million contracted HIV in 2020 (WHO, 2021).

In order to achieve the proposed global 95-95-95 targets set by UNAIDS, efforts must be intensified to avoid a projected 7.7 million HIV-related deaths in the next ten years, in part because of an increase in HIV infections caused by COVID-19 service disruptions and a sluggish public health support for HIV care (WHO, 2021).

The symptoms of HIV amongst individuals differ depending on the stage of infection. Numerous HIV-infected persons are not aware of their status up until the advanced stage of infection, yet they tend to be most contagious in the early stages. Early on after the original infection, they may not exhibit any symptoms or may only have flu-like symptoms such as headache, fever, sore throat, or rash.

Progressively, HIV weakens the body defense and without treatment and proper care, the sequelae is development of severe opportunistic illnesses such as TB, cryptococcal meningitis, pneumococcal pneumonia, severe bacterial infections and cancers such as lymphomas, Kaposi

sarcoma and cervical cancer. However, HIV/AIDS has taken a new course since the introduction of HAART.

The lung has been the most frequently affected organ by HIV/AIDS according to the early descriptions of HIV/AIDS. Many patients develop a respiratory problem during the history of HIV infection, primarily of an infectious nature and these account for significant causes of HIV-related morbidity and mortality. Patients frequently succumb to OIs), respiratory infections and their accurate diagnosis still remains a challenge (1). Lung cancer is a leading non-AIDS defining cancer as well as the most frequent cause of cancer deaths in HIV-infected persons.

The stage at which lung cancer is diagnosed is an important determinant in the survival of a patient. Late diagnosis of this cancer is common. Sputum cytology screening trials have been shown to detect lung cancers at an early stage enabling curative surgical treatment increasing 5-year survival rates in the screened arm (2).

Cytologic diagnosis in general has advanced, and its value is closely comparable to histology. For patients with respiratory symptoms, sputum cytology is the simplest and least invasive method to obtain a cytologic diagnosis (3).

Sputum cytology has not been able to prevent lung cancer in the same way that the Pap smear has prevented cervical cancer (4). However, the general principle that early diagnosis is the best hope for cure, or at least better survival, still holds true. The estimated probability of survival for early stage lung cancer is over 90% at five years. Moreover, close to 40% of all lung cancers can be detected in this favorable stage by present radiologic and cytologic screening techniques (5). Respiratory cytology (exfoliative/aspiration) plays the role of detection and classification of pulmonary disease, with emphasis on neoplastic disease, so that appropriate and timely therapy can be instituted (4).

In addition to lung cancer, MTB and HIV morbidities are still a major health concern in Kenya. MTB is a bacterial infection caused by the bacterium *Mycobacterium tuberculosis* and is a leading cause of death among HIV patients. It is an indicator for HIV staging. WHO insists on strong coordination of programs and integrating service delivery for the two infections. Kenya comes in at position 13 out of the 22 countries with high TB burden (6).

PCP, an opportunistic fungal infection caused by *Pneumocystis jirovecii* is a major cause of morbidity and mortality among HIV/AIDS patients and is a worldwide problem. It is the frequent most opportunistic respiratory infection in HIV/AIDS patients (7). Even though the prevalence of PCP among HIV-infected persons has decreased in developed countries, the occurrence of AIDS-related PCP in developing countries is still frequent and poorly controlled. In HIV cases, more so late presenters who are not aware of their HIV status in the early phase of infection and who most times present with very low CD4+ counts, there is a high possibility of contracting PCP(8).

# 2.0 LITERATURE REVIEW

## 2.1.1 Broad Overview of HIV positive patients with respiratory symptoms

AIDS refers to the emergence of certain malignancies, infections, or other severe long-term clinical manifestations. In the first early days after initial infection, HIV patients might not exhibit any symptoms or may just have an influenza-like illness with fever, headache, rash or sore throat (WHO, 2021). However, many of them develop a respiratory problem during the course of HIV infection, mainly of infectious etiology. These are major causes of morbidity and death amongst HIV-infected patients.

## 2.1.2 HIV negative patients with respiratory symptoms

In cases of HIV negative individuals, immune status might still play a role in opportunistic neoplastic changes and opportunistic infections of the lung (9). Lung cancer is commonest among adults >60years of age than amongst young adults of HIV-ve status (2). Among cigarette smokers who are the population most at risk for lung cancer, HIV+ve population still have an elevated cancer risk in comparison to the general population (10). Opportunistic fungal infections like cryptococcosis are not uncommon among HIV-ve individuals but their immune status is an advantage as the infection severity is not as in HIV+ve patients and also response to treatment of the two groups varies (9)

## 2.2 Respiratory tract Anatomy and Cytology

**Upper respiratory tract**: squamous cells, ciliated columnar cells

**Lower respiratory tract:** trachea, bronchi, basal/reserve cells, goblet cells, neuroendocrine cells, non-ciliated cuboidal/columnar cells (Clara cells), ciliated columnar cells, terminal bronchioles, , alveoli, type I and II pneumocytes, alveolar macrophages (11). Squamous epithelial cells that regularly exfoliate from the oral cavity and throat, columnar cells that frequently exfoliate from the tracheobronchial tree and occasionally from the upper respiratory tract, bronchiolar cells, and alveolar pneumocytes are the typical epithelial components of sputum. Squamous cells are the predominant cell type in sputum, whereas ciliated columnar cells and goblet cells are more prevalent in bronchial specimens and BALs. Alveolar pneumocytes and bronchiolar cells won't likely be detected unless they are hyperplastic or unusual (12).

### 2.2.1 Squamous Cells

Squamous cells, especially originating from the oral cavity, are frequently seen in sputum specimens. There're also intermediate cells that are round-to-oval vesicular and superficial cells with pyknotic nuclei and an orangeophilic cytoplasm. Occasional anucleate squames and parabasal cells could as well be seen. Parabasal cells released in cases of infection, inflammation and ulceration may be confused with metaplasia. Masses of anucleate squames could be produced due to chronic mucosal irritation with leukoplakia (12).

### 2.2.2 Ciliated Bronchial Columnar Cells

A bronchial cell has a columnar or prismatic shape-ending in a tail. The nucleus is positioned toward this tail and it has a finely granular chromatin pattern with one or more small nucleoli. Longitudinally, the nucleus could look as if it is wider in diameter than the entire cell really there is a thin cytoplasm rim between the cytoplasm outline and the nuclear membrane. There are cilia that have a terminal plate. In BALs, FNAs bronchial washings, aspirates, or brushings ciliated columnar cells are seen. Except in cases of extensive damage to the respiratory epithelium or in post-bronchoscopy specimens, ciliated columnar cells should not be frequently seen in sputum(12).

Microorganisms and environmental toxins are examples of insults irritating the bronchial epithelium. This alteration causes them to exhibit increased nuclear size (up to 10 to 20 times), coarse chromatin pattern and one or more enlarged nucleoli. Saito and associates have explained atypical reparative changes in bronchial cells after brushing. Multi-nucleation is also a common response to irritation; however, the nuclei here are not increased in size and they are mirror images of each other. Even though they are seen in cases of various insults, the most common is after instrumentation (12).

### 2.2.3 Goblet Cells

These are mucus-producing bronchial cell in the bronchial epithelium lining that is less frequently encountered. They are identified by the presence of one or more mucus-filled vacuoles. They are common in chronic tracheobronchial disease, such as bronchiectasis, asthmatic and chronic bronchitis patients (12).

### 2.2.4 Epithelial Cells from Bronchioles and Alveoli

Conventional light microscopy of cytologic specimens without disease won't let the examiner appreciate these different cell types although using some modern laboratory techniques can enable differentiation of subtypes of terminal bronchiolar and alveolar cells. They are rarely present in cytologic material in their normal forms. They are comparatively small and, when in cytologic material, they look like rounded single cells with finely vacuolated cytoplasm and centrally located nuclei that have single or two small nucleoli. Some have cilia and this morphology causes their interpretation to be alveolar macrophages (12).

## 2.3 Probable causes of respiratory symptoms in HIV+ve and HIV-ve patients

### 2.3.1 Malignant transformation

Lung cancer is the major cause of cancer mortality in the world for both sexes, resulting in about 1.2 million deaths annually according to the WHO in 2020. It is the third most frequent cancer among HIV-infected patients, following KS and NHL in prevalence (10). Lung cancer risk is two to four times higher in HIV-infected ones than in the healthy population, even after modifying for other aspects such as smoking intensity and duration (13).

The raised risk of lung cancer in HIV-infected patients is not quite explained by smoking; other numerous factors are possibly vital in promoting lung cancer; the highly probable oncogenic role of HIV, recurrent respiratory diseases, HIV-induced immune compromise and HIV- linked reduction in immune surveillance. HIV could also enable amplified susceptibility to tobacco carcinogens (13). However for the HIV-ve population, smoking is the most common risk factor for lung cancer among other minor risk factors like chronic pulmonary diseases (14).

### 2.3.2 Inflammatory reactions among both groups

In cases of infection, trauma and hypersensitivity, inflammation occurs as it is the body's way of responding to insults. The process is complex as it entails various mechanisms to fight pathogens and repair tissue (15). Inflammation in the lungs is primarily brought on by pathogens or exposure to toxins, pollutants, irritants, and allergens. Several different kinds of inflammatory cells are activated during the process (15). Each changes other inflammatory cells by releasing cytokines and mediators. Clinically, acute inflammation is shown in cases of pneumonia and ARDS, but cases of asthma and chronic obstructive lung disease (COPD) are more likely to have chronic inflammation (15).

Microscopically, inflammation is characterized by vasodilation, increased vascular permeability and inflammatory cell infiltration. Inflammation aims at destroying, getting rid of walling off and confining the injurious agents (15). Inflammatory cells include eosinophils, lymphocytes, macrophages, mast cells neutrophils and dendritic cells (15). Acute lung inflammation has neutrophils as the predominant cell type involved while chronic inflammation entails majorly lymphocytes and macrophages (15).

Epithelial, endothelial, and mesenchymal cells also participate in the inflammation process in addition to the inflammatory cells. All cell types can create cytokines. They play endocrine, paracrine, or autocrine roles in the regulation of inflammation and immunity. They are either pro-inflammatory or anti-inflammatory. Pro-inflammatory activate the immune system and take part in the acute inflammatory response e.g TNF-α and IL-1β. Anti-inflammatory cytokines are produced by alveolar macrophages, and they reduce lung inflammation. ARDS may be triggered by an imbalance of pro- and anti-inflammatory cytokines. In a study conducted at Mbagathi District Hospital, Kenya, inflammatory changes were seen in 57% of the studied HIV/AIDS patients' sputum (16).

### 2.3.3 Bacterial infections

Gram-positive *Staphylococcus aureus, Streptococcus pneumoniae* and Gram-negative *Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli* and *Mycobacterium tuberculosis* are some of the commonest respiratory bacterial opportunistic infections (17). *Mycobacterium tuberculosis* is the most common. HIV-infected persons are at a high risk of contracting active TB from reactivated latent and exogenous infection. HAART use has been discovered to be linked with up to an 80% decrease in the risk of TB. However, HIV-positive persons on HAART are still at high risk of TB in comparison to HIV-negative individuals (17). In the HIV-ve population, MTB and other respiratory bacterial infections are also still of major concern. MTB is mostly due to poor containment especially in slums and poor settlements where there is overcrowding, use of public means of transport and lack of awareness and sensitization (16).

### 2.3.4 Fungal infections

*Cryptococcus neoformans, Pneumocystis jirovecii pneumonia* and *Aspergillus fumigatus* are the main pulmonary fungal microbes that can cause life-threatening invasive diseases in immunocompromised individuals such as HIV/AIDS positive patients. These infections are not

easily found in the target organs in healthy people (9). Upon fungal encounter, macrophages, dendritic cells(DC) and recruited neutrophils launch the first line of defense by phagocytosis and releasing of cytokines (9).

The most typical infection that characterizes AIDS is PCP caused by the *Pneumocystis jirovecii* species. Lethal cryptococcal meningitis is caused by Cryptococcus, which primarily affects the lungs before attacking the brain through circulation. Around 1 million people with AIDS are affected by cryptococcosis, which can lead to life-threatening cryptococcal meningoencephalitis and cause more than 600,000 fatalities worldwide each year (18). Cryptococcosis is not uncommon among HIV-ve individuals but their immune status is an advantage as the infection severity is not as in HIV+ve patients and also response to treatment of the two groups varies (9).

## 2.4 Sputum cytology

### 2.4.1 Introduction

Respiratory tract cytology is now revolutionized (4). To sample the respiratory tract for cytologic evaluation, various methods can be utilized (4). These include Sputum collection, Bronchial brushing, washing or aspirates, bronchoalveolar lavage (BAL) and fine needle aspiration (FNA) (4). These are helpful in the clear-cut visualization and localizing of tumours in the lungs (11). Sputum collection is the simplest non-invasive method to obtain specimen provided there is a productive cough (13)

Sputum is different from saliva as it is made up of cells that line the respiratory passages. For any patient with respiratory symptoms, sputum cytology would be the simplest and least invasive method to obtain a cytologic diagnosis (13). It would efficiently play an initial diagnostic role as even its value is closely comparable to histology (3). Sputum cytology trials carried out in the 1980s found more early-stage lung cancers fit for curative treatment (characteristically surgery), and also an improved 5-year survival for the screened.

Later, Saccomanno et al. collected sputum samples from workers in uranium mines believed to be at great risk for developing lung cancer(19). Their sputum was established to contain atypical cells and these patients developed lung cancer over time. The association between abnormal sputum cytology and lung cancer occurrence has successively been confirmed in other studies (20)

Sputum cytological examinations identify malignancies early in their initial stages as the small lesions could exfoliate malignant cells(21). These are identifiable by microscopy months or years before increasing in size to show on X-rays or result in symptoms of primary or metastatic disease (21). Squamous cell malignancies are typically central in origin and exfoliated cells can easily be recognized. Adenocarcinomas are often peripheral and hardly exfoliate hence imaging techniques best identify them (20).

Sputum cytology has not been able to prevent lung cancer in the same way that the Pap smear has prevented cervical cancer (4). However, early diagnosis is the best hope for cure, or at least better survival is a principle that still holds true. The estimated probability of survival for early stage lung cancer is over 90% at five years. Moreover, close to 40% of all lung cancers can be detected in this favorable stage by present radiologic and cytologic screening techniques (5). Respiratory cytology (exfoliative/aspiration) plays the role of detection and classification of pulmonary disease, mainly neoplastic disease, so that appropriate and timely therapy can be instituted (4).

### 2.4.2 Some Factors Affecting Diagnostic Reliability

**Number of Specimens**: Three satisfactory sputum specimens detect, on average, about 60% of cases of lung cancer (22). Three bronchial cytologic specimens can detect up to 90% of cases. Of the tumours that can be diagnosed with routine exfoliative respiratory cytology (i.e, sputum and bronchial cytology), roughly half to three-quarters of cases are diagnosed on the first specimen, another 15% to 20% on the second, and about 10% on the third (22). Few additional lung cancers are detected after five consecutive specimens have been found to be negative. Unfortunately, a single specimen is so unreliable in diagnosing even advanced cancer, that as many as every other patient with cancer in a screening program could be given a falsely reassuring benign diagnosis. The optimum number of sputum for cytology specimens is three to five (22).

**Location of Tumour:** Both bronchial and sputum cytology can detect central tumours in a high proportion of cases (23). A peripheral tumour, however, has fewer diagnostic cells in sputum; It is difficult to obtain the cells bronchoscopically, but bronchial cytology is generally better than sputum cytology in diagnosing peripheral lesions (23).

**Size of Tumour:** There is a rough association between the size of the tumour and the number of diagnostic cells i.e, the larger the tumour, the more cells, and therefore, the more likely that the lesion can be diagnosed by cytologic methods (23). Similarly, higher-stage tumours are more likely to be diagnosable cytologically (23). Smaller tumours may not exfoliate sufficient numbers of cells to make a diagnosis (23). However, sometimes large tumours can be more difficult to diagnose than smaller ones (23). For example, a large tumour can obstruct the bronchus, resulting in a decreased harvest of malignant cells in the sputum or via the bronchoscope. Also, large tumours may yield only necrotic debris. Thus, there may be an optimum tumour size for diagnosis, roughly between 3 to 6 cm(23).

**Type of Tumour and Degree of Differentiation**: Cell type influences both diagnostic sensitivity and specificity. Squamous cell carcinomas, particularly of the well-differentiated or keratinizing type, and small cell carcinomas are the most accurately classified primary lung cancers (4). Bronchoalveolar carcinoma (BAC) and adeno-squamous carcinoma are among the least commonly detected and least accurately classified lung carcinomas, while adenocarcinoma and large cell carcinoma fall in (23). Of major clinical importance, the accuracy of bronchial cytology in distinguishing small cell carcinoma from non-small cell carcinoma approaches 100. However, sputum cytology may misclassify a few cases of small cell carcinoma (23).

Generally, sputum cytology is more reliable for squamous cell carcinoma, while bronchial cytology is more reliable for adenocarcinoma (11). Metastatic cancer is usually diagnosed at a lower rate than primary lung cancer (23). Exfoliative respiratory cytology may be unreliable in the diagnosis of benign tumours because of submucosal growth or failure to shed diagnostic cells(23). Due to decreased intercellular cohesion, poorly differentiated tumours tend to shed more cells than well-differentiated tumours (23). Thus, poorly differentiated carcinoma may be more readily detected than well-differentiated cancers, cytologically. There may also be some discrepancies between the cytologic and the histologic grading of a tumour and tumours are well known to be inhomogeneous, and sampling can be a problem in any biopsy (4).

### 2.6 Problem statement

Most HIV +ve patients may initially present with AIDS-defining illnesses; fungal, bacterial, parasitic and viral. Pulmonary infections in particular continue to be a leading cause of morbidity and mortality. Prompt recognition and appropriate management of these opportunistic

infections/complications is imperative to decrease morbidity and mortality related to these conditions (24).

Neoplastic changes in the respiratory tract e.g lung cancer have also been reported in this population; 2-4 times greater risk. It is the third most frequent cancer among HIV-infected persons, coming after KS and NHL in incidence. Unlike palpable masses that could be diagnosed early before progression to invasive disease, lung cancer is in most cases detected late in advanced stages. (13).

Sputum cytology is rarely requested in our set up yet the production of significant amounts of sputum is often indicative of pulmonary disease. Most tests done on sputum in cases of productive cough in HIV -ve and HIV +ve patients have mainly focused on the opportunistic *Mycobacterium tuberculosis* (MTB). A few studies have been done in the past on neoplastic changes, fungal and bacterial OIs in HIV positive patients. The studies have confirmed a correlation between HIV/AIDS and these opportunistic infections. However, over time, there have been great changes in general healthcare and especially in the HIV/AIDS dynamics. It's therefore necessary to do a study on the respiratory opportunistic infections and neoplastic conditions in this population to inform on the landscape of these co-infections today.

### 2.7 Justification

Lung cancer is difficult to diagnose in the early stages before progression to invasive disease. Sputum cytology would be significant in early detection of neoplasia and identification of other causes of respiratory symptoms. A study to compare the respiratory tract cytology of two different groups (HIV +ve & HIV -ve patients) would be significant in providing information on OIs and neoplastic conditions in HIV +ve patients.

Studies have been done in the past confirming a shift in the landscape of AIDS co-infections and conditions such as lymphomas, Hepatitis B and C. The sputum cytological findings at this point would inform on the current landscape of respiratory infections/neoplasia in HIV/AIDS patients seen at Kenyatta National Hospital.

### 2.8 Research Question

What are the sputum cytological findings in HIV positive and HIV negative patients with productive cough attending the CCC and Chest Clinic in KNH?

## 2.9 Objectives

### 2.9.1 Broad Objectives

To describe sputum cytological findings in HIV positive and HIV negative patients with productive cough at the CCC and Chest Clinic in KNH

### 2.9.2 Specific Objectives

1. To describe the cytomorphological findings in sputum of HIV positive and HIV negative patients with productive cough.
2. To determine the prevalence of neoplastic changes in the sputum of HIV positive and HIV negative patients with productive cough in KNH
3. To identify infective causes of respiratory symptoms in HIV positive and HIV negative patients with productive cough.
4. To compare the sputum cytologic findings between the two groups

# 3.0 STUDY METHODOLOGY

## 3.1 Study Design

This was a cross-sectional descriptive study. This study type involved studying two different groups of participants to determine any differences in sputum cytological findings among HIV +ve patients and HIV–ve patients. Clear-cut conclusions were possible after a comparison of findings between the two groups. Comparison is an essential component of epidemiologic investigation (25).

## 3.2 Study Site

The study was conducted within Nairobi County at KNH CCC, Chest Clinic and the UON microbiology laboratory. KNH CCC and KNH chest clinic are referral clinics for patients with respiratory conditions with HIV and those without HIV respectively. KNH is situated in the capital city of Kenya and is a national tertiary referral hospital. Its exact location is to the west side of Upper Hill 3.5km west of the CBD. The hospital complex is 45.7 acres. It was founded in the year 1901, the oldest hospital in Kenya. It has a bed capacity of roughly 2000 and it has different departments.

The CCC and the Chest Clinic are open five days a week and these two departments serve a large population. UON and CCC microbiology laboratories are best suited for cyto-preparation of the sputum as sputum processing is done under a biosafety cabinet for protection from potentially infectious aerosols according to good clinical laboratory practice (GCLP) and the KNH guidelines.

## 3.3 Study Population

The study population was HIV positive patients as well as HIV negative adults with productive cough attending the CCC and the Chest Clinic in KNH respectively. HIV negativity was ascertained from the patient's health records. HIV testing is a basic requirement for all patients attending clinics at KNH provided they consent especially initially when they begin their clinic attendance. Provided the results were from tests conducted within 1 year from the period of this study, these were recruited in the HIV negative group. If the patients were uncertain of their HIV status and they fit the recruitment criteria, a test was requested. Pre-test counselling was then done by a counsellor at the KNH VCT. Those who did not consent to an HIV test were excluded from this study.

The sampled population in both groups was of the same age, socio-economic status and an equal number was sampled for both groups.

Enrolment for both HIV +ve and HIV -ve patients was done after reception of the patients in the chest clinic and CCC respectively as they were being triaged by the nurse in charge. At this stage, recruitment was done after an explanation of the study, patients consenting in writing and then completion of the study questionnaire.

### 3.4 Selection criteria

#### 3.4.1 Inclusion criteria

- All HIV positive patients with productive cough that consented to participate
- HIV negative patients attending the Chest Clinic with a productive cough that consented to participate

#### 3.4.2 Exclusion criteria

- Patients that were unable to provide a suitable deeply coughed-up sputum specimen
- Patients with known respiratory tract cancers

### 3.5 Sample Size

The formula for sample size estimation for two independent samples was used - (dichotomous variable)

$n = \{p(1-p) + p(1-p)\} (Z/E)^2$

n = estimated sample size

p = (0.036) prevalence/incidence as in one previous study, HIV infection was associated with increased lung cancer risk (risk ratio 3.6; (26)

Z = 1.96(95% confidence level used),

E = 0.05 which is the expected precision.

$= \{0.036 (1-0.036) + (1-0.036)\} (1.96/0.05)^2$

= 106.65

= 53 HIV +ve, 53 HIV -ve patients

### 3.6 Laboratory Methods

#### 3.6.1 Requirements

1. Biosafety cabinet for sputum processing as it's a highly hazardous specimen, especially in cases of *Mycobacterium tuberculosis.*
2. Sputum containers for sputum collection by the study participants.
3. Centrifuge for centrifugation of the specimen in tightly corked centrifuge tubes
4. Refrigerator for storage of the tightly corked specimen.
5. Microscopic slides for making smears for microscopic examination.
6. Routine stains; H&E, Pap stain and special stains; ZN, PAS, Giemsa and Gram stain.
7. Others: Microscope, gloves, oil immersion, 95% Ethanol

#### 3.6.2 Sample collection protocol

For every recruited participant, a spot sputum specimen was provided and one/two more containers were added when they were able to get an early morning deep cough mostly within a day or two of the first specimen collection. These participants were instructed to make deep coughs to obtain sputum into provided wide-mouthed leak-proof containers after rinsing or brushing their mouths. They were to ensure not to spit saliva into the collection container but deep cough/expectorated sputum and tightly cork the containers before delivering them to the laboratory. The ideal number of cups of sputum specimen cytology is 3-5 even as by the KNH guidelines. For this research however, patients provided a minimum of two containers of sputum specimen and this provided specimen was accepted after macroscopic confirmation of adherence to instructions to ensure deeply coughed sputum and not saliva. This is because KNH is a referral facility with patients from all over the country and five container specimens was challenging to obtain. Therefore, two containers of sputum were the acceptable number of sputum containers but after very clear instructions to ensure high-quality specimen.

They gave one spot specimen after rinsing their mouth and two cups of sputum specimen were brought in on the third day after the 1$^{st}$ specimen which for most was manageable as they were still staying not far away from the facility; to attain the optimum sputum collected on three different days. For the specimens collected away from the facility, they were advised to do it away from people and in a well ventilated area and they carefully try to minimize aerosol spread. Within the facility, for the spot sputum specimen, there was an area in the CCC that's reserved

for the same and also for the chest clinic which is within other clinics, there's a separate clinic that is an extension of the chest clinic with a sputum collection room. Therefore, those were the areas preferred for patients to collect sputum.

After specimen collection and secure capping, the sputum cups were placed in biohazard bags and then well-sealed before being packed in another carrier bag and then delivered to the facility before being taken in cool boxes to the microbiology laboratory biosafety cabinet from the patients' respective clinics.

### 3.6.3 Sample processing

This was strictly carried out in a biosafety cabinet. For each provided specimen, six smears were prepared. Two were immediately fixed in 95% Ethanol and stained in H & E and Pap stain. The other four were air-dried and stained in ZN, Giemsa, Gram stain and PAS.

### 3.6.4 Laboratory procedures:

Pick and smear: The sputum specimens were visually inspected for any blood or solid particles and then the appropriate portions were selected from the specimen to make the smear. Then the smears were immediately fixed in 95% ethanol before staining in Pap stain and H & E. Other slides were intermittently air-dried for ZN, Giemsa, Gram stain and PAS stains.

Biosafety: This was assured by use of PPEs and patients were well educated on how to properly collect the specimen without spreading aerosols and contaminating surfaces and tightly cap the specimen containers so there won't be any leakage.

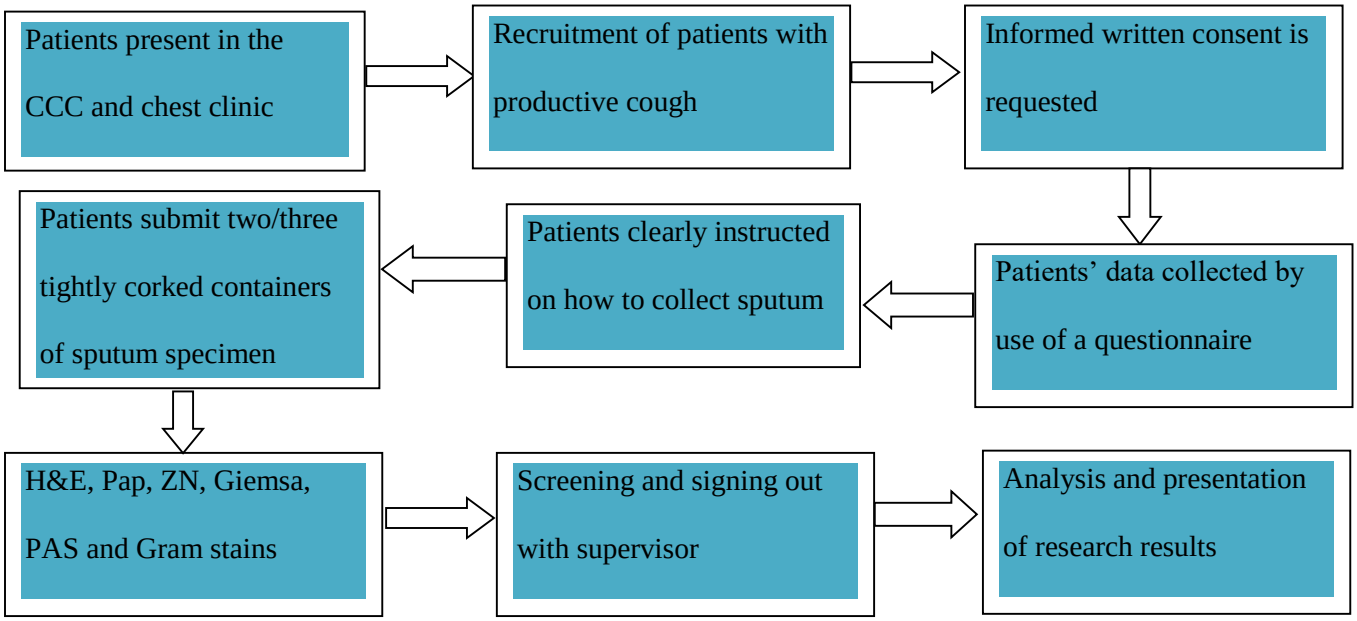
The containers used for sputum collection were disposed by incineration after treatment with 2% hypochlorite solution.

### 3.6.5 Quality Assurance

Participants were instructed accordingly to ensure expectorating quality samples for good results. Salivary and leaking specimen were rejected. For quality sputum specimen in cytology, alveolar macrophages are the proof of a quality specimen that has been expectorated from deep in the lungs and not just saliva/ upper respiratory tract material. This was ensured in all specimen reported as satisfactory for evaluation during microscopy. All specimens were clearly labelled and accompanied by lab request forms. An individual's specimens were mixed, homogenized,

smears made and fixed immediately, others air-dried; this was in a biosafety cabinet and then staining was done on the air-dried and well-fixed smears as appropriate. During staining with ZN, a negative and a positive control accompanied the patients' smears for quality assurance purposes.

### 3.7 Research Process Flow chart



### 3.8 Data Analysis and Presentation

Results were clearly and precisely recorded in hard copy and in excel work sheet. All the data was summarized using Microsoft excel 2016. Data analysis was done using SPSS version 20. Frequency of each of the measured variables in each of the two groups was calculated. This gave distribution of the cytomorphological features and infective causes among the two groups under study.

Data was then presented in pie charts, tables and bar charts. Multivariate logistic regression was used to help predict the relationship between the independent and dependent variables.

Dependent variables: Cytomorphology findings, inflammatory changes, fungal, bacterial, parasitic infections.

Independent variables: Age, sex, HIV status (HIV+ve, HIV-ve)

### 3.9 Ethical Consideration

Ethical clearance was obtained from the KNH-UON ethical research committee. Patient consent was sought prior to enrollment and utmost confidentiality was assured throughout the study period. Results were sent to patients' file in the usual manner to inform on further management.

They did not pay for additional tests requested for this study. Study participants were free to withdraw from the research at any time without being coerced and were not discriminated from other services in the hospital.

Confidentiality for all information from the patients was maintained. Identification codes and not patient's names were used. All handwritten copies were kept safe in a lockable cabinet, and the soft copies kept safe from unauthorized persons by password protection. Communication of the test results was from the attending clinic doctors during clinics.

### 3.10 Study Results Dissemination

Study findings were shared with the UON Human Pathology Department and the Department of Medicine, KNH. The results were also shared for patients' benefit with their clinicians in their respective clinics for review in their next clinic visits. This dissertation will form part of the University of Nairobi's Research Repository and will be written up for a publication in a scientific journal.

## RESULTS

This study investigated the sputum cytological findings in HIV +ve and HIV-ve patients at KNH that had productive cough.

### Clinical History

Among study participants in this study, 53 (50%) were HIV positive patients with productive cough with 3 (2.8%) of these being smokers. Half the patients (50%) were HIV negative with productive cough of which 35 (33%) presented with a history of chronic cough and 8 (7.5%) of them reported being smokers (table 1).

**Table 1: Clinical History in the study population**

| Group | Clinical history | Frequency | Percentage |
|---|---|---|---|
| HIV -ve patients | Asthma | 11 | 10.4 |
| | COPD | 3 | 2.8 |
| | Chronic Cough | 27 | 25.5 |
| | Chronic Cough Smoker | 8 | 7.5 |
| | Pneumonia | 4 | 3.8 |
| HIV +ve patients | RVD With Productive Cough | 50 | 47.2 |
| | RVD With Productive Cough, Smoker | 3 | 2.8 |
| | Total | 106 | 100.0 |

**Age distribution among the study participants**

In the sample population of the 106 participants, the age range 26-35 was the most frequent with 40 (37.7%) patients. The least number of participants were in the age group 65 and above with only 4 (3.8%) participants.

**Table 2: Age distribution among the study participants**

| Age range | Frequency | Percentage |
|---|---|---|
| 18-25 | 16 | 15.1 |
| 26-35 | 40 | 37.7 |
| 36-45 | 23 | 21.7 |
| 46-55 | 18 | 17.0 |
| 56-65 | 5 | 4.7 |
| >65 | 4 | 3.8 |
| Total | 106 | 100.0 |

**Age Distribution by Gender**

As seen from the bar graph below, participants in this study were mostly between the ages of 26-35 with females being more than males in this age group (24 females and 16 males). 36-45 was the second highest age group in the study and in this particular age group, males were more than females (15 males and 8 females)

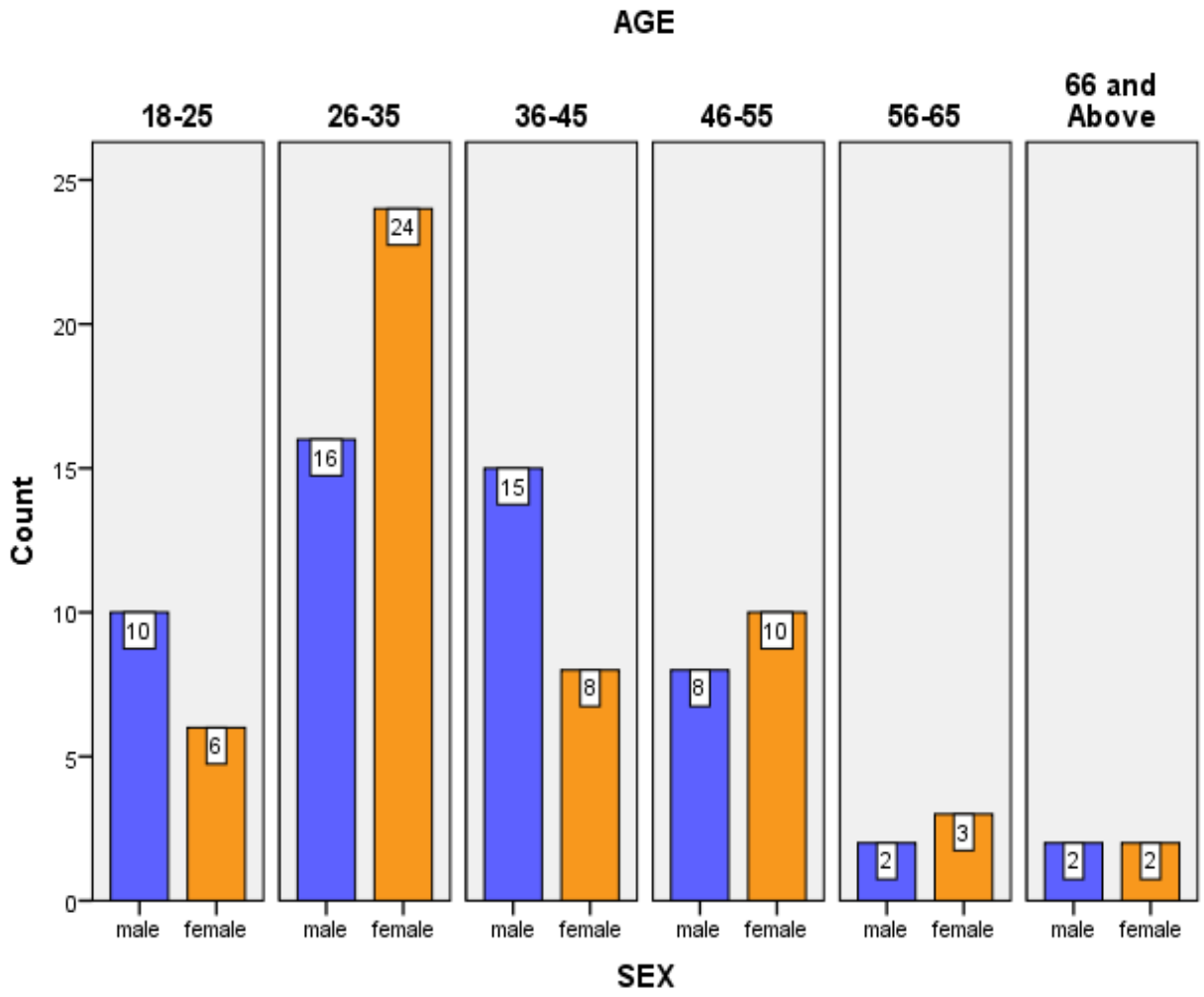



*Figure 1: Age Distribution by Gender*

**Cytomorphology**

The sputum cytomorphologic findings were categorized as unsatisfactory for evaluation, negative for malignancy, inflammatory changes and ASCUS. They were as summarized in table 3 below. Majority, 87 (82.1%) of the study participants had their sputum negative for malignancy while a total of 9 (9.4%) had inflammatory changes. Four (3.7%) of the participants in this study had atypical squamous cells of uncertain significance (ASCUS) and no obvert case of malignancy was detected. Of the 4 ASCUS cases, only one was HIV +ve.

**Table 3: Cytomorphology distribution among the HIV +ve & HIV -ve patients**

| Cytomorphological finding | Frequency | Percentage |
|---|---|---|
| Unsatisfactory for Evaluation | | |
| HIV -ve patients | 2 | 1.9 |
| HIV +ve patients | 4 | 3.8 |
| Total | 6 | 5.7 |
| Negative for malignancy | | |
| HIV -ve patients | 44 | 41.5 |
| HIV +ve patients | 43 | 40.6 |
| Total | 87 | 82.1 |
| Inflammatory changes | | |
| HIV -ve patients | 4 | 3.8 |
| HIV +ve patients | 5 | 4.7 |
| Total | 9 | 8.5 |
| ASCUS | | |
| HIV -ve patients | 3 | 2.8 |
| HIV +ve patients | 1 | 0.9 |
| Total | 4 | 3.7 |

**Prevalence of Neoplasia among the two groups**

Generally, there were no cases of obvert malignancy and no single case of suspicious for malignancy. There were only cases of atypia and the incidence was generally low among both groups; at 2.8% in HIV –ve patients and at 0.9% among HIV +ve patients.

As from table 4 below, of the four ASCUS cases, three were HIV –ve and only one was HIV +ve.

**Table 4: Prevalence of Neoplasia amongst HIV +ve & HIV –ve patients**

| Cytomorphological finding | Frequency | Percentage |
|---|---|---|
| Negative for malignancy | | |
| HIV -ve patients | 50 | 47.2 |
| HIV +ve patients | 52 | 49.1 |
| ASCUS | | |
| HIV -ve patients | 3 | 2.8 |
| HIV +ve patients | 1 | 0.9 |
| Suspicious for malignancy | | |
| HIV -ve patients | 0 | 0 |
| HIV +ve patients | 0 | 0 |
| Malignant | | |
| HIV -ve patients | 0 | 0 |
| HIV +ve patients | 0 | 0 |
| Total | 4 | 3.7 |

**Infectious causes of productive cough**

Fungal infections, AFB, other various bacterial and parasitic infections were the infective causes of productive cough that were tested for. *Candida spp.*, AFB and other bacterial infections were the findings as shown in the tables (5-7) and figures (2-4) below.

**Acid Fast Bacilli (AFB)**

Of all the study participants, 9 (8.5%) tested positive for AFB.

**Table 5: AFB findings**

| Finding | Frequency | Percentage |
|---|---|---|
| Negative | 97 | 91.5 |
| Positive | 9 | 8.5 |
| Total | 106 | 100.0 |

**AFB distribution among HIV +ve and HIV -ve patients**

The findings as shown in the pie chart below showed that 49 (92.4%) of HIV negative persons had tested negative for AFB with only 4 (7.6%) testing Positive for AFB. The second pie chart for HIV Positive individuals showed that 48 (90.6%) had tested negative for AFB while 5 (9.4%) tested positive for AFB. HIV positive patients that tested positive for AFB exceeded the HIV negative participants who tested positive for AFB by one participant.

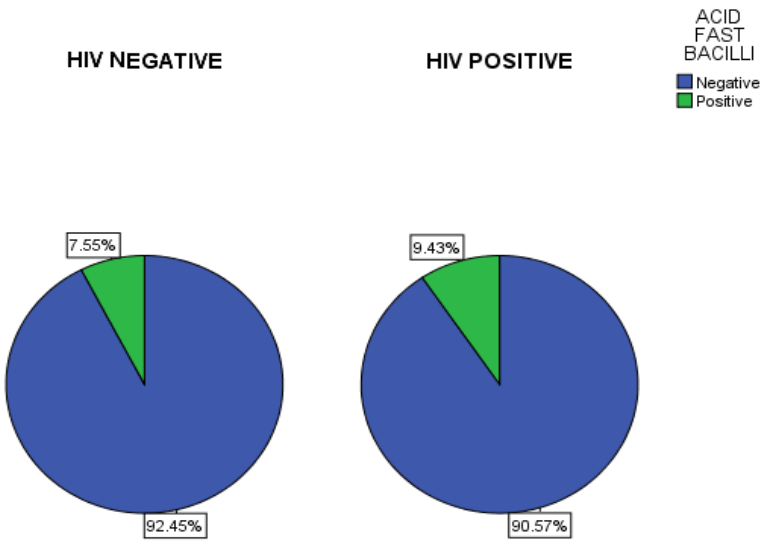



*Figure 2: AFB distribution among HIV +ve and HIV -ve patients*

**Fungal Infections**

Seven (6.6%) of all the participants tested positive for Candida Spp.

**Table 6: Candida spp. findings**

| Finding | Frequency | Percentage |
|---|---|---|
| Negative | 99 | 93.4 |
| Candida spp. positive | 7 | 6.6 |
| Total | 106 | 100.0 |

**Candida spp positive findings among HIV +ve and HIV -ve patients**

The findings showed that 2 (3.8%) HIV negative patients tested positive for Candida spp. and 5 (9.43%) of the HIV Positive patients tested positive for Candida spp.

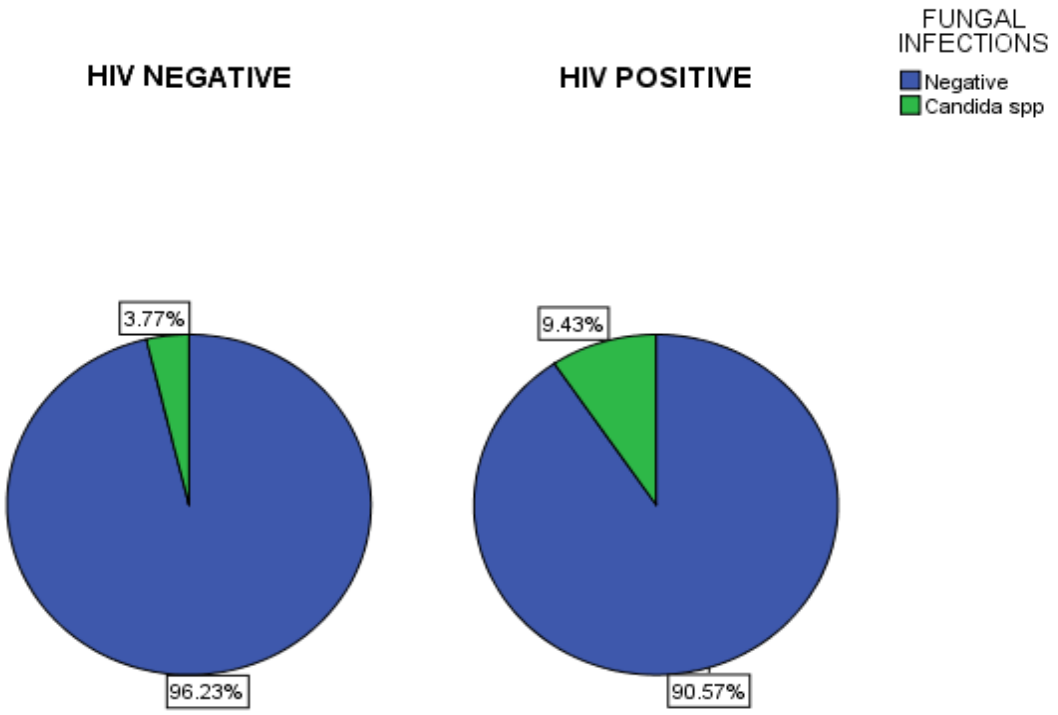



*Figure 3: Candida spp findings distribution among HIV +ve and HIV -ve patients*

**Bacterial Infections**

In twenty seven (25.5%) of these study participants, no bacterial forms were identified on Gram stain. Twenty one (19.8%) of the patients had only gram positive cocci in clusters and 20 (18.9%) had only gram-positive cocci in chains stained. Twenty three (21.6%) of the patients had Gram positive diplococci with 15 (14.1%) of these being mixed with other bacteria. Others had mixed bacterial forms of all the above in their specimen. Two patients had Gram negative bacilli in chains while one patient had Gram positive club-shaped bacteria.

**Table 7: Distribution of identified bacteria**

| Identified bacteria | Frequency | Percentage |
|---|---|---|
| GRAM POSITIVE COCCI IN CLUSTERS | 21 | 19.8 |
| GRAM POSITIVE COCCI IN CHAINS | 20 | 18.9 |
| GRAM POSITIVE DIPLOCOCCI | 8 | 7.5 |
| GRAM POSITIVE COCCI IN CLUSTERS, GRAM POSITIVE COCCI IN CHAINS & GRAM POSITIVE DIPLOCOCCI | 4 | 3.8 |
| GRAM POSITIVE COCCI IN CLUSTERS & GRAM POSITIVE COCCI IN CHAINS | 12 | 11.3 |
| GRAM POSITIVE COCCI IN CHAINS & GRAM POSITIVE DIPLOCOCCI | 10 | 9.4 |
| GRAM POSITIVE COCCI IN CLUSTERS & GRAM POSITIVE DIPLOCOCCI | 1 | .9 |
| GRAM NEGATIVE BACILLI IN CHAINS | 2 | 1.9 |
| GRAM POSITIVE CLUB-SHAPED BACTERIA | 1 | .9 |
| NEGATIVE | 27 | 25.5 |
| Total | 106 | 100.0 |

**Distribution of the bacteria identified among HIV +ve and HIV -ve patients**

On bacterial infections 2.83% of HIV negative had gram positive cocci in clusters & gram positive cocci in chains as compared to 8.49% for the same on HIV positive persons.

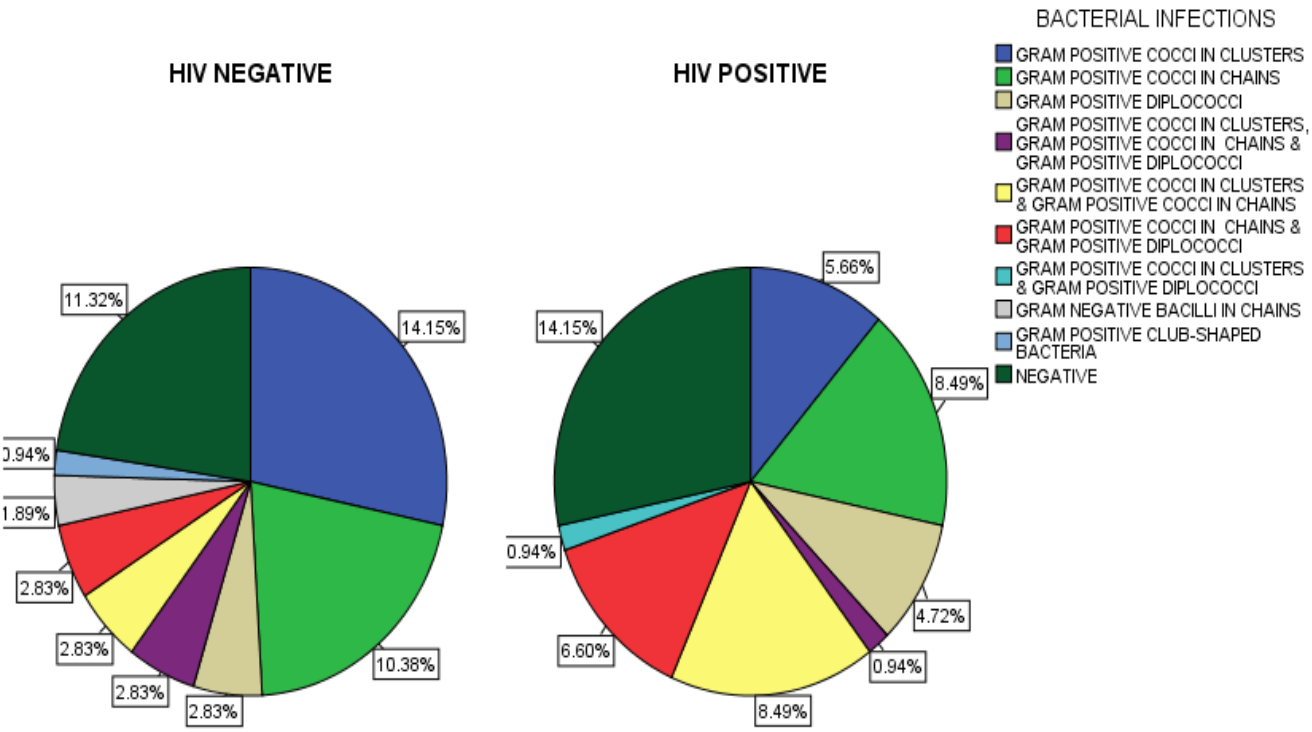



*Figure 4: Distribution of the bacteria identified among HIV +ve and HIV -ve patients*

**Parasitic infections**

All the patients sampled had tested negative for parasitic infections; males, females, HIV +ve and HIV -ve patients.

**Positive findings**

To attain this study's objectives, six stains were done on the sputum smears. H&E, Pap stain, ZN, Giemsa, PAS and Gram stain all to screening for various conditions. H&E and Pap stain were for demonstration of any inflammatory and neoplastic cellular changes and ASCUS, inflammatory changes and negative were the findings. ZN was for demonstration of AFB such that the findings were either positive or negative for AFB. The use of Giemsa aimed at demonstrating parasites and the results from this study were all negative for any parasitic infections in the participants' sputum. PAS staining was for demonstration of fungal elements and the findings were positive or negative for fungal infection whereby the diagnosed fungal organism in this study was Candida spp. Gram stain was meant to help identify the bacteria as Gram positive or Gram negative.

| **STAIN** | **FINDINGS** | |
|---|---|---|
| H&E, Pap stain | **Positive** | **Negative** |
| | ASCUS<br>Inflammatory changes | Negative |
| ZN stain | AFB Positive | AFB Negative |
| PAS stain | Candida spp. | Negative |
| Giemsa | None | All negative for parasitic infection |

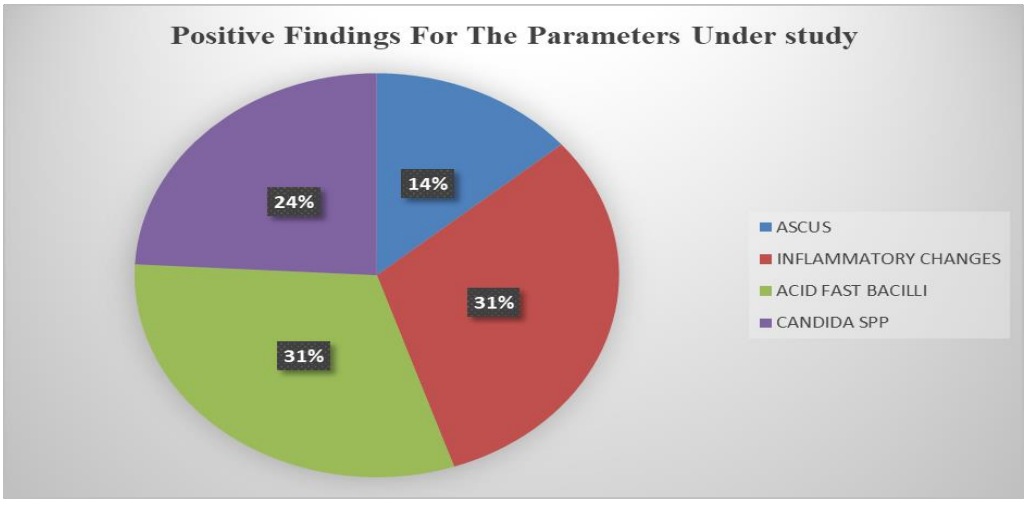



Figure 5: Positive Findings for the Parameters under study

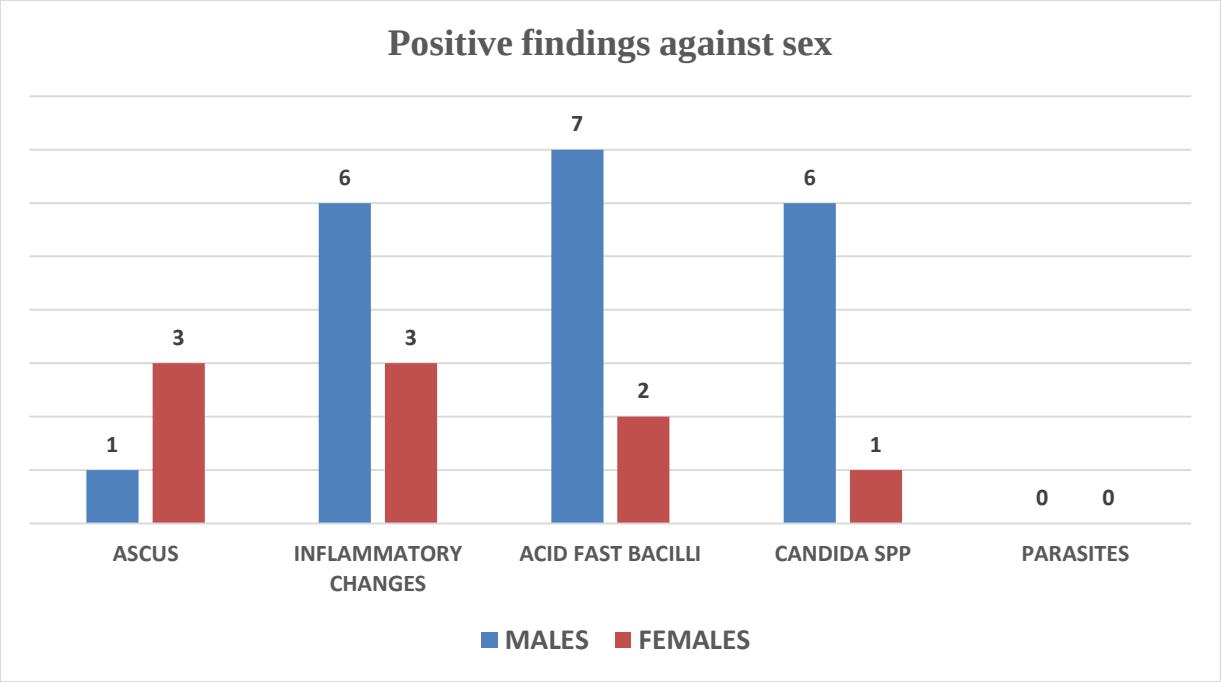



*Figure 6: Relationship between sex and the Positive findings*

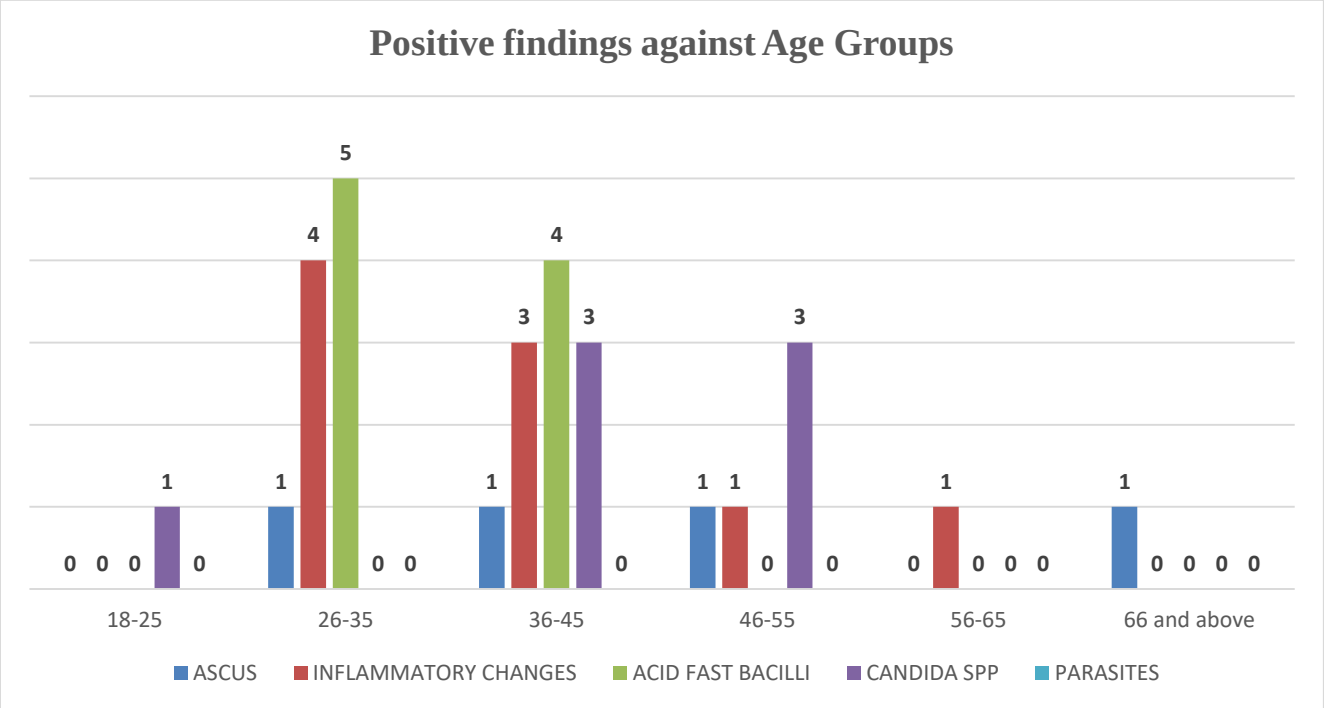



*Figure 7: Relationship between Age groups and Positive findings*

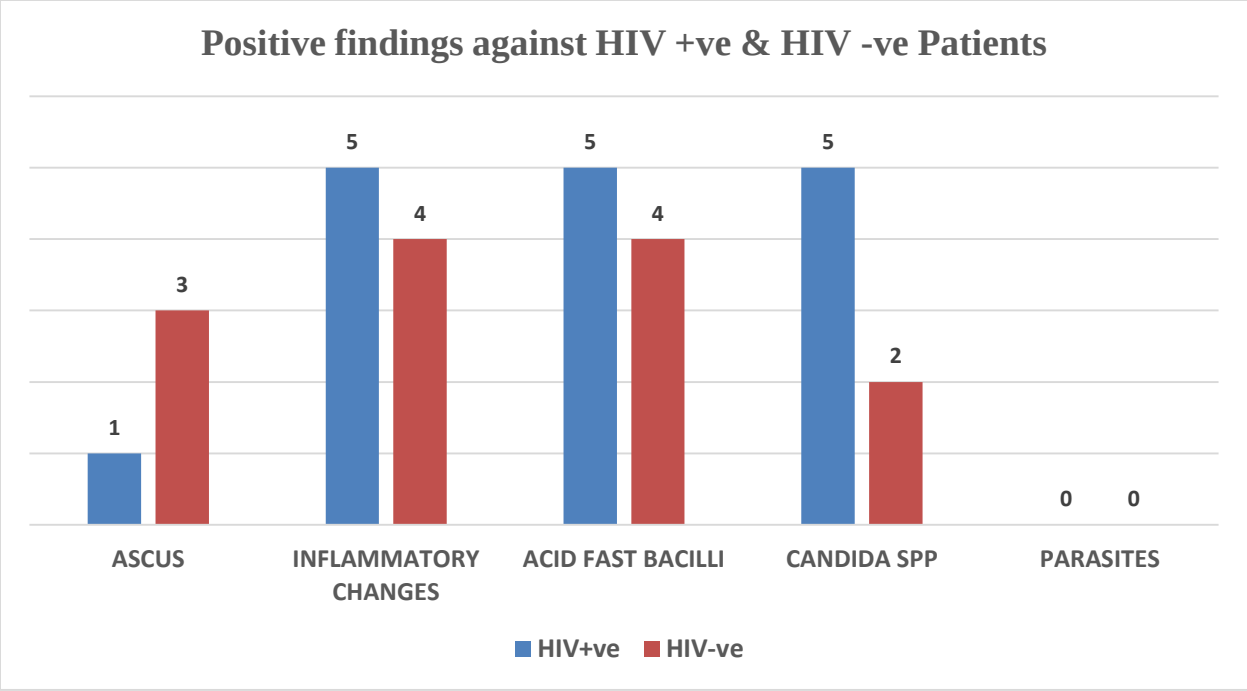



*Figure 8: Relationship between HIV +ve & HIV -ve patients Against Positive Findings*

**Data Analysis**

Multivariate logistic regression was used to come up with a mathematical formula that could be used in predicting relationships between the dependent and independent variables.

**Table 8: Descriptive Statistics**

Descriptive statistics was done to check on the characteristics of the variables under study. Bacterial infections for example had a mean of 4.93 implying a high number of the sputum specimens analyzed tested positive for a certain bacterium on Gram stain. Fungal infections had a mean of 0.07 implying that the specimen that tested positive for fungal infection were not as may and there was no single parasite stained in these specimens.

| Finding | HIV +ve/HIV -ve | Mean | Std. Deviation | N |
|---|---|---|---|---|
| CYTOMORPHOLOGY | HIV NEGATIVE | 0.30 | 0.749 | 53 |
| | HIV POSITIVE | 0.36 | 0.857 | 53 |
| | Total | 0.33 | 0.801 | 106 |
| ACID FAST BACILLI | HIV NEGATIVE | 0.08 | 0.267 | 53 |
| | HIV POSITIVE | 0.09 | 0.295 | 53 |
| | Total | 0.08 | 0.280 | 106 |
| FUNGAL INFECTIONS | HIV NEGATIVE | 0.04 | 0.192 | 53 |
| | HIV POSITIVE | 0.09 | 0.295 | 53 |
| | Total | 0.07 | 0.250 | 106 |
| BACTERIAL INFECTIONS | HIV NEGATIVE | 4.45 | 3.603 | 53 |
| | HIV POSITIVE | 5.42 | 3.325 | 53 |
| | Total | 4.93 | 3.484 | 106 |
| PARASITIC INFECTIONS | HIV NEGATIVE | 0.00 | 0.000 | 53 |
| | HIV POSITIVE | 0.00 | 0.000 | 53 |
| | Total | 0.00 | 0.000 | 106 |

**Multivariate test table** – Pillai's trace row was used to check for significance of various variables under study. 0.024 was the significance value as from the table below and 0.024 is less than the P value which was 0.05 implying there was significance among the variables. This means that majorly, the dependent variables happened from a cause and not by chance i.e the independent variables for example, had an effect on some of the dependent variables.

**Table 9: Multivariate Tests[a]**

| Effect | | Value | F | Hypothesis df | Error df | Sig. | Partial Eta Squared |
|---|---|---|---|---|---|---|---|
| Intercept | Pillai's Trace | 0.700 | 58.897[b] | 4.000 | 101.000 | 0.000 | 0.700 |
| | Wilks' Lambda | 0.300 | 58.897[b] | 4.000 | 101.000 | 0.000 | 0.700 |
| | Hotelling's Trace | 2.333 | 58.897[b] | 4.000 | 101.000 | 0.000 | 0.700 |
| | Roy's Largest Root | 2.333 | 58.897[b] | 4.000 | 101.000 | 0.000 | 0.700 |
| HIV +ve/HIV -ve | Pillai's Trace | 0.031 | .806[b] | 4.000 | 101.000 | 0.024 | 0.031 |
| | Wilks' Lambda | 0.969 | .806[b] | 4.000 | 101.000 | 0.024 | 0.031 |
| | Hotelling's Trace | 0.032 | .806[b] | 4.000 | 101.000 | 0.024 | 0.031 |
| | Roy's Largest Root | 0.032 | .806[b] | 4.000 | 101.000 | 0.024 | 0.031 |

a. Design: Intercept + HIV +ve/HIV -ve

b. Exact statistic

**Levene's test** – This was to assess the equality of variance for the variables under study. From the table below, the significance values as below (0.484, 0.490, 0.018 and 0.248) were all greater than 0.05. This implied that homogeneity assumption of the variance was met and that the model was efficient.

**Table 10: Levene's Test of Equality of Error Variances[a]**

| Finding | F | df1 | df2 | Sig. |
|---|---|---|---|---|
| CYTOMORPHOLOGY | 0.494 | 1 | 104 | 0.484 |
| ACID FAST BACILLI | 0.479 | 1 | 104 | 0.490 |
| FUNGAL INFECTIONS | 5.752 | 1 | 104 | 0.018 |
| BACTERIAL INFECTIONS | 1.350 | 1 | 104 | 0.248 |
| PARASITIC INFECTIONS | 0.000 | 1 | 104 | . |

Tests that the error variance of the dependent variable is equal across groups.

a. Design: Intercept + HIV +ve/HIV -ve

**Parameter estimates** – As by table 12 below, this summarized the effect of each independent variable on the dependent variables. The interpretation of coefficient here is based on signs of coefficient. Parasitic infections had an intercept of 0 and 0 significance meaning no effect. The other variables however had a positive effect shown by the B values; 0.358, 0.094, 0.094 and 5.415; Positive significance correlation. This aided come up with a formula that could be used in predicting effect of listed independent variables on the dependent variables.

$$\underset{B}{\{Y1-E\}} + \underset{B}{\{Y2-E\}} + \underset{B}{\{Y3-E\}} + \underset{B}{\{Y4-E\}} + \underset{B}{\{Y5-E\}} = X$$

$$= \underset{B}{\{0.358-E\}} + \underset{B}{\{0.094-E\}} + \underset{B}{\{0.094-E\}} + \underset{B}{\{5.415-E\}} + \underset{B}{\{0-E\}} = X$$

**Table 11: Parameter Estimates**

| Dependent Variable | Parameter | B | Std. Error | t | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound |
| CYTOMORPHOLOGY | Intercept | 0.358 | 0.111 | 3.243 | 0.002 | 0.139 | 0.578 |
| | [HIV +ve/HIV -ve =0] | -0.057 | 0.156 | -0.362 | 0.718 | -0.367 | 0.253 |
| | [HIV +ve/HIV -ve =1] | 0[a] | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| ACID FAST BACILLI | Intercept | 0.094 | 0.039 | 2.442 | 0.016 | 0.018 | 0.171 |
| | [HIV +ve/HIV -ve =0] | -0.019 | 0.055 | -0.345 | 0.731 | -0.127 | 0.089 |
| | [HIV +ve/HIV -ve L=1] | 0[a] | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| FUNGAL INFECTIONS | Intercept | 0.094 | 0.034 | 2.757 | 0.007 | 0.026 | 0.162 |
| | [HIV +ve/HIV -ve =0] | -0.057 | 0.048 | -1.170 | 0.245 | -0.153 | 0.039 |
| | [HIV +ve/HIV -ve =1] | 0[a] | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| BACTERIAL INFECTIONS | Intercept | 5.415 | 0.476 | 11.371 | 0.000 | 4.471 | 6.359 |
| | [HIV +ve/HIV -ve =0] | -0.962 | 0.673 | -1.429 | 0.156 | -2.298 | 0.373 |
| | [HIV +ve/HIV -ve =1] | 0[a] | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| PARASITIC INFECTIONS | Intercept | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | [HIV +ve/HIV -ve =0] | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| | [HIV +ve/HIV -ve =1] | 0[a] | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| a. This parameter is set to zero because it is redundant. | | | | | | | |

**Photomicrographs**

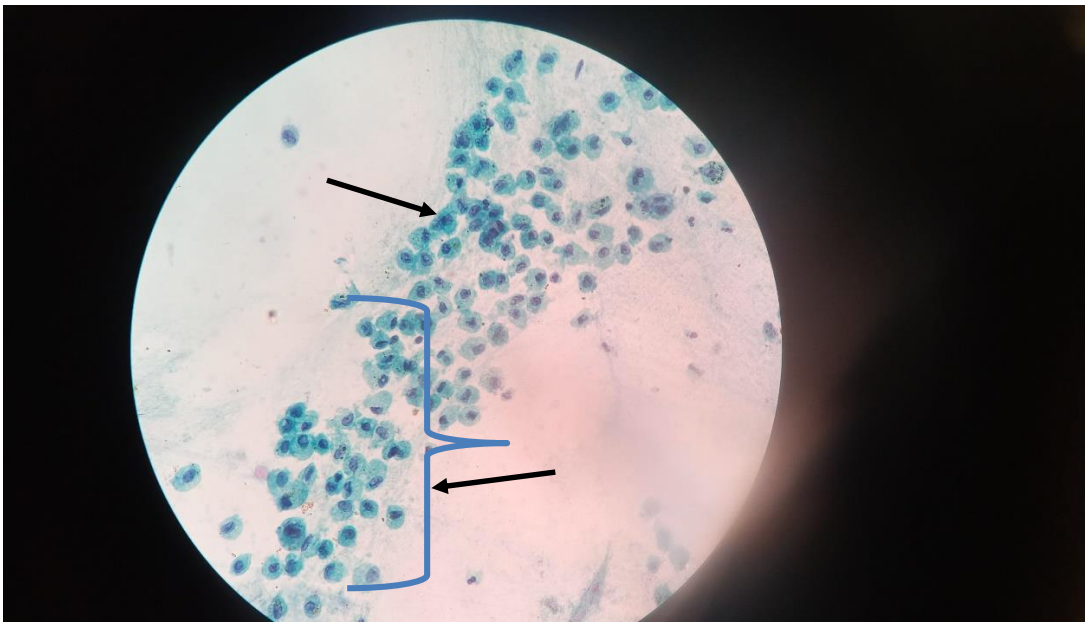
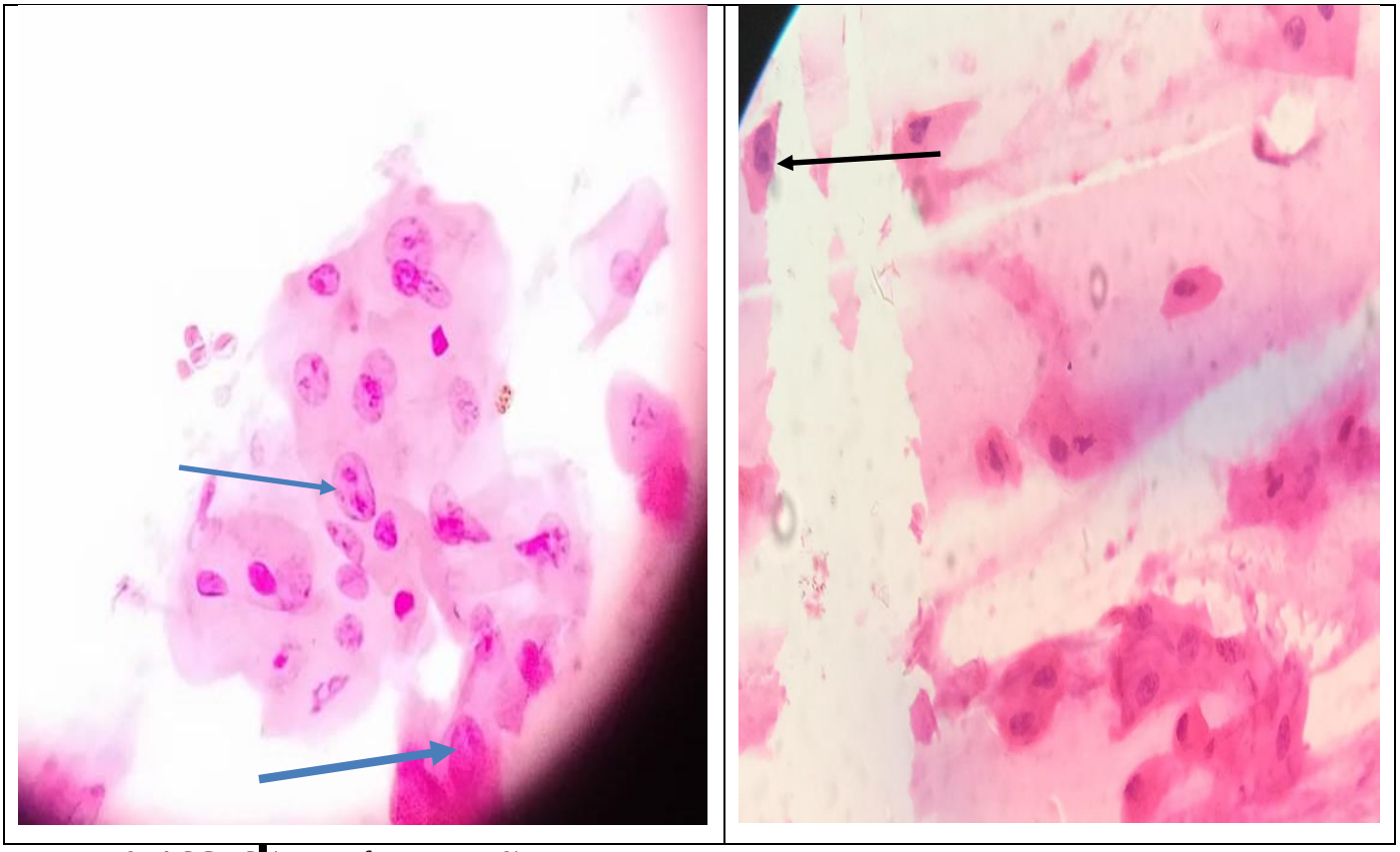


Figure 9: Alveolar macrophages (Magnification x10)



Figures 10: ASCUS (Magnification x40)

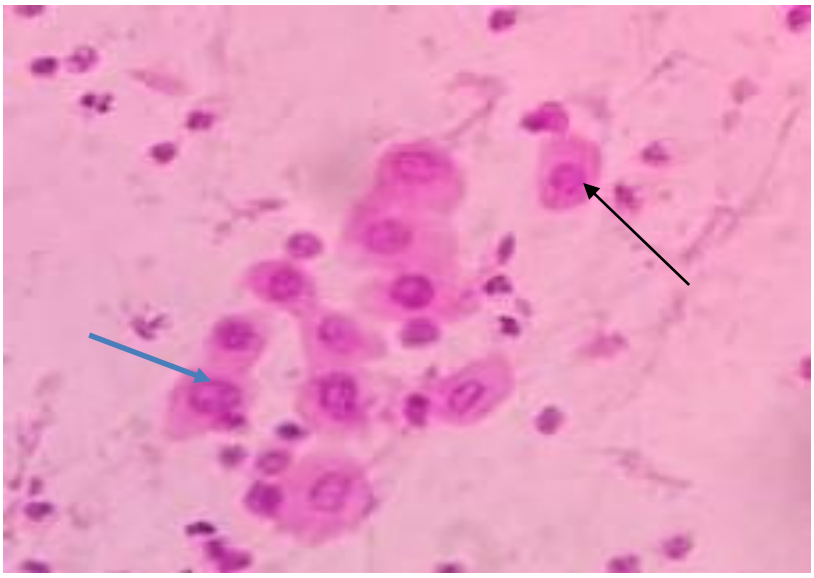


Figure 11: Inflammatory changes (Magnification x40)

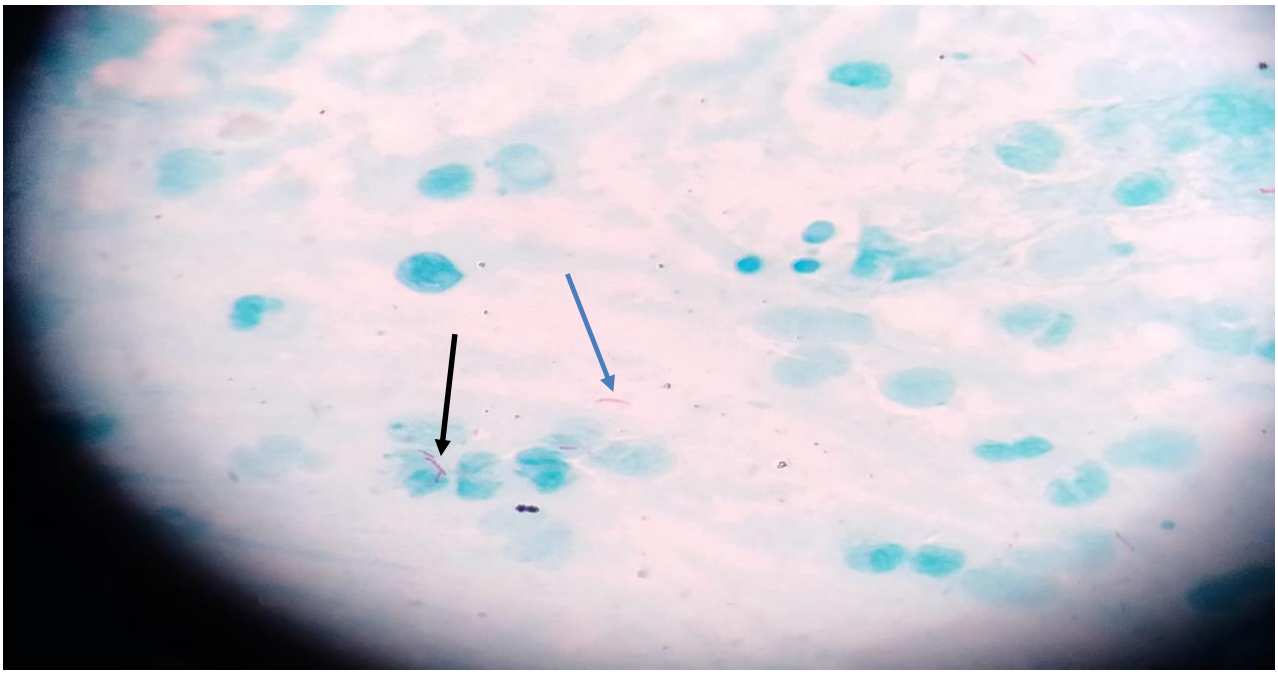


Figure 12: Acid fast bacilli (Oil immersion x100)

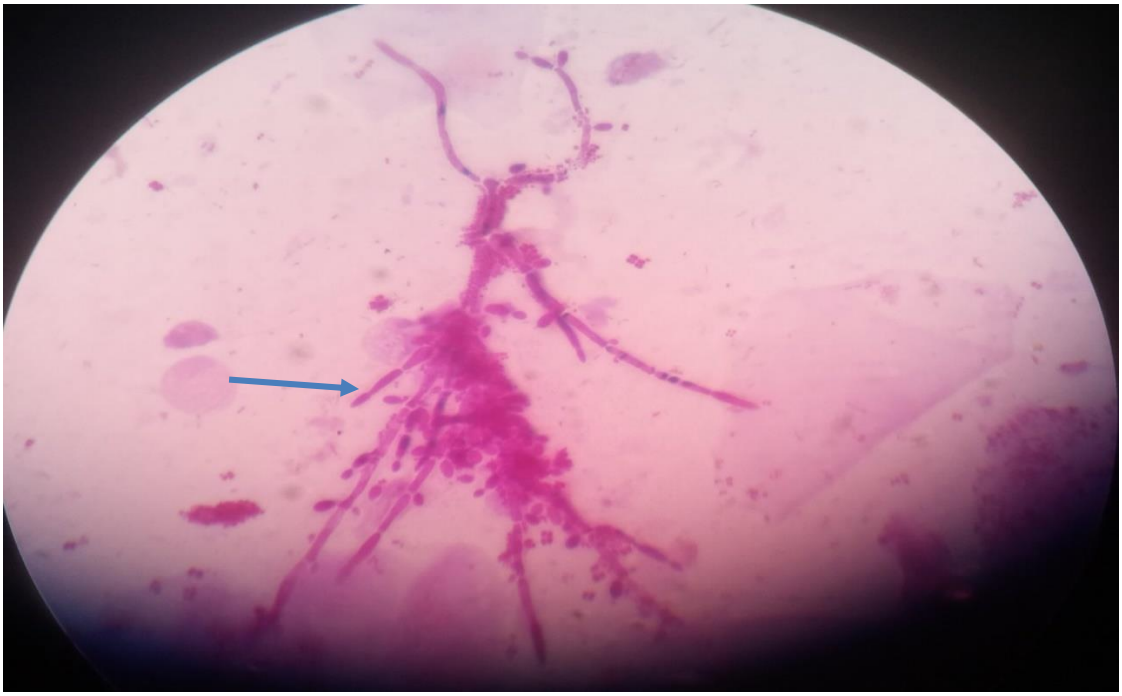


Figure 13: Candida (Septate hyphae) (Magnification x40)

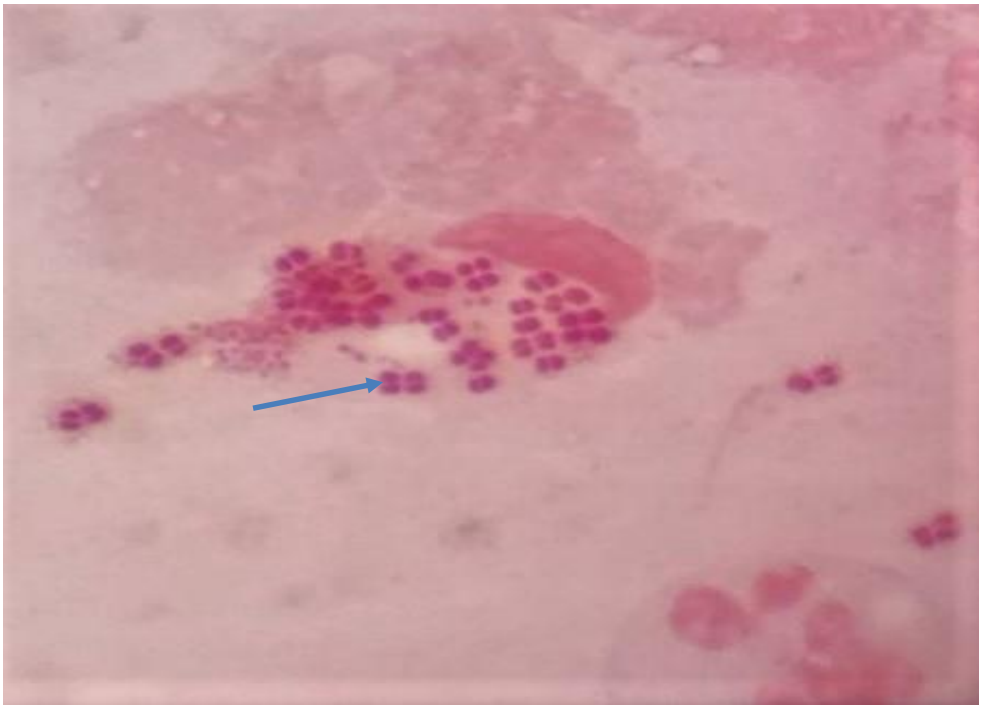


Figure 14: Gram positive diplococci (Oil immersion x100)

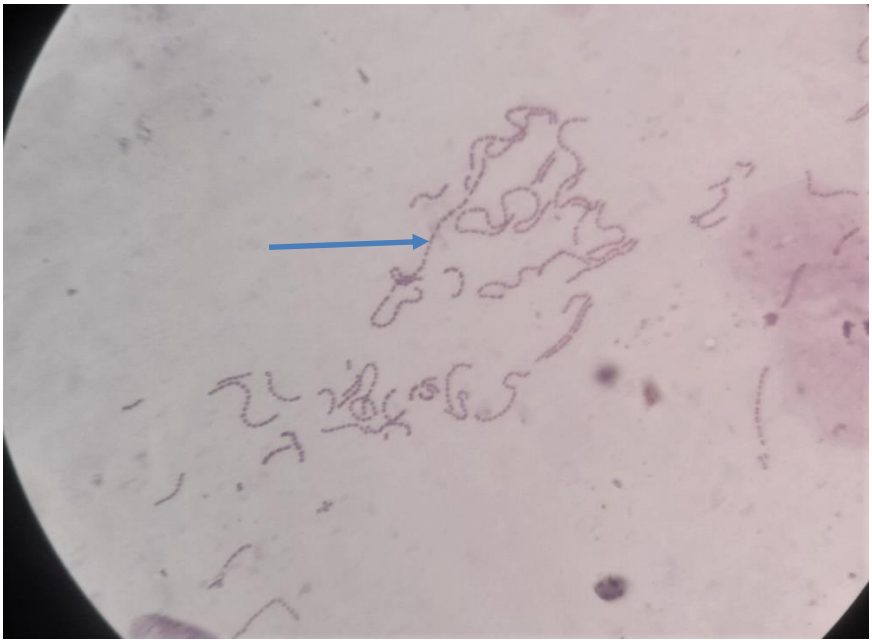


Figure 15: Gram positive cocci in chains (Oil immersion x100)

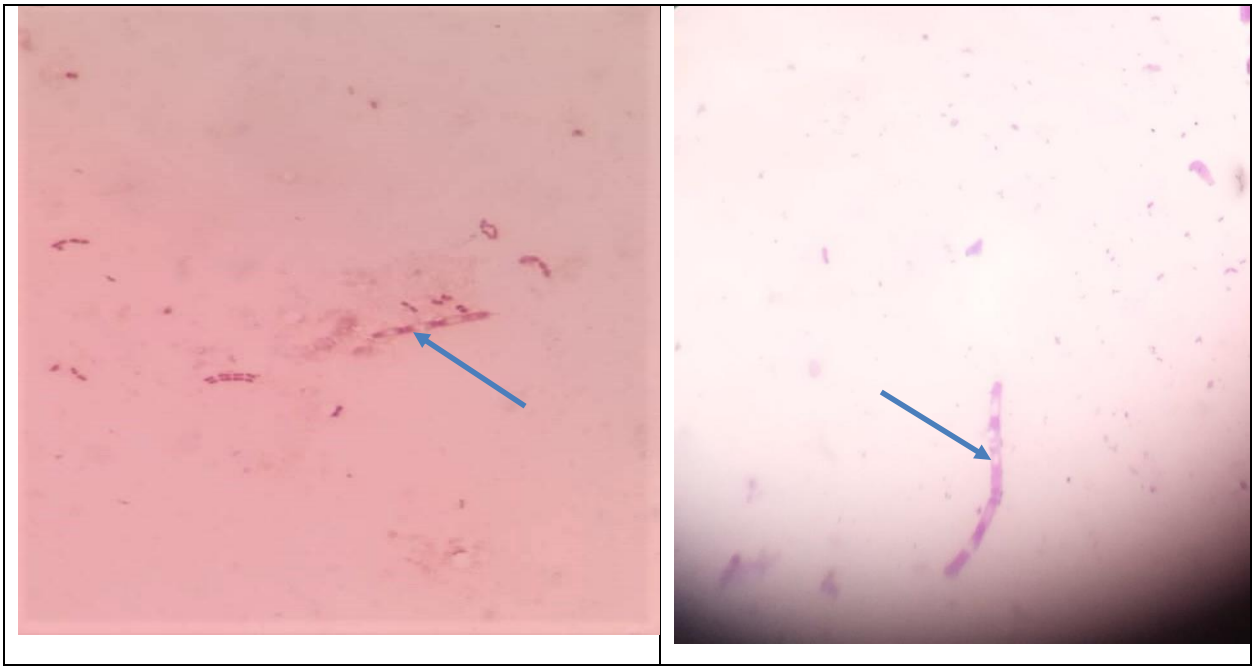


Figure 16: Gram positive club-shaped bacterium (Oil immersion x100)

# DISCUSSION, CONCLUSION, RECOMMENDATIONS

## 5.1 Discussion

This cross-sectional descriptive study was done to determine the pattern of neoplastic changes and infections in HIV +ve patients compared with HIV –ve patients in Kenya. Sputum spontaneously produced by deep cough was the specimen used in this study. The specimens were brought without any fixative. Delay was avoided as degeneration takes place after 8-10 hours of collection. Koss (2006) suggested that specimens with high mucous content, like sputum might be preserved for 12-24 hours, if refrigerated. Sputum is easily accessible as it's obtained by a non-invasive procedure. It's simple to process, cost effective and accurate. In a study by Ammanagi AS. et al., among 13 squamous cell carcinoma patients, sputum cytology was positive for malignancy in 10 cases. Thus, the sensitivity of sputum cytology was 76.9% for squamous cell carcinoma and 40% for adenocarcinoma (22). This level of sensitivity justified this study's objective of describing cytomorphological findings using sputum of individuals with a productive cough.

A total of 106 HIV-infected patients aged 18-75 years who attended KNH CCC and chest clinic during the study period were studied. Those aged 26-35 were the majority;40 (38%). This compared well with similar studies done by Karuga et al. with those aged 31-40 being 42% (16) and Saha et al. where the majority of the patients were 31-40 (43%) years (27).

In this study, 34 (32%) of the patients were unemployed, 72 (68%) were small-scale business persons and in various forms of employment. Studies have reported persistent high unemployment rates among persons living with HIV/AIDS in the era of HAART, ranging from 45% to 65%. This is different from this study majorly due to the empowerment of individuals in developing nations and the rising desire to improve the quality of their lives and therefore a job seeking culture or small business start-ups among a majority.

The most common cytological findings in this study were inflammatory changes and AFB at 8.5% each. This is consistent with Karuga et.al whose study also found Inflammatory changes as the most common cytological change in the sputum of individuals with productive cough (16).

Generally, the shift in the landscape of HIV co-infection with respiratory neoplastic conditions and respiratory infections has changed over time due to the use of HAART. According to this

study, there was a low incidence in the assessed parameters in HIV/AIDS patients; there was no major difference in the incidences between HIV+ve individuals and HIV-ve individuals. There was no correlation between the assessed parameters and the HIV status of the study participants as had been seen in previous studies like one by Kirk GD et al. that found a strong correlation between HIV infection and an increased risk of lung cancer

### 5.1.1 Cytomorphology

In this study, there were no cases of obvert malignancy or suspicious for malignancy. Just like Karuga et al. study that reported 4% ASCUS cases (16), there were 4 (3.7%) ASCUS cases in this study half of whom were smokers and only one of the four was HIV +ve. This similarity can be explained by the fact that a common population (Both studies in Kenya) was sampled in both studies. This however does not concur with a cohort study by Kirk GD. et al. where lung cancer was higher among HIV-infected patients than among HIV-uninfected participants. Smoking is the major etiologic agent of lung cancer and the study found that heavier smoking among HIV-infected persons resulted in higher rates of lung cancer. Importantly, they presented strong evidence that HIV infection contributes to lung cancer, independent of smoking status. After adjusting for individual smoking exposure, they identified a statistically significant ~3.5-fold elevated risk for lung cancer associated with HIV infection (26). This disparity with the cohort study could be explained by the fact that this current study was done at a time HAART use has greatly improved and only 11 (10%) of the sampled participants were smokers. Moreover, the low ASCUS incidence (3.7%) could be explained by the fact that the majority of the study participants in this study were aged 25-40. However, lung cancer is most prevalent among individuals aged >65 which was the least age group (4 patients) in this study (28).

### 5.1.2 Inflammatory changes

Inflammatory changes, the commonest finding in this study; 8.5% - consistent with Karuga et.al, whose study had inflammatory changes as the commonest (16). This was characterized by squamous cell inflammatory changes including metaplasia, binucleation, conspicuous nucleoli and evenly increased nuclear cytoplasmic ratio. The relatively high number of inflammatory changes among these patients could be attributed to infections in the respiratory tract the most common being bacterial pneumonia. It is reported to be 5-15 times more common in HIV-

infected patients than in HIV negative patients, and is the common cause of pneumonia requiring hospitalization in HIV-infected patients (24).

Inflammatory changes also occur due to several reasons that are not specific to a single infection/ complication. They could be due to various pathogens as well as toxins, pollutants or allergens hence they are the most common finding (15).

### 5.1.3 ACID FAST BACILLI

For demonstration of the AFB, ZN method was used in this study as recommended by the World Health Organization (WHO) for screening patients since it is simple and quick at picking most of the infectious patients. Mycobacterium culture is the gold standard method for isolation of tubercle bacilli, but it is time consuming and was not used in this study. Direct sputum smears were done on microscopic slides and ZN staining was done. This method was relatively sensitive as AFB were stained in a number of sputum specimens of the study participants with productive cough and other MTB-related clinical signs.

Nine (9) (8.5) of the 106 study participants tested positive for AFB. This was first time diagnosis for these participants meaning no previous positive ZN test and they were not on anti-TB treatment. Of these nine, five were HIV +ve and four were HIV –ve. According to Lazarus DG et al., the use of HAART has been found to be associated with more than an 80% reduction in the risk of TB. However, HIV-positive patients on HAART remain at high risk of TB compared to HIV-negative patients, and this risk remains appreciable even among those with a good response to HAART (24). This is confirmed in this study as well because the number of AFB positive patients is slightly higher among HIV+ve individuals than among HIV-ve individuals

Kenya is one of the countries identified as having a high TB burden and this explains the relatively high prevalence of AFB in this study though it's lower than other studies; Saha et al. study found AFB cases at 35% in India. Yassin et al. found 19% of AFB out of the studied HIV +ve patients in Ethiopia (29). Other studies in Kenya & SA had AFB at 27-34%. The major cause of the difference in AFB findings between this study and the others is the fact that HAART use has improved and the viral loads of the patients sampled in this study were not as high as those of the participants in the previous studies. Another reason is that this current study didn't sample only HIV positive participants but had HIV negative participants as well.

The fact that endogenous reactivation of TB is common among HIV/AIDS individuals also accounts for a rather high prevalence of TB among HIV/AIDS patients as indicated by Githui W.A et al. (30).

### 5.1.4 Fungal infections

Candida spp. is the only reported opportunistic fungal infection in this study. Seven (6.6%) of the 106 patients had Candida infection. This is in contrast with studies conducted by Karuga et al. in Kenya which had 14% (16), Saha et al. where Candida spp. was found to be 53% and Esebelahie et al. in Nigeria; 52%. Esebelahie et al. analyzed three different types of specimen and the study used microbiological techniques which are more accurate in identifying fungal organisms, unlike this current study that used only PAS staining for the identification. This study reported no cases of PCP though it is a common fungal infection in HIV/AIDS positive patients as reported in other studies. If HIV +ve patients respond to HAART with immunologic improvement, they have a substantially lower risk of developing PCP. However, the effect of HAART on endemic fungal infections of the lung is hard to determine, as the incidence of this infection has never been fully elucidated (24)

Culture is the gold standard in the identification of fungal species thus increasing sensitivity of the test but culture was not part of the methodology in this study. Special stain Periodic Acid Schiff (PAS) was used as an ancillary test to make a diagnosis for fungal infections. This could be the other reason for the lower incidence of candida among the study participants in this study, unlike other previous studies.

### 5.1.5 Bacterial infections

In one study from Johns Hopkins, the use of HAART was associated with a 45% reduction in the risk for bacterial pneumonia (31). In this study, the most common bacteria was Gram positive cocci in clusters with 21 (19.8%) patients having pure infection but 38 (35.8%) patients in total when including mixed infection cases. Gram positive cocci in chains was 20 (18.9%) patients in pure infection but 46 (43.4%) patients in total; in addition to those with mixed infection. Twelve (11.3%) patients had mixed infection of Gram positive cocci in clusters and Gram positive cocci in chains. There also was Gram positive diplococci with 8 (7.5%) patients having pure infection but 23 (21.6%) co-existing with other bacteria. There was one case of Club-shaped bacteria and 2 cases of Gram negative rods. According to a study by Segal LN. et al., the commonest causes

of bacterial pneumonias were *Streptococcus pneumoniae*, then *Haemophilus influenzae* followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa* came fourth (31).

The reason for this variation in bacterial infections identified in the two studies is the method used to identify the bacteria. In the previous study, molecular methods on bronchoscopic material were used while in this current study, Gram staining was done on sputum. Molecular methods are more sensitive and specific.

### 5.1.6 Parasitic infections

In this study, no single parasite or Giemsa-demonstrated microorganism like PCP was isolated from the sputum specimen. This is in contrast to a study by Walker J. et al. that demonstrated PCP cysts and trophozoites with the use of Giemsa stain (32).

### 5.1.7 Mixed infection

Mixed infection was found in two (1.9%) of the 106 samples. The co-infections in this study population were AFB and Candida spp.. Many patients with TB usually have co-infection with Candida species due to increased use of broad-spectrum antibiotics and immunosuppressive drugs. There was also frequent coinfection of most of the other conditions with various bacterial species. This compares with Karuga et al. who also found mixed infections in 4% of the patients (16).

## 5.2 Study limitations

Ideally, for sputum cytological diagnosis, five specimens collected on five consecutive days is considered the ideal optimum for diagnostic accuracy. The KNH guidelines for sputum collection for cytological diagnosis also agrees with this practice. The only limitation in these guidelines is the general fact that sputum won't be 100% accurate as it majorly detects central lesions as opposed to peripheral lesions. For this study, it was difficult to access participants able to report back to submit their specimens every day for five consecutive days. To alleviate this challenge, such that this study's outcome is improved, clear instructions, adequate and optimal specimens were ensured. This was accomplished by ensuring proper sputum cup labelling, a clearly filled-out accompanying request form and giving clear guidelines to the patients. These guidelines included ensuring rinsing of the mouth 4-5 times or brushing of teeth when collecting from home. Then they were to take several deep breaths and then cough up from deep in the

lungs. The coughed-up material (approximately 5mls or one tablespoon) was then expelled into the provided sputum cup then capped securely so there is no specimen leakage.
The likelihood of study participants being able to collect 2-3 containers of specimen was still a major consideration in this study.

### 5.3 Conclusion

Of the positive findings as outlined under study findings, AFB and Inflammatory changes were the commonest findings in this study (8.5%)

3.7% of the findings in this study accounted for ASCUS (4 out of all the 106 study participants and only one was an HIV +ve patient). Lung cancers were therefore uncommon in this study population that comprised both HIV +ve and HIV -ve patients, with productive cough.

Of the atypical findings and infective causes of productive cough categorized in this study, there was no difference in findings between HIV-ve and HIV +ve patients. It was therefore concluded from this study that there was no correlation between cytological findings and HIV status.

### 5.4 Recommendation

Sputum cytology should continue to be the standard of care for patients with productive cough, both HIV positive and HIV negative.

Careful evaluation of sputum for epithelial lesions, although rare, is recommended so that lesions are not overlooked and missed. However, routine screening for intraepithelial lesions is not recommended.

Sputum cytology can also be utilized in the identification of other infectious causes of productive cough just as a complement to microbiological and mycological tests.

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# APPENDICES

## 1.0 Participant explanation and Consent form

My name is Alice Bitengo, a postgraduate student pursuing MSc clinical cytology at the University of Nairobi. I am conducting a research at KNH and here are the details on my study to help you decide whether or not to participate in the study. I will also provide a copy of this form for you. Feel free to ask any questions in regard to your health or any risks associated with the study.

### Research topic

Sputum cytological findings in HIV negative and HIV positive patients at KNH

### Study objective

The study aim is to describe sputum cytological findings in HIV positive and HIV negative patients with productive cough. Sputum cytology is a simple to perform technique, it's inexpensive, non-invasive, and ensures timeliness; short turn-around time.

### The benefit to the participant

You will benefit from this study by early detection of any transformation in the respiratory tract; ruling out malignancies or other infectious causes of the productive cough.

### Study protocol

After consenting, there will be filling in of a well-detailed questionnaire with the assistance of the PI or the research assistant. It will require filling in of personal information and a few health-related details in a simple easily understood language.

You will be provided with two sputum containers in which to make deep coughs of sputum into. You will be advised on how to ensure safety during sputum collection so as no aerosols are spread. You are advised to then tightly cork the containers, place in the provided biohazard polythene bags and we'll receive them. In a biosafety cabinet in the UON microbiology lab, smears will be made on four glass slides and two fixed and two air-dried prior to staining and examination under a microscope. Finally, results will be discussed with your doctor and a copy given to him for filing.

**Risk**

The procedure is non-invasive and no complications are expected to occur.

**Compensation**

No incentives will be given to participate and no monetary rewards will be given for participation.

**Confidentiality**

Utmost confidentiality for all information you will give to us will be maintained. We will use identification codes and not your name. All hand written copies will be kept safe in a lockable cabinet and the soft copies kept safe from unauthorised persons by password protection. Communication of the test results will be from your doctor as you attend the clinic.

**Withdrawal from the study**

Participating in this study is a voluntary decision and if you do not wish to continue with the research, you are free to decline. However, not at the later stages when data collection is complete and data analysis is underway. Your decision will not affect the treatment and care provided to you at the hospital.

**Contact information**

If you have further questions or concerns about the research, you can ask any of the study personnel

Principal Investigator:

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Telephone no. +254725539207

Email address: bitengoa@students.uonbi.ac.ke

**Supervisors**

Prof. Lucy W. Muchiri (+2547)

Dr Joseph Ndung'u (+2547)

For further information about your rights as a participant, you are free to contact;

Prof M. L. Chindia

The secretary KNH/UON Ethics and Research Committee.

Tel 27263 Ex-44102, Email: uonknh_erc@uonbi.ac.ke

I (Name)……. I have had the opportunity to read this consent form. I have discussed the study details with the Principal investigator and have understood the outcomes from participating in this study. I am aware that the researcher will make an effort to keep my identity confidential. Therefore, I can withdraw from this study without it costing me quality clinical services and the care I am entitled to in this hospital. Therefore, I freely give informed consent and voluntarily agree to participate in the study.

Participant signature……. Date…….

Nurse/ Lab technologist (witness)…… Date …….

Principal investigator…… Date……..

**1.1 Maelezo ya mshiriki na fomu ya idhini**

**Mada ya utafiti**: Matokeo ya kitologia ya makohozi miongoni mwa wagonjwa wasio na virusi vya UKIMWI na walio na virusi vya UKIMWI katika hospitali ya Kenyatta.

Jina langu ni Alice Bitengo, mwanafunzi wa kuhitimu anayesomea MSc cytology katika Chuo Kikuu cha Nairobi. Ninafanya utafiti huko KNH na hapa kuna maelezo juu ya utafiti wangu kukusaidia kuamua ikiwa utashiriki katika utafiti huu au la. Pia nitatoa nakala ya fomu hii kwa ajili yako. Jisikie huru kuuliza maswali yoyote kuhusu afya yako au ikiwa kuna hatari zozote unahofia zinazohusiana na utafiti.

Kusudi la utafiti ni kuelezea matokeo ya kitologia ya makohozi miongoni mwa wagonjwa wasio na virusi vya UKIMWI na walio na virusi vya UKIMWI katika hospitali ya Kenyatta. Hili kufanya kipimo hiki, ni rahisi kupata makohozi, si ghali, na inachukua muda usio mwingi kupata matokeo.

**Faida kwa mshiriki**

Utafaidika na utafiti huu kwa kugundua mapema sababu ya kikohozi chenye tija na ikiwa yapo mabadiliko yoyote katika njia ya kupumua; mwishowe kutoa uamuzi iwapo mabadiliko hayo ni kutokana na saratani au magonjwa mengine ya kuambukiza.

**Itifaki ya utafiti huu**

Baada ya kukubali, kutakuwa na kujaza dodoso lililo na maelezo kwa upana kusaidwa na mtafiti mkuu au msaidizi wa utafiti. Itahitaji kujaza habari za kibinafsi na maelezo machache yanayohusiana na afya kwa lugha rahisi inayoeleweka kwa urahisi.

Utapewa vyombo viwili vya makohozi ambavyo unaweza kufanya kikohozi kirefu ndani. Utashauriwa jinsi ya kuhakikisha usalama wakati wa ukusanyaji wa makohozi ili hakuna erosoli zinazoenea. Unashauriwa basi funika kwa kukaza vyombo vizuri, weka mifuko ya nailoni ya sampuli hatari iliyotolewa na tutapokea. Kutoka kwa makohozi, smears zitatengenezwa katika kabati ya usalama wa viumbe katika maabara ya microbiologia ya chuo kikuu cha Nairobi kwenye slaidi nne za glasi. Mbili zilizowekwa kwenye fixative na mbili kukaushwa kwa hewa kavu kisha baadaye uchunguzi chini ya darubini., Mwishowe, matokeo yatajadiliwa na daktari wako na nakala atakayopewa aweke kwa faili yako.

**Hatari**

Utaratibu katika utafiti huu sio vamizi na hakuna shida zinazotarajiwa kutokea.

**Fidia**

Hakuna motisha itakayopewa kushiriki na hakuna thawabu za fedha zitakazopewa kwa ushiriki.

**Usiri**

Usiri kabisa kwa habari yote utakayotupa utadumishwa. Tutatumia nambari za kitambulisho na sio jina lako. Nakala zote zilizoandikwa ngumu zitahifadhiwa salama katika kabati linaloweza kufungwa, na nakala laini iliyohifadhiwa salama kutoka kwa watu wasioidhinishwa na ulinzi wa nywila. Mawasiliano ya matokeo ya kipimo yatakuwa kutoka kwa daktari wako unapohudhuria kliniki.

**Kuacha kushiriki katika utafiti**

Kushiriki katika utafiti huu ni uamuzi wa hiari na ikiwa hutaki kuendelea na utafiti, uko huru kuacha kushiriki. Walakini, sio katika hatua za baadaye wakati ukusanyaji wa data umekamilika na uchambuzi wa data unaendelea. Uamuzi wako hautaathiri matibabu na utunzaji utakaokuwa ukipewa hospitalini.

**Maelezo ya mawasiliano**

Ikiwa una maswali zaidi au wasiwasi juu ya utafiti, unaweza kuuliza wafanyikazi wowote wa utafiti.

**Mpelelezi Mkuu**

Alice Bitengo

Anwani ya mwili P.o Box 2181-00202, KNH Nairobi.

Simu hapana. + 254725539207

Anwani ya barua pepe: bitengoa@students.uonbi.ac.ke

**Wasimamizi**

Prof Lucy W. Muchiri (+ 254722703364 )

Dk Joseph Ndung'u (+ 254722673749 )

Kwa habari zaidi juu ya haki zako kama mshiriki, uko huru kuwasiliana;

Prof M. L. Chindia

Katibu wa KNH / UON Kamati ya Maadili na Utafiti.

Simu 27263 Ex-44102, Barua pepe: uonknh_erc@uonbi.ac.ke

Mimi ( Jina )……. Nimepata nafasi ya kusoma fomu hii ya idhini. Nimejadili maelezo ya utafiti na mpelelezi mkuu na nimeelewa matokeo kutoka kwa kushiriki katika utafiti huu. Ninajua kuwa mtafiti atafanya bidii kuweka kitambulisho changu kuwa cha siri. Kwa hivyo, naweza kujiondoa kwenye utafiti huu bila kunigharimu huduma bora za kliniki na utunzaji ambao nina haki katika hospitali hii. Kwa hivyo, ninatoa idhini ya habari kwa hiari na kwa hiari kukubali kushiriki katika utafiti.

Saini ya Mshiriki……. Tarehe…….

Mtaalam wa Muuguzi / Maabara (shahidi) …….

Mpelelezi mkuu……..

**2.0 Data collection tool: Study questionnaire**

Patient identification: Date: ……

Study number: ……

1. Age: ( )
2. Marital status:
   Single: ( ) Married: ( )
3. Occupation:
   Unemployed: ( ) Employed: ( ) Self-employed: ( )
4. Contacts:
5. Education level
   Primary: ( ) Secondary: ( ) University/College: ( ) Never attended: ( )
6. Do you smoke cigarettes? YES ( ) NO ( )
   If YES, for how long now: ( )
7. Any known underlying disease or long-term condition: ( )
8. Are you currently on any treatment? YES ( ) NO ( )
   If YES, for how long now ( )
9. Any known underlying respiratory condition: ( )
10. Family history of a known respiratory condition: YES ( ) NO ( )
    If YES, which one ( )
11. Period now since the cough started: ( )
12. Phlegm thickness: Salivary: ( ) Mucopurulent: ( ) Bloody:( )

Laboratory technologist: …… Sign:……..

Date: …….

Principal investigator: ……..……. Sign: ……..

Date: …….

**2.1 Zana ya kukusanya data: Hojaji ya masomo**

Kitambulisho cha mgonjwa: Tarehe: ……

Nambari ya utafiti: ……

1. Umri: ( )

2. Hali ya ndoa:

Sijaoa/Sijaolewa: ( ) Nimeoa/nimeolewa: ( )

3. Kazi:

Sina ajira: ( ) Nimeajiriwa: ( ) Nimejiajiri: ( )

4. Nambari ya simu:

5. Kiwango cha elimu

Msingi:( ) Sekondari:( ) Chuo Kikuu/Chuo:( ) Sijawahi kuhudhuria:( )

6. Je, unavuta sigara? NDIYO ( ) HAPANA ( )

Kama NDIYO, kwa muda gani sasa: ( )

7. Ugonjwa wowote wa msingi unaojulikana au hali ya muda mrefu: ( )

8. Je, unatumia matibabu yoyote kwa sasa? NDIYO( ) HAPANA ( )

Kama NDIYO, kwa muda gani sasa ( )

9. Hali yoyote inayojulikana ya msingi ya kupumua: ( )

10. Historia ya familia ya hali inayojulikana ya kupumua: NDIYO( ) HAPANA( )

Kama NDIYO, ipi ( )

11. Kipindi sasa tangu kikohozi kuanza: ( )

12. Unene wa kikohozi: Matemate( ) Kikohozi kizito: ( ) Damu:( )

Mtaalamu wa teknolojia ya maabara: …….

Saini: …….. Tarehe: …….

Mpelelezi mkuu: …….

Ishara: …….. Tarehe: …….

**3.0 H & E staining procedure**

1. After proper fixation in 95% Ethanol, the smears are stained in Hematoxylin for 4 minutes

2. After rinsing in tap water, blue by treatment in scotts' tap water for 30 seconds then rinse in water

3. Decolourize in 0.05% acid alcohol for 30seconds

4. Then stain in 1% Eosin for 1 minute

5. Rinse in 95% Ethanol then dehydrate (in ascending grades of alcohol), clear and mount

**4.0 Papanicolaou staining procedure**

**Regressive Method**

1. Make smears and fix when still wet in 95% Ethanol

2. Hydrate smears by passing through descending grades of alcohol (95%, 70%, 50%).

3. Rinse in distilled water.

4. Stain in Harris hematoxylin for 4 minutes

5. Rinse in H2O

6. Differentiate in 1% acid alcohol (1-5 sec).

7. Blue in Scott’s tap water for 30 seconds.

8. Rinse in 95% alcohol (2 changes)

9. Stain in orange G for 2 minutes

10. Rinse in 95% alcohol (2 changes)

11. Stain in eosin azure for 3 minutes

12. Rinse in 95% alcohol (2 changes)

13. Dehydrate in absolute alcohol ()

14. Clear in xylene

15. Mount in DPX

**5.0 PAS staining procedure**

1. Deparaffinize and hydrate to water.

2. Oxidize in 0.5% periodic acid solution for 5 minutes.

3. Rinse in distilled water.

4. Place in Schiff reagent for 15 minutes (Sections become light pink color during this step).

5. Wash in lukewarm tap water for 5 minutes (Immediately sections turn dark pink color).

6. Counterstain in Mayer's hematoxylin for 1 minute.

7. Wash in tap water for 5 minutes.

8. Dehydrate and coverslip using a synthetic mounting medium.

**6.0 Ziehl Neelsen staining procedure**

1. Make smear on a slide

2. Stain in Carbol Fuchsin for 15 minutes.

3. Rinse slide in $H_2O$.

4. Decolorize in Acid-alcohol for 5 seconds.

5. Rinse slide in $H_2O$.

6. Stain in Methylene blue for 30 seconds.

7. Rinse slide in $H_2O$

8. Blot slide dry with bibulous paper.

**7.0 Gram staining procedure**

1. To the air-dried smears, apply the primary stain (crystal violet) for 1 minute
2. Rinse in water
3. Add the mordant (Gram's iodine) for 1 minute
4. Rinse in water
5. Decolourize in acetone for 20 seconds then rinse in water
6. Counterstain with safranin for 1 minute then rinse in water and allow to dry then mount

**8.0 Giemsa staining procedure**

1. Flood the air-dried smears with filtered Giemsa stain for 15minutes

2. Rinse in water

3. Let to dry then mount

**9.0 Laboratory reporting form for sputum cytology**

**Name of facility: …… Date: ……..**

**Name of patient: …….. Age: ……. Sex: M ( ) F ( )**

**Address: …… Patient no.:**……

**Reason for examination:** Productive cough

**Dates of sputum collection: …….**

| **Date of examination** | **Specimen** | **Visual inspection (S, M, B)** | **Cytomorphological findings (H&E,Pap)** | **ACID FAST BACILLI** | **Fungal organisms (PAS)** | **Bacterial infections (Gram stain)** | **Parasitic infections (Giemsa)** |
|---|---|---|---|---|---|---|---|
| | **I** | | | | | | |
| | **II** | | | | | | |
| | **III** | | | | | | |

- **S – Salivary, M- Mucopurulent, B- Blood-stained**

**Principal investigator Date: …….. Signature: …….**

**Supervisor Date: …….. Signature: …….**

## 10.0 RESULTS REPORTING CRITERIA

### 10.1 Cytomorphology

**Table 12: : Cytomorphology reporting criteria**

| **Diagnosis** | **Microscopy** |
|---|---|
| Unsatisfactory for evaluation | No cellular material obtained |
| Inflammatory changes | Smears which only infectious organisms are identified and no atypia in the line of malignancy |
| Atypical squamous cells of undetermined significance | Epithelial cells or any other diagnostic material present and exhibiting some sort of nuclear atypia that's not outright suspicious for malignancy |
| Suspicious for malignancy | Specimens showing atypical features with a significant malignancy risk |
| Malignant | Specimens with definitive diagnosis of malignancy |

### 10.2 Bacterial infections

Gram stain was performed for identification of bacterial infections as routine cytopathological stains are inadequate in classification of bacteria into Gram positive or Gram negative.

ZN was also done for the identification of *AFB.*

**Table 13: Bacterial infections reporting criteria**

| **Diagnosis** | **Microscopy** |
|---|---|
| Negative for bacterial infection | No organism seen |
| Positive for Gram positive bacterial infection | Gram positive cocci//diplococci/bacilli |
| Positive for Gram negative bacterial infection | Gram negative bacilli/ cocci/diplococci |
| Positive for AFB | Pink rods after ZN staining |

### 10.3 Fungal organisms

PAS staining was carried out to identify fungal organisms in the sputum specimens.

**Table 14: Fungal organisms reporting criteria**

| **Diagnosis** | **Microscopy** |
|---|---|
| Negative for fungal organisms | No yeast, no hyphae |
| Candida spp | Small budding yeast, pseudohyphae |
| *Aspergillus fumigatus* | Hyphal structures with regular branching |
| PCP | Cup-shaped with central dark/ foamy alveolar casts |

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Ref: KNH-ERC/A/48

30th January, 2023

Alice Bitengo Nyagaka
Reg. No. H56/38544/2020
Dept. of Human Pathology
Faculty of Health Sciences
University of Nairobi

Dear Alice,

**RESEARCH PROPOSAL: SPUTUM CYTOLOGICAL FINDINGS IN HIV POSITIVE AND HIV NEGATIVE PATIENTS WITH PRODUCTIVE COUGH AT THE KENYATTA NATIONAL HOSPITAL (P721/09/2022)**

This is to inform you that KNH-UoN ERC has reviewed and approved your above research proposal. Your application approval number is **P721/09/2022.** The approval period is 30th January 2023 – 29th January 2024.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by KNH-UoN ERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to KNH-UoN ERC 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH-UoN ERC within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to KNH-UoN ERC.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

**DR. BEATRICE K.M. AMUGUNE**
**SECRETARY, KNH-UoN ERC**

c.c. The Dean, Faculty of Health Sciences, UoN
The Senior Director, CS, KNH
The Assistant Director, Health Information Dept., KNH
The Chairperson, KNH- UoN ERC
The Chair, Dept. of Human Pathology, UoN
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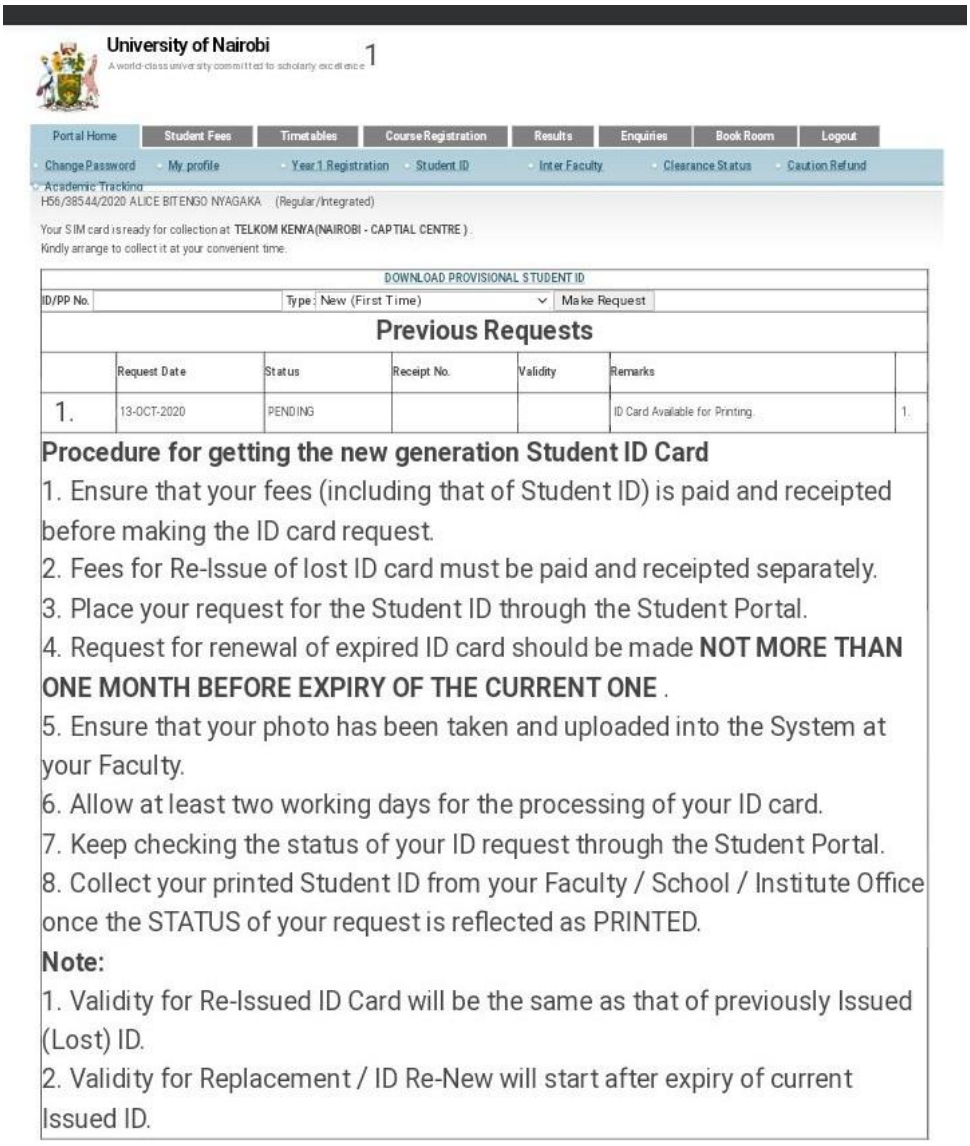

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