# CYTOLOGICAL FINDINGS OF SKIN LESIONS AMONG HIV INFECTED PATIENTS ATTENDING THE COMPREHENSIVE CARE CLINIC AT KENYATTA NATIONAL HOSPITAL, NAIROBI.

By

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**DECLARATION**

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

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**DEDICATION**

I dedicate this dissertation to my sponsor Dr. Tom Albright and his family for encouraging, loving, supporting and believing in me over the years I have spent in my studies. God bless you for the sponsorship you awarded me, you just added to the medical – world a Cytologist; this rare field of study.

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## TABLE OF CONTENTS

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AIDS | Acquired Immune Deficiency Syndrome |
| ART | Anti-Retroviral Therapy |
| BCC | Basal Cell Carcinoma |
| CCC | Comprehensive Care Clinic |
| CMV | Cytomegalovirus |
| CBCL | Cutaneous B cell lymphoma |
| CD4 | Cluster of Differentiation 4 |
| DPX | Di-n-Phthalate in Xylene |
| DNA | Deoxyribonucleic acid |
| ERC | Ethics and Research Committee |
| E.A | Eosin Azure |
| FNA | Fine Needle Aspiration |
| FNG | Fungal |
| HIV | Human Immunodeficiency Virus |
| HAART | Highly Active Antiretroviral Therapy; |
| HSV | Herpes Simplex Virus |
| HPV | Human Papilloma virus |
| H&E | Heamatoxylin and Eosin |
| HHV8 | Human Herpes Virus 8 |
| IRIS | Immune Reconstitution Inflammatory Syndrome |
| KNH | Kenyatta National Hospital |

| | |
|---|---|
| KOH | Potassium Hydroxide |
| KS | Kaposi's sarcoma |
| MRSA | Methicillin-Resistant *Staphylococcus Aureus* |
| MCC | Merkel cell Carcinoma |
| NADC | Non-AIDS-defining cancers |
| NEG | Negative |
| N/C Ratio | Nuclear Cytoplasmic Ratio |
| NSI | Non-specific inflammation |
| OI(s) | Opportunistic Infection(s) |
| O.G | Orange G |
| PAS | Periodic Acid Schiff |
| PPE | Pruritic Papular Eruption |
| SOP | Standard Operating Procedures |
| SPSS | Package for Social Science |
| SD | Standard deviation |
| SCC | Squamous Cell Carcinoma |
| UoN | University of Nairobi |
| Yrs | Years |

## LIST OF TABLES AND FIGURES

## LIST OF TABLES



## LIST OF FIGURES

## LIST OF APPENDICES

**ABSTRACT**

**Background**: According to WHO report of 2011, there were 34.0 million people living with Human immunodeficiency virus (HIV) in the world. Sub-Saharan Africa remains most severely affected, with South Africa carrying the largest burden. In 2012, Kenya showed that approximately 5.6% of the adult population was HIV infected. Cytopathology of skin lesions in HIV infection has been poorly studied in sub-Saharan Africa. HIV infected individuals are living longer due to HAART. The spectrum of cutaneous malignancies has expanded to include both AIDS defining cancers as well as non AIDS defining cancers (NADC).These skin conditions impair patients' quality of life. Using exfoliative cytology, this study highlighted on specific infections, inflammations, ulcerations and neoplasms likely to be encountered in this setting and has also given the current shift in the pattern of HIV related diseases thus improving on patient care.

**Objective:** To describe the cytological findings of skin lesions in HIV infected patients that attended CCC at Kenyatta National Hospitals, Nairobi.

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

**Setting:** Recruitment and sample collection was done at CCC at KNH. Sample processing was done at UON cytology laboratory.

**Study Population:** HIV infected patients in the CCC at KNH who had skin complaints and met the inclusion criteria.

**Method:** One hundred (100) patients were recruited and group counselled on the importance of the study. A structured questionnaire was used to collect socio-demographic information and clinical history. Cytology sampling techniques: touch preparations, FNA and scrapings were used to collect the material from the lesions. Three smears of the

collected material were made. Two were stained with Papanicolaou and heamatoxylin respectively, and the other air dried for special stain. In this study Periodic acid Schiff and Ziehl Neelsen stains were used as warranted. The principal investigator examined the preparations then reviewed them with the pathologists. A tie breaker was sought for controversial cases.

**Results:** All participants were black. The female to male ratio in this study was 3:1 (73% & 27% respectively. The peak age group was 41 to 50 years (36%) with mean age of 41.47yrs, (SD of 11.46). Majority hailed from Nairobi County. Cytological findings; 41% were negative for cytological lesions, 32% were inflammatory (Nonspecific inflammation 21%, viral 6% and 5% fungal), 5% were malignant (KS 2% and SCC 3%) and for the purpose of analysis 22% were grouped as others. There was a significant difference on skin cytology and clinical findings at 95% confidence interval because the p value was less than 0.001 and the chi square statistics was 1089. There was no evidence of significant difference in cytological findings of patients on and not on HAART.

**Conclusion:** The commonest cytological changes were inflammatory. Malignant skin lesions are rare presentations in HIV infection among the studied population.

**Recommendations:** Cytology is a valuable, affordable and a quick diagnostic tool that should be incorporated in the routine management of HIV-related skin lesions.

## 1.0 INTRODUCTION

In 2011, World Health Organisation (WHO) reported that there were 34.0 million people living with HIV in the world, up from 29.4 million in 2001, this has resulted to continuing new infections (1). Although the burden of the epidemic continues to vary considerably between countries and regions, Sub-Saharan Africa remains most severely affected, with nearly one in every twenty adults (4.9%) living with HIV and accounting for 69% of the people living with HIV worldwide (2). South Africa is the leading country with HIV infection in Sub-Saharan Africa, followed by Nigeria and Kenya appearing third. In 2012, Kenya showed that approximately 5.6% of the adult population is HIV infected (3).

Skin manifestations are frequently the first sign of HIV infection and approximately 90% of individuals with HIV/AIDS are diagnosed with skin disease at some point during the course of their illness (4). A study carried out in India by Harminda et al found a prevalence of 87.6% dermatological disorders. Skin diseases contribute a large proportion of morbidity in these patients and HIV itself is also associated with increased morbidity and mortality (5, 6). There is a great over-lap between HIV infection and skin diseases, for a plethora of neoplastic, infectious, and reactive conditions occur on the skin throughout the course of HIV disease. The increased number and severity of these skin disorders is associated with the declining immunity, drug reactions and they may also arise from opportunistic infections secondary to the declined immunity (7).

Skin diseases of HIV infected patients has been poorly studied in sub-Saharan Africa, where the burden of the infection is high and where more than two-thirds of all HIV positive people in the world live (8). A recent study carried out in Nigeria (2011) by Salami et al found that 5.18% of

HIV infected patients had skin problems. The prevalence of cutaneous manifestations however, appears to be higher in other African countries. For example, Mohammad et al reported a prevalence of 41.7% among HIV positive police officers in Dar-es-salaam. It is however lower than the 68.8% observed by Josephine et al, (2006) in Cameroon. Unlike in Nigeria and that conducted in Tanzania, the Cameroon study population included children.

When compared to prevalence studies done outside Africa, the prevalence is much higher. In India, skin diseases affect 80% to 98% of HIV infected persons as reported by Singh et al, in 2009 and Sharma et al, in 2004. Reasons attributed to the differences in prevalence may include different study designs; the socio economic status; the prevailing environmental conditions of different countries and the HAART status of patients under study.

Patients' life expectancy has been prolonged after the advent of the HAART and skin cancers are becoming more frequent. Cancer is a significant cause of morbidity and mortality in people infected with HIV; in fact 30% to 40% will develop a malignancy during their lifetime (9). Moreover, the spectrum of cutaneous malignancies encountered has expanded to include both AIDS defining cancers as well as non AIDS defining cancers (NADC).The majority of cancers affecting HIV positive people are AIDS defining for example Kaposi's sarcoma (KS) (4). Increased use of HAART has led to a decrease in the incidence from 15.2 per 1000 patient-years to 4.9 per 1000 patient-years. Prior to the widespread use of HAART, KS was the most common malignancy in HIV infected patients. In the early 1980's, KS was the AIDS defining illness in approximately 30% of infected individuals; this later dropped to 15% in the late 1990's. Furthermore, the incidence rates for KS are five times lower in HIV infected patients who have received HAART compared to those patients who have not (9).The standardized incidence ratio

is that people with HIV are 3,640 times more likely to develop KS than the general population (10).

In most cases, HIV associated skin diseases can be easily recognized on clinical grounds. When diagnostic difficulty is encountered, prompt diagnosis can often be established through careful cytological and histological examination of clinical specimens although microbiological tests remain vital (11). The most common approaches to skin lesions are punch biopsies and shave biopsies, but cytological methods have also been used (4). Cytology is simple, fast, inexpensive, non-invasive and reliable method of diagnosing many skin diseases (12).

The aim of this study is to describe the cytological findings of skin lesions in HIV infected patients.

## 2.0 LITERATURE REVIEW

### 2.1 NORMAL CYTOLOGY OF THE SKIN

The skin consists of keratinized squamous epithelium and the underlying dermis with connective tissue and appendages. Maturation is from nucleated basal, spinous, granular and a nuclear coneocytes'. Normally, the only cells that exfoliate from the surface are anucleate squames. A few inflammatory cells, such as lymphocytes, histiocytes, and mast cells maybe be present (13). All the components of the skin may be affected either by inflammatory or neoplastic disorders that may be either benign or malignant (14).

### 2.2 COMMON SKIN INFECTIONS.

#### 2.2.1 Viral

Common viral cutaneous infections in skin of HIV infected patients include: herpes simplex (HSV), herpes zoster, *molluscum contagiosum, cytomegalovirus* (CMV), warts, verruca vulgaris, and hairy leukoplakia. In a study done by Salami et al, in Nigeria (2011), viral skin conditions accounted for the biggest share of the cutaneous pathology at 37.1% with Herpes Zoster and plain viral warts accounting for the highest cutaneous viral lesions at 27% and 15.4% respectively of the viral skin manifestations. Also Josephine et al study, herpes zoster was the highest viral skin condition with 28.1% among their cohort of patients. Herpes zoster is also the commonest viral skin pathology reported from patients in Togo and Dar-es-salaam (8).

Although cytological examination has low sensitivity (approximately 60-70%), (15) skin scraping of a lesion or a Tzanck preparation may reveal cytological appearances characteristic of herpes virus infection, such as multinucleated giant cells and epithelial cells containing intranuclear inclusion bodies (14).

*Varicella zoster virus* infection can occur at any stage. The incidence is usually higher among patients with low CD4 cell counts and during the four months after initiating potent antiretroviral therapy (3). The cytological features include characteristic intra-nuclear inclusion bodies with multinucleate giant cells, also known as Tzanck syncytium, nuclear moulding, margination of the chromatin and ballooning degeneration of nuclei (14). Both verruca vulgaris and condylomata acuminata are common in HIV disease (16). In cytologic studies, warts show numerous parakeratotic cells with or without intranuclear inclusions and koilocytic changes (13).

Although systemic CMV infection is common in HIV infected patients, mucocutaneous lesion is rare and usually occurs as genital ulcers which can be associated with herpes. Molluscum contagiosum is caused by pox virus. The exact incidence of molluscum in HIV infected persons has not been documented yet, but some studies have estimated that 5 to 18% of untreated HIV infected patients develop molluscum lesions at some point in their clinical course (13). Smears of these lesions are easily obtained by squeezing the lesion and expelling its core on a slide (4). Cytology smears show "molluscum bodies" which are large, rounded, homogeneous cytoplasmic inclusions, measuring up to 35 μm in diameter that completely fill the cell, pushing the pyknotic nucleus to the periphery (13).

### 2.2.2 Fungal

Common superficial fungal infections such as, tinea pedis, tinea corporis, cryptococcosis histoplasmosis and onychomycosis are common in HIV infected patients (17). In recent years, the prevalence of dermatophytosis in HIV patients has been observed to be increasing hence, it is important for dermatologists to have a good knowledge of cutaneous manifestations of fungal infections in those infected with HIV (8). In Salami et al, study (2011) fungal infections

appeared second after viral infections with the main fungal pathology being Tinea corporis occurring in 52.9% of patients followed by seborrhoeic dermatitis occurring in 23.5% of the patients. The prevalence of Tinea corporis found in this study is similar to the 53.7% reported by Kaviarasan et al, and 21% seborrhoeic dermatitis reported by Sivayathorn et al. Dermatophyte infections can be readily diagnosed based on the history, physical examination, and potassium hydroxide (KOH) microscopy of the cornified cells (squames) scraped from the surface of a lesion. (14) The smears may be inspected unstained or stained with methylene blue or other rapid stains.

### 2.2.3 Bacterial

Bacterial skin infections caused by *Staphylococcus*, bacillary angiomatosis, tuberculosis, *M leprae* and syphilis are extremely common in HIV infection. In Nigeria (2012), Ukonu et al, found the prevalence of bacterial infection to be 2.5 % (18). This is similar to Salami et al study (2011) carried out in the same country which found a prevalence of 2.9 % in bacterial infection (8). Tuberculosis may present as Lupus vulgaris ulcers and diagnosis can be achieved from scrapings.

Bacillary angiomatosis is an infectious disease of skin and viscera characterized by angiomatous lesions. Histology shows lobular capillary proliferation. Warthin-Starry stain demonstrates clumps of bacilli. Syphilis is being identified in the gay and HIV populations (19). Salami et al, study (2011) found syphilitic infection to be the most common bacterial infection. Syphilitic cytological smears are analysed using silver stains or dark back ground microscopy for the presence of spirochetes (14).

### 2.2.4 Arthropod

In early HIV disease, scabies has a classic presentation with erythematous, pruritic papules and burrows in the finger webs, wrists, and anogenital region. Cytological material is acquired by squeezing material from the burrows and examining directly for the mites or their excreta (20). A study carried in Nigeria by Ukonu et al, (2012) found the prevalence of parasitic infestations to be 2.2 % with scabies leading with 1.1% (18). However in Atraide et al, study carried out in the same country, onchocerciasis was the commonest with prevalence 0.7% (21).

## 2.3 COMMON INFLAMMATORY DISORDERS

The skin is the site of numerous inflammatory conditions but cytologic approaches to diagnosis of this group of diseases have not been explored, except for some infectious disorders. Common inflammatory conditions include: xerodema ichthyosis, seborrhoeic dermatitis, pruritic papular eruption, eosinophilic folliculitis, psoriasis, atopic eczema. Eosinophilic folliculitis is the most common pruritic follicular eruption that mainly affects adult HIV infected men having sex with men. Histological examination shows a mixed inflammatory infiltrate with a predominance of eosinophil and lymphocytes surrounding and invading the follicular and sebaceous epithelia, resulting in destruction of sebaceous gland (14).

Early in the HIV epidemic, it was clear that pruritus was a marker of HIV infection (22). Many cases have been labelled as "pruritic papular eruption"(PPE) especially among HIV infected Africans, residing in regions with high mosquito densities. The prevalence of PPE has been estimated to range from 12 – 46% and has reached as high as 58% in countries neighbouring Kenya (21). However, a recent Kenyan study (2013) by Ramadhan et al, found only 20 (5%) HIV infected patients with PPE (23). Although this prevalence appears to be extremely low, it is comparable to that observed by Budhavari et al, (2007), in South Africa which found a PPE prevalence of 6.9%. Recent studies suggested that PPE is seen more commonly among patients

on HAART probably occurring as part of an immune reconstitution syndrome (23). Only 1–3% of the general population have seborrhoeic dermatitis. Seborrhoeic dermatitis occurs in 20–85% of patients with HIV infection (20). In a study by Harminder et al, Seborrheic dermatitis which had a prevalence of 74.2% was the most common dermatological disorder (5).

## 2.4 COMMON BENIGN NEOPLASMS

Bullous conditions associated with blistering maybe the best suited to cytologic examination, particularly because the fluid within the vesicles can be easily obtained. Blistering conditions include pemphigus vulgaris and pemphigoid, autoimmune conditions, which contains atypical parabasal squamous cells, which are round single cells with a perinuclear halo and a prominent nucleolus. It is preferable to sample the base of such lesions and to do so in the early stages of presentation to avoid the degeneration or secondary bacterial infection that may occur. Giant cells may be present and the cytologic atypia seen may mimic that of a malignancy or herpes (14).

## 2.5 MALIGNANT TUMOURS.

### 2.5.1 AIDS defining

In the course of HIV infection 30% to 40% of patients will develop a malignant lesion (9). HAART prolongs patient's life expectancy making skin cancers become more prevalent (10). Majority of cancers in HIV infection are AIDS defining for example Kaposi's sarcoma and non-Hodgkin Lymphomas (4).

In a study by Salami et al in Nigeria (2011) Kaposi sarcoma accounted for the bulk of neoplasms with a prevalence of 5.7% while an unpublished Kenyan study by Rogena et al, from 2001 to 2011 KS had a prevalence of 48% was found (36). However, this is different from Ramadhan et-al study who found only one (0.25%) KS (23). KS prevalence in Kenya appears to be extremely

low compared to 1.6% in Tanzania found by Mohammad et al, (2003) and 9.9% in Cameroon found by Josephine et al, (2006).

While most clinicians today perform biopsies for KS, FNA has previously been shown to be useful in the diagnosis of KS (6). Cytologically, KS is characterized by moderately cellular and bloody specimens, overlapping spindle cells, and ill-defined cytoplasmic borders. Nuclear crush artefact can be present. Smaller groups of loosely cohesive spindle-shaped cells and individual spindle cells with cytoplasm are also seen. The cells might display cytoplasmic vacuoles, cytoplasmic hyaline drops, and hemosiderin granules. The nuclei are fusiform to cigar shaped with inconspicuous nucleoli and fine chromatin pattern. Red blood cells are frequent (14).

Lymphomas are more common in people with HIV infection. Skin is one of the common sites for extra-nodal lymphomas in patients with AIDS with T cell type being more common than B cell type. In a case study carried out in Brazil (2010) by Luis De Carolis *et al* presented five patients with cutaneous involvement in the setting of HIV/AIDS disease. Two of them were primary cutaneous non-Hodgkin lymphomas. All were Cutaneous B cell lymphoma (CBCL); three were immunoblastic, one was plasmablastic, and the other was a Burkitt's lymphoma (24).

### 2.5.2 Non AIDS defining

Non AIDS defining cancers like Squamous cell carcinoma (SCC), basal cell carcinoma (BCC) and Merkel cell carcinoma (MCC) are becoming more common. In the Wendy et al, study cohort of 6,560 HIV-positive and almost 37,000 HIV negative subjects drawn from members of Kaiser Permanente Northern California from 1996 to 2008, 0verall HIV positive subjects had a 2.1-fold higher risk for basal cell carcinomas (BCC) and a 2.6-fold higher risk for squamous cell carcinomas (SCC), compared to HIV negative subjects. In Ukonu study (2012), a prevalence of 0.3% in BCC and SCC and 0.1% melanoma was found (21).

SCC cytological features show presence of pleomorphic neoplastic cells arranged in clusters and single cells. The clusters do not display palisading as seen in basal cell carcinoma. The neoplastic cells have hyperchromatic nuclei, and irregular chromatin distribution. The cytoplasm might be keratinized and form intercellular bridges and squamous pearls. Small, anucleated, keratinized cells may also be found (12).

Basal cell carcinoma tumour is characterized by slow, but relentless growth, but only rarely metastasizes (14). The cytologic examination shows large, tight clusters of crowded atypical, basaloid cells, with very high N/C ratio (13). Some clusters have smooth borders along the edges. The nuclei are small, round to oval, hyperchromatic, and do not mould. Nucleoli are usually inconspicuous, but can be prominent. Mitotic figures can be present, but are not atypical. Atypical bowenoid nuclei are occasionally present. Basal squamous whorls, similar to pearls, may be seen. Pigmented lesions demonstrate cytoplasmic melanin pigment in tumour cells and macrophages. Occasional cases have a small number of cells containing neurosecretory granules. Pink amorphous material is often present in the background (24).

Merkel cell carcinoma cytologic preparation shows cellular specimen with small to medium sized cells in a predominantly single-cell pattern; Uniform round to oval nuclei with delicate nuclear membranes and finely granular chromatin pattern, scant cytoplasm, and frequent mitotic figures and individual cell necrosis (12).

### 2.5.3 Metastatic cancers

Metastatic cancers are likely to increase in HIV infected patients (8). The most common sites of origin include the lung, breast, melanoma, squamous cell carcinoma, and gastrointestinal tumours. They can present as either ulcerated or subcutaneous tumour masses and might be the

initial presentation of the disease. The morphologic features are similar to those of the primary site, although the yield might be low (12).

## 2.6 JUSTIFICATION

HIV/AIDS incidence has a paralleled increase in infectious, neoplastic and reactive skin conditions. These HIV related diseases are a major public health burden and decrease quality of life in patients. As is common with many Sub Saharan Africa countries, Kenya too suffers a high HIV burden. In any given community, describing the patterns and distribution of HIV related skin diseases is important for planning appropriate skin care of HIV infected patients.

Although HIV/AIDS is endemic in Kenya, it is not clear what the burden of skin disease is since largely this data on HIV related skin diseases is unavailable. No cytology based studies have been carried out in our set up. Therefore a cytology based study will help in describing the patterns and distribution of HIV related skin diseases. Punch or shave biopsies are the most common approaches that have been used on skin lesions. However, cytological methods such as FNA, touch preparations and scrapings can also be of great importance in diagnosis of cutaneous lesions. Cytology, which is simple, fast, inexpensive, non-invasive and reliable diagnostic method, could be extremely beneficial in situations where even a simple 2 mm punch biopsy may be considered inappropriate as in highly vascular or haemorrhagic lesions. The aim of this study is to describe the cytological findings of skin lesions in HIV infected patients.

## 2.7 RESEARCH QUESTION

What are the cytological findings of skin lesions in HIV infected patients attending CCC at KNH?

## 2.8.0 0BJECTIVES

### 2.8.1 General objective

To describe the cytological features/findings of skin lesions in HIV infected patients attending CCC at KNH.

### 2.8.2 Specific Objectives

**Primary**

1. To describe the cytological patterns of skin lesions in HIV infected patients attending CCC at KNH.

2. To compare the cytology of skin lesions in HIV infected patients attending CCC at KNH with clinical diagnosis.

**Secondary**

1. To compare cytological findings of skin lesions in HIV infected patients on and those not on HAART.

## 3.0 MATERIALS AND METHODS

### 3.1 STUDY DESIGN

A descriptive cross-sectional cytological study on skin disorders in HIV infected patients attending CCC at KNH.

### 3.2 STUDY AREA

The study was conducted at Kenyatta National Hospital (KNH),Comprehensive Care Clinic (CCC) and the University of Nairobi/KNH Cytology Laboratories. KNH is the largest teaching and referral hospital in the country and has an established care unit for HIV patients. The Comprehensive Care Centre in KNH has been providing comprehensive care and treatment to HIV positive clients since 2002. It is a public referral and teaching hospital; one of only two in the country. It has since grown to a client base of 10,583 patients as of September 2011. The clinic enrolled 1,103 new patients between September 2010 and September 2011, 682 (62%) females and 421 males (38%), translating to about 93 new patients per month. The clinic provides care to over 8,000 HIV infected patients of whom female patients account for 60%. The daily patient flow is an average of 100 patients. The services offered in the CCC include anti-retroviral therapy provision for those who are eligible, identification and treatment of opportunistic infections and counselling and nutrition support for the patients.

### 3.3 STUDY POPULATION

One hundred HIV infected patients (adult and children) who had skin complaints and met the selection criteria, children who gave assent and parents/guardians who agreed to give consent.

## 3.4 STUDY DURATION

The study was carried out for one month (March 2014 -April 2014).

## 3.5 SELECTION CRITERIA

### 3.5.1 Inclusion criteria

1. Adult male and female HIV infected patients who had known and emerging skin lesions and gave an informed consent.
2. HIV infected children who had known and emerging skin lesions and gave assent. (13-17 years) and whose parents/ guardians gave consent.

### 3.5.2 Exclusion criteria

1. HIV infected patients who declined to give consent.
2. Individuals who had skin cancer before they were diagnosed with HIV infection.

## 3.6 SAMPLE SIZE CALCULATION

Samples size was calculated using prevalence of 5.18% obtained from a study done in Nigeria (2011) by Salami et al. (8)

The Fisher's formula was used.

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

Where;

n= is the required sample size

Z= Z statistic for a level of confidence or normal distribution critical value set at 1.96 which corresponds to 95% confidence interval

P=expected prevalence or proportion estimated to a particular characteristic 5.18% of population

$d^2$=degree of precision. This study used 0.1degree of precision.

$$n = \frac{1.96^2 \text{x } 0.518 \text{ x } 0.482}{0.1 \text{x} 0.1}$$

n=95.915532

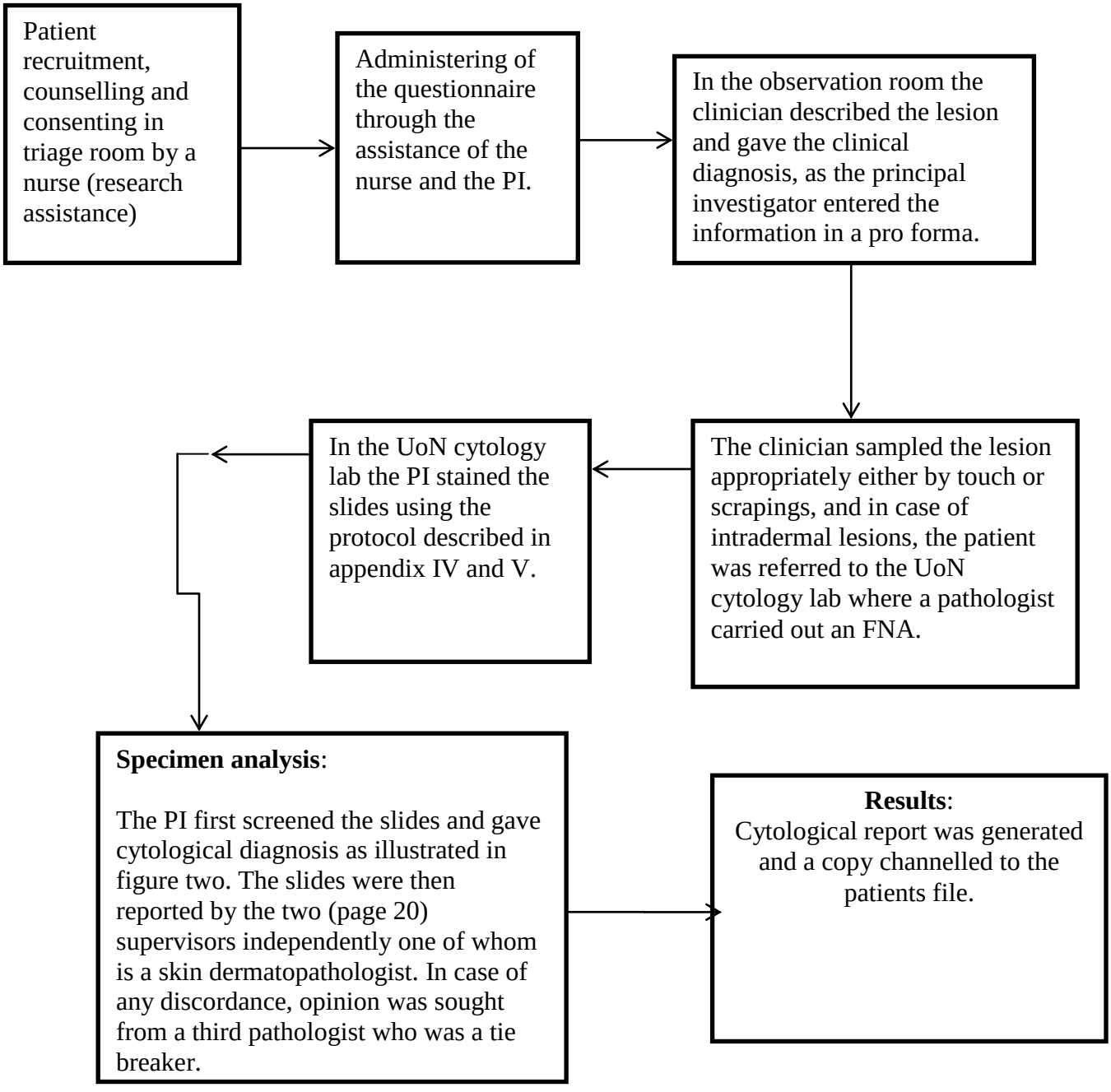
n= 96

However, for this study, due to time and financial constraints a lower degree of precision set at 10 % was used. This being a descriptive study a minimum sample size of thirty (30) is sufficient.

### 3.7.1 **RECRUITEMENT, SPECIMEN COLLECTION, ANALYSIS AND CYTOMORPHOLOGY INTERPRETATION**

### 3.7.1General procedure for recruitment and specimen collection

Figure 1



### 3.7.2 Participants recruitment, specimen collection and processing procedure

At the triage room of the CCC, potential participants were recruited and group counselling done on the importance of the research. They were then assisted to voluntarily give consent/assent by the research assistant (nurse). The participants were then interviewed and their demographic information entered in a preformatted questionnaire by the Principle investigator (PI). All patients' notes were treated with confidentiality. Each participant was given a specific research number. A tag was put on the patient's file as a sign of identification. The identified patient was seen by the clinician and then directed to a specific room where the sample was collected. The clinician described the lesion and gave a clinical diagnosis as the principal investigator entered this information in a pro forma. The lesion was then sampled appropriately either by touch or scrapings or in case of intradermal lesion; the patient was referred to the UoN cytology lab where the pathologist carried out an FNA. Three smears were made, two fixed in 95% alcohol and one air dried. One smear was stained with PAP stain and the other with H&E stain as stipulated in appendix IV and V. The air dried smear was stained with special stains to assist reach a definitive diagnosis when needed. The principal investigator first screened the slides and gave a cytological diagnosis as illustrated in figure two. The slides were then reported by the two supervisors independently. For controversial cases, opinion was sought from a third pathologist who was a tie breaker. The final report was written and a copy channelled to the patients file.

### 3.7.3 Touch preparations

For lesions that were sampled using touch preparations, a normal saline swab was used to wipe the lesion prior to making of the smear .Three slides were made; two were fixed immediately in 95% alcohol and stained with Papanicolaou and Haematoxylin and Eosin staining respectively,

and one air dried for special stains. Periodic acid Schiff (PAS) was used to stain cases where fungal organisms were suspected and Ziehl Neelsen (ZN) was used for suspected mycobacterium.

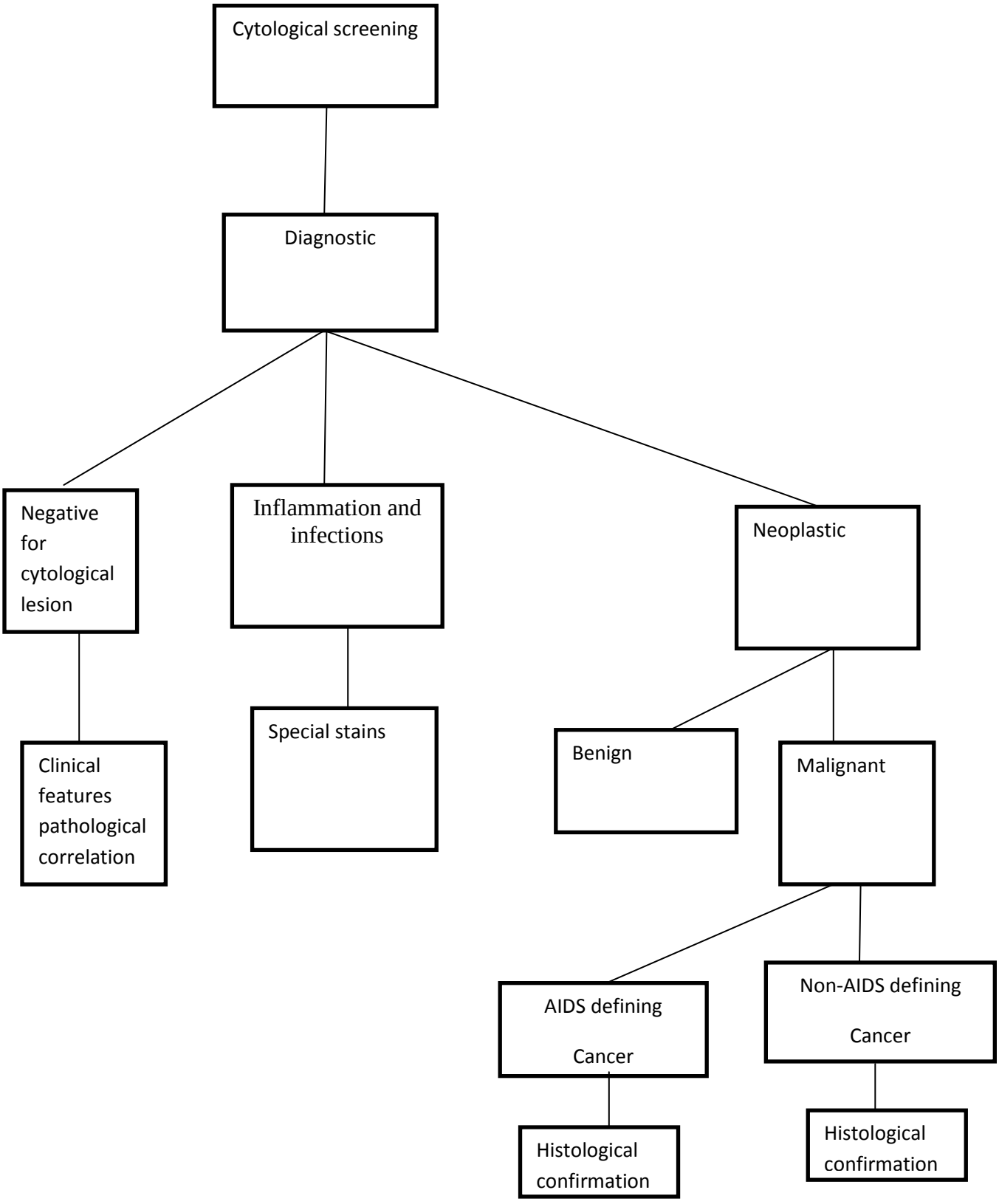
### 3.7.4 Scrapings

Lesions with skin surface involvement were evaluated by scrapping. (12) A simple scalpel blade was used to scrape the surface of the lesion until material appeared on the blade's surface. The material was then smeared thinly across a slide. Three slides were made, two fixed immediately in 95% alcohol and stained with Papanicolaou and haematoxylin staining respectively and one air dried for special stain.

### 3.7.5 FNA

Intradermal lesions forming masses were referred to the UoN cytology lab. The pathologist or registrar on duty performed FNA using a small calibre needle (gauge 23). Obtained material was smeared onto three slides; two were fixed immediately in 95% alcohol for Papanicolaou and Haematoxylin and Eosin staining and one air dried for special stains.

**Figure 2: Cytological Screening**



### 3.8 QUALITY ASSURANCE

Prior specimen collection, slides were well labelled with the patients study number using a pencil on the frosted end. A normal saline swab was used to wipe the lesion to reduce contamination. For skin scrapings, slides were first immersed in a container filled with 95% ethyl alcohol, using a scalpel blade, the samples were obtained from the edges of the surface of the lesion in order to obtain a more representative sample, of suspected fungal organisms, then a smear was done on the prefixed slide and immediately re-immersed in the fixative For wet lesions, care was taken not to distort the cells by touching the lesion gently using a slide and avoiding dragging of the slide across the lesion. FNA's were taken by experienced pathologists who do the procedures on day to day bases. The specimens were then transported to the laboratory and in order to avoid compromising the diagnostic integrity of specimens and diagnostic material being washed away, especially for skin scrapings, the slides were removed from the fixative and air dried until when enough batch for staining was reached The specimens were processed and all reagents prepared at UoN cytology laboratory in accordance with Standard Operating Procedures used in the laboratory. Stains were filtered before each use and deteriorated stains discarded and replaced where necessary. On microscopic examination, the slides were first screened by the principal investigator, and then signed out together by the two supervisors (one of whom is a skin specialist) independently. For discordant cases, a third senior and experienced pathologist was sought to break the tie.

### 3.9 BIOSAFETY

Detailed protocols and systems for infection control specific to the CCC were adhered to during the entire period of the study. They included:

- Good personal hygiene practices ( use of skin disinfectants, washing and drying hands before and after every patient)
- use of personal protective equipment ( which included wearing of gloves, lab coats and face masks)
- There was safe use and disposal of sharps as well as contaminated wastes.
- Smears were fixed immediately to immobilize any infectious micro-organism and minimize the risk of transmission of infectious diseases.

### 3.10 ETHICAL CONSIDERATION

Permission to conduct the study sought from KNH/UON ERC. Relevant information about the study: the title, objective of the study, type of specimen, benefits, risks, treatment, voluntarism, follow up, researcher information and any other information were provided to the patient to help them make an informed decision whether or not they wanted to participate voluntarily. Those that chose to participate were required to sign a consent form before they took part in the study. For those who cannot read and write, the information was read to them and they were required to put a thumbprint under a witness. Individuals below 18 years were requested to assent before the sample was collected. Data collected from the questionnaires and in hard cover register was kept in lockable cabinets where only the researcher had access, to maintain confidentiality. Information stored in soft copies was protected from access from unauthorized persons by a password. All records were identified by study ID numbers only to maintain participant confidentiality; subjects' names and other personal identifiers were not disclosed to any person apart from to the physician seeing the patient. A result was sent to the clinician through the patients' files to inform further clinical management. Open biopsy was recommended in cases where malignancy was suspected.

### 3.11 DATA MANAGEMENT

Data was coded, entered and managed in a predesigned Microsoft Access database. Entries were done continuously in the course of the study and data cleaning was performed and analysis was done using SPSS version 20.0.software.

The characteristics of the participants were summarized into means/medians and proportions for continuous variables and categorical variables respectively. Categorical variables were compared using Chi square. Results were then presented in graph, charts, percentages and tables. A p-value of **<0.05** was considered significant. At the end of the study, all the specimens and data will be stored as per regulations practiced by the Department of Anatomic pathology University of Nairobi after which it will be destroyed.

***Variables***

- **Independent variables -**Age, Gender, HIV status, Occupation, Residence, Use of HAART.

- **Dependent variables –** Cytological findings.

## 4.0 RESULTS

### 4.1.1 Demographic characteristics

A total of 109 eligible participants were enrolled into the study. Nine patients were excluded from the study due to inadequate specimen sampling. The remaining 100 patients met the inclusion criteria and were evaluated for the requirements of the study. of these 73% were female and 27% were males giving a female: male ratio of 3:1 (Table 1) More than a half of the population (58%) had secondary level of education, 73% were residents of Nairobi county and the other 27% from other counties. There were two main occupation types, business 31% and employment 32%. A few were students (4%) and the majority were unemployed 33%.

| **Gender** | **Frequency (n=100)** |
|---|---|
| Male | 27 |
| Female | 73 |
| **Age group** | |
| <20 | 7 |
| 21-30 | 11 |
| 31-40 | 30 |
| 41-50 | 35 |
| 51-60 | 12 |
| >60 | 4 |
| **Residence** | |
| Nairobi | 73 |
| Non Nairobi | 27 |
| **Education level** | |
| Non formal | 4 |
| Primary | 15 |
| Secondary | 59 |
| Tertiary | 22 |
| **Occupation** | |
| Student | 4 |
| Business | 31 |
| Employed | 32 |
| Unemployed | 33 |

| | |
|---|---|
| **Treatment** | |
| Patient on HAART | 90 |
| Not on HAART | 10 |
| **Duration of HAART** | |
| >1 | 15 |
| 1-5 | 50 |
| 6-10 | 23 |
| >10 | 2 |

**Table 1: Demographic characteristics of study population (n=100)**

### 4.1.2 Gender and Age distribution

Majority of participants were female with 73% and male 27%. Female male ratio= 3:1.

A pie chart showing the percentage of female and male.

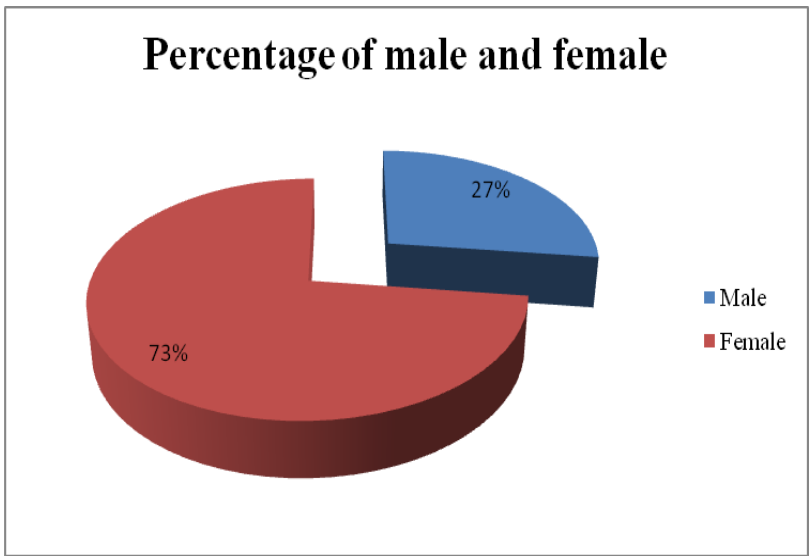



**Figure 3: Percentage of female and male (n=100)**

Peak age groups; 41 to 50 years (36%), followed by 31 to 40 (30%). Among the participants, children accounted for 7 %. Those aged between 21 to 30 years had 11%, a percentage that was very close to 12% accounted by those aged between 51 to 60 years. Only a few patients (4%) were of greater than sixty (60) years of age. Age range: 62 years, Mean age: 41.47 (SD +/_11.46) a Median: 41 and Mode: 40.

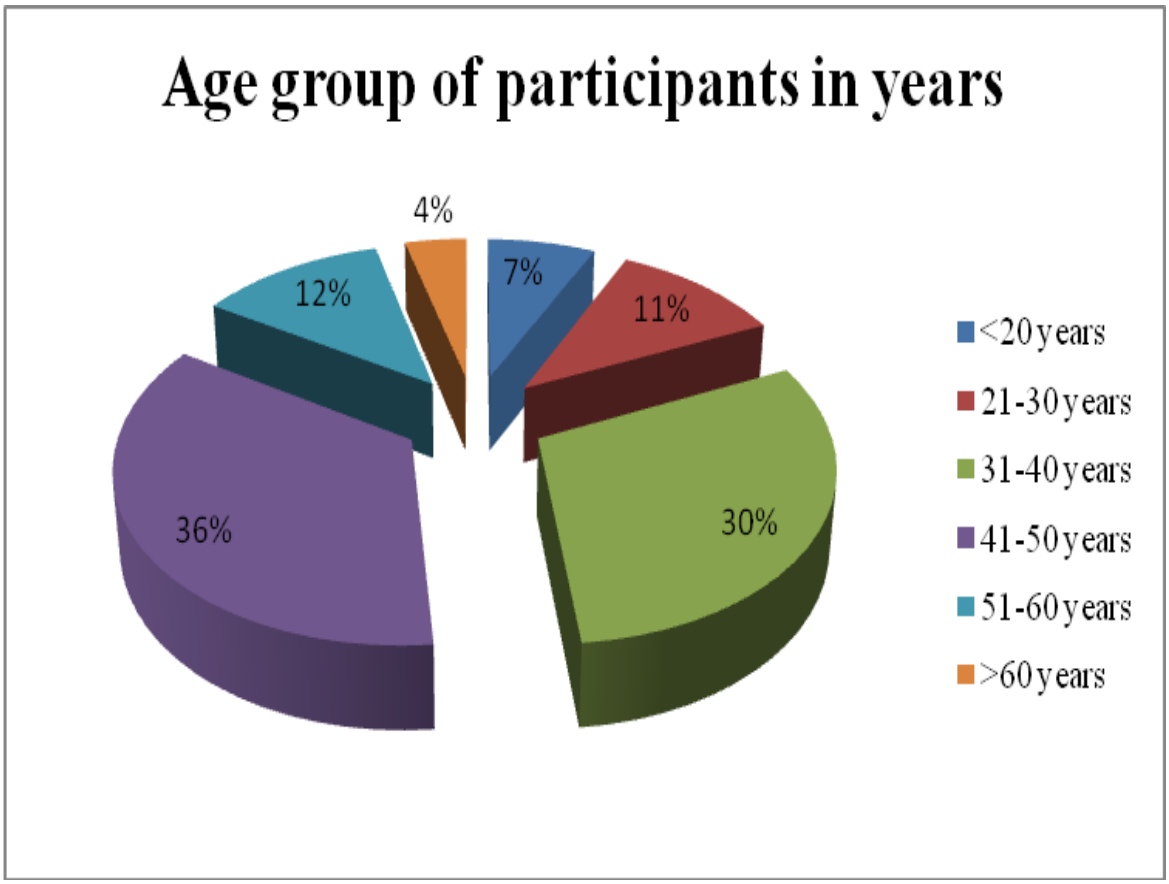



**Figure 4: Age (years) group of participants in years (n= 100)**

In all age groups female were the majority. Between the two peak age groups (31 to 40 and 41 to 50), the number of females and males participants were almost the same. In the age group between 21-30 years no male patient participated in this study.

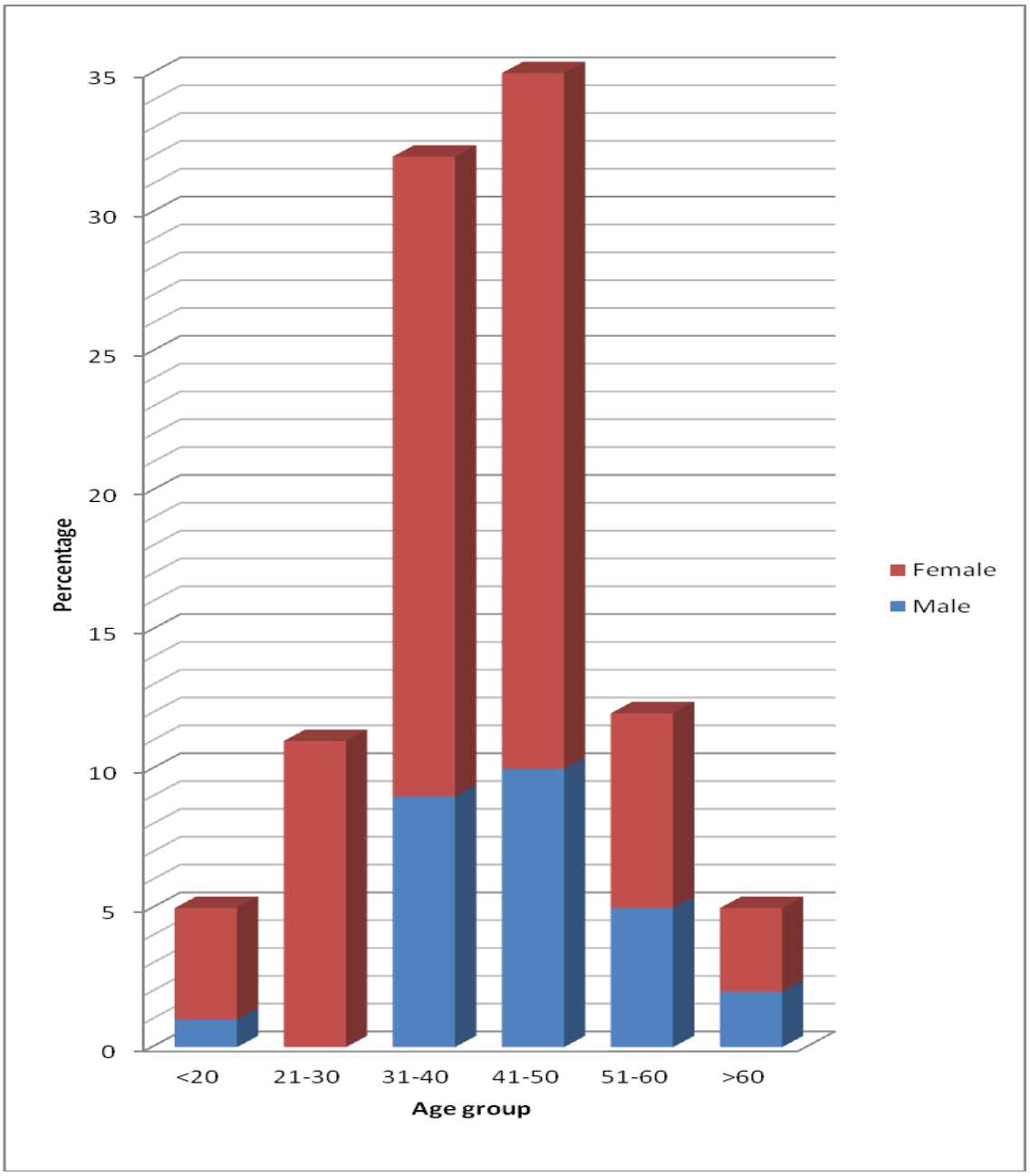



**Figure 5: Age group distribution against gender. (n=100)**

### 4.2 Cytological Findings

Most of the samples were negative for cytologic lesions (41%) followed by nonspecific inflammation 21%. Malignant neoplasms were 5%, of which 3% were SCC (non- Aids defining) and 2% KS (AIDS Defining). For the purpose of analysis Pruritic papular eruptions with 7% were grouped as others as well as drug reaction, eczema, atopic dermatitis and acne with 2% respectively. Also, seborrheic dermatitis, allergic cellulitis, prurigo, osteomyelitis, ichthyosis, psoriasis and keloids had 1% respectively.

| **Cytomorphology** | **Frequency (n=100)** |
|---|---|
| **Negative** | **41** |
| **Inflammation** | **32** |
| Non-specific inflammation | 21 |
| Fungal | 5 |
| Bacteria | 1 |
| Viral | 5 |
| **Malignant** | **5** |
| Kaposi Sarcoma | 2 |
| Squamous cell carcinoma | 3 |
| **Others** | **22** |
| PPE | 7 |
| Seborrheic dermatitis | 1 |
| Drug reaction | 2 |
| Eczema | 2 |
| Allergic | 1 |
| Atopic dermatitis | 2 |
| Cellulitis | 1 |
| Prurigo | 1 |
| Osteomyelitis | 1 |
| Acne | 2 |
| Ichthyosis | 1 |
| Psoriasis | 1 |
| Keloids | 1 |
| **Total** | **100** |

**Table 2: Cytological findings of skin diseases**

### 4.3 Clinical diagnosis of skin conditions

Clinically majority of skin abnormalities were diagnosed as PPE (20%), followed by 17 % given to fungal infection. Allergic reactions appeared third with 7%. For neoplastic conditions 5 % of the cases were KS while 4% were malignant lesions.

**Table 3 Clinical diagnosis of skin conditions**

| **Fungal conditions** | **Frequency( n=100)** |
|---|---|
| Psoriasis | 3 |
| Fungal infection | 17 |
| Ichthyosis | 1 |
| Atopic dermatitis | 1 |
| Seborrheic dermatitis | 3 |
| **Viral conditions** | |
| Molluscum contagiosum | 1 |
| Herpes zoster | 3 |
| Herpes simplex | 3 |
| Warts | 3 |
| **Bacterial conditions** | |
| Cellulitis | 1 |
| Osteomyelitis | 1 |
| Folliculitis | 1 |
| **Neoplastic conditions** | |
| Malignancy | 4 |
| Kaposi sarcoma | 5 |
| **Others** | |
| Skin rash | 3 |
| Abscess | 1 |
| Allergic reaction | 7 |
| Drug reaction | 3 |
| Eczema | 5 |
| Trauma | 3 |
| Ring worms | 1 |
| PPE | 20 |
| Pruligo | 1 |
| Boil | 1 |
| Acne | 3 |
| Scabies | 1 |
| Ulcer | 3 |
| Keloids | 1 |

3. **Comparison of clinical diagnosis with cytomorphological findings** (**Table 4)**

There was a significant difference between skin conditions on clinical findings and the corresponding cytology results at 95% confidence interval. The p value was less than 0.001 and the chi square statistics was1089. When clinical diagnosis of others as a category was compared with cytology, still, there was a significant difference on skin condition reported and cytology results since the p value was 0.004 which is less than 0.05 at 95% confidence interval.

| | **Cytomorphological findings** | | | | | | |
|---|---|---|---|---|---|---|---|
| **Clinical conditions** | **NEG** | **NSI** | **FNG** | **KS** | **SC** | **Others** | **Total** |
| Skin rash | 2 | 0 | 1 | 0 | 0 | 0 | 3 |
| Herpes zoster | 1 | 2 | 0 | 0 | 0 | 0 | 3 |
| Herpes simplex | 0 | 3 | 0 | 0 | 0 | 0 | 3 |
| Abscess | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Warts | 0 | 0 | 0 | 0 | 0 | 3 | 3 |
| Seborrhoeic dermatitis | 0 | 0 | 0 | 0 | 0 | 3 | 3 |
| Malignancy | 0 | 1 | 0 | 1 | 2 | 0 | 4 |
| Allergic reaction | 3 | 2 | 0 | 0 | 0 | 2 | 7 |
| Drug reaction | 2 | 0 | 0 | 0 | 0 | 1 | 3 |
| Eczema | 2 | 0 | 1 | 0 | 0 | 2 | 5 |
| Kaposi sarcoma | 2 | 2 | 0 | 1 | 0 | 0 | 5 |
| Psoriasis | 2 | 0 | 0 | 0 | 0 | 1 | 3 |
| Trauma | 0 | 3 | 0 | 0 | 0 | 0 | 3 |
| Ring worms | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Fungal infection | 12 | 1 | 3 | 0 | 0 | 1 | 17 |
| Cellulitis | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| PPE | 10 | 2 | 0 | 0 | 0 | 8 | 20 |
| Prurigo | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Osteomyelitis | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Boil | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Acne | 1 | 0 | 0 | 0 | 0 | 2 | 3 |
| Ichthyosis | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Scabies | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Folliculitis | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Ulcer | 0 | 1 | 0 | 0 | 1 | 1 | 3 |
| Keloids | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Molluscum Contagiosum | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Atopic dermatitis | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| **Total** | 41 | 21 | 5 | 2 | 3 | 28 | 100 |

### 4.4 Cytology and HAART

Pearson chi square test was 1.452 with a p value of 0.919. This is greater than 0.05 at 95% confidence interval ( at 5% level of significance) showing that there was no evidence of significant difference in cytology results among HIV patients who were on HAART and those who were not on HAART. Patients on HAART were 89% while 11% were not on HAART. For those patients on HAART, negative for cytological lesions accounted for 37%, non-specific inflammations 19%, while others had 24%. Both neoplastic conditions Kaposi s sarcoma (2%) and Squamous cell carcinoma (3%) were found in patients on HAART.

**Table 5: Cytology and HAART**

| CYTOLOGY | HAART USE | | TOTAL | CHISQUARE | P-VALUE |
|---|---|---|---|---|---|
| | Yes | No | | | |
| Fungal | 4 | 1 | 5 | 1.452 | 0.919 |
| KS | 2 | 0 | 2 | | |
| NEG | 37 | 4 | 41 | | |
| Non Specific | 19 | 2 | 21 | | |
| Others | 24 | 4 | 28 | | |
| SC | 3 | 0 | 3 | | |
| **Total:** | **89** | **11** | **100** | | |

.

**Period of HAART use in years.**

Most of the patients had taken HAART for more than one year but less than five years, followed by those who had taken HAART for over five years but less than 10 yrs.The least had taken HAART for over 10 yrs.

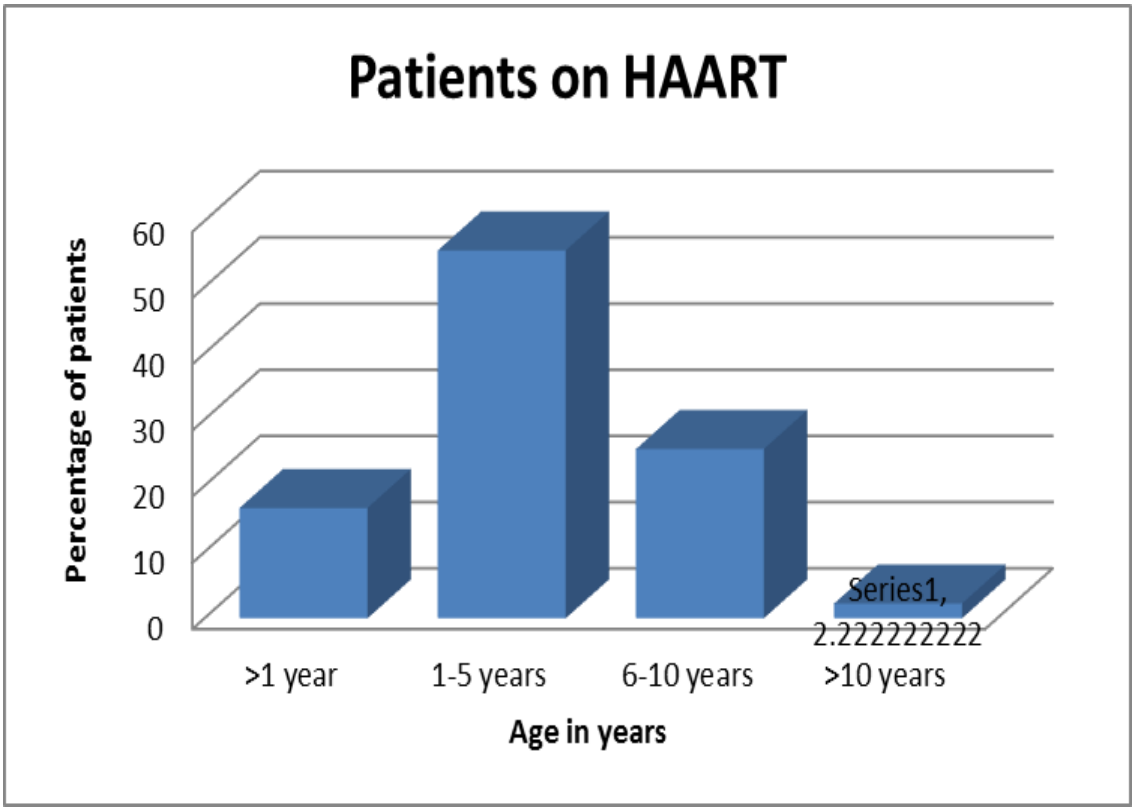



**Figure 6: Comparison of Patients on HAART in years**

## 5.0 DISCUSSION

A total of 109 patients recruited in the study met the inclusion criteria, of these nine patients were excluded from the study due to inadequate specimen sampling. One hundred patients (100) who clinically had skin lesions were studied, of which 59% were confirmed cytologically to have lesions. Females were the majority with 73%. A similar observation was made by Josephine et al 2006 where female (61.5%) were the majority (30). However, in other studies done outside Africa a predominance of men was noted. In India a study done by Sen et al 2009 males 68.9% (210 cases) outnumbered females 31.1% (95 cases) (31). The reason for this discrepancy is currently unknown: In the Kenyan context, the gender variance observed may reflect the national HIV prevalence where women are twice affected as men (National AIDS Control Council of Kenya 2009) (36) It may also mean that women are more skin health conscious and do seek treatment more often than men as observed in Cameroon by Josephine *et al.,* (2006) (30).

In this study 41% of the cases were negative for cytological lesion, 32% were inflammatory (nonspecific inflammation 21%, viral 5% and fungal 5%). Salami et al (8) reported 24% fungal lesions which was higher than the current study. Low prevalence in the present study can be attributed to the different nature of the two studies; diagnosis on Salami study was based on the clinical features while the present study was based on cytology study.

Viral infections were found to be 6%; Warts, Herpes Zoster, Herpes Simplex and Molluscum contagiosum were one per cent respectively. This is in sharp contrast with studies done elsewhere, Salami et al from Nigeria, found 37% viral; Warts 34%, Herpes Zoster 27%, Herpes Simplex 15.4 % and Molluscum contagiosum were 7.7% (8). Josephine et al in Cameroon showed 28% herpes zoster (30). Viral warts encountered in this study were all found in HIV

adult patients. Previously, it had been noted in Nigeria by Umoru et al and Salami et al. that, viral warts appear commonly in HIV patients' particularly in paediatric patients (32, 8). The current study had 3% peadiatric patients. The difference may be due to the fact that, the study was not carried during a school holiday when most of the paediatrics are booked in for the clinic.

Drug reactions in this study accounted for 2%. In Cameroon Josephine et al reported 3%. (30). However, this is much lower than 11% reported in by Salami et al (8). Salami also notes that, because cotrimoxazole and nevirapine are two core drugs necessary for the care of these patients, this type of reactions have continued to be seen despite better prevention of its occurrence.(8). Non ulcerated lesions due to drug reactions have mainly dermal features which are not accessible on superficial collection methods and this could explain the reasons as to why superficial scrapings were not diagnostic.

The prevalence of KS in this study was (2%).This is almost similar to Ramadhan et al (2013) study in Kenya which reported one case of KS (0.3%) and Mohammad *et al* (2003) in Tanzania who reported 1.6% (23,33). Cutaneous Kaposi sarcoma prevalence in Kenya appears to be low compared to Salami et al (2011) study which reported 5.7% while Josephine *et al* (2006) found 9.9%. (8, 30) This may be a reflection of the different geographical locations of the study populations However, Rogena et al had 48% in a study on AIDS associated malignancies from 2001 to 2011(36). In this study, the prevalence was higher probably because their study covered a longer period of time (10yrs).

Lymphomas (AIDS defining) have also been identified elsewhere. In an unpublished study by Rogena et al, cutaneous NHL accounted for 4.6 %. In this study, a case of lymphoma was confirmed by histology after being suspected clinically but cytology was not conclusive. Cytology has been reported to be poor in diagnosis of lymphomas (14). Lymphoma is a dermal

lesion, and this could have been the reason why cytology was not conclusive particularly if the lesions are not ulcerated.

Squamous cell carcinoma (Non AIDS defining) accounted for 0.3% of all the lesions. Ukonu et al, study in Nigeria (2012), reported 0.3% SCC (18). A study done in Northern California, Wendy et al. showed a 2.6-fold higher risk for squamous cell carcinomas, compared to HIV negative subjects. This is in contrast with the zero prevalence reported from Bangkok by Pallangyo et.al (34) and in Singapore by Chan (35). Stage of disease for the patients was not recorded in this study and this could explain the reason for the differences. This could also be attributed to the fact that, HAART has increased life expectancy giving a higher chance for the patients to be affected by non- AIDS defining cancers too.

This study describes a positive correlation between clinical impression and cytological findings of the skin lesions. The concordance for inflammatory conditions is much lower than that of neoplasms. This suggests that cytological methods can be utilized as primary diagnostic test.

All the five cases of malignancies were in patients on HAART, although there was no statistically significant difference in cytological findings of patients on and not on HAART. Patients not on HAART were very few though, 11%.This could have been due to the fact that, by the time patients are reporting for treatment for the first time, they already had the skin lesions due to the low immunity. Patients on HAART also live longer and have higher chances of developing neoplasms.

## CONCLUSION

Most of the positive cytological findings were as a result of inflammation whereas malignant skin lesions were rare.

## RECOMMMENDATIONS

1. Continuous medical education on skin specimen collection, processing and reporting should be extended to the clinicians involved in management of dermatologic conditions among HIV infected patients.
2. Cytology is a valuable, affordable and quick diagnostic tool that should be incorporated in the routine management of HIV-related skin lesions.

## STUDY LIMITATIONS

For non-specific microscopic findings such as inflammatory skin disorders where exclusion of fungal infection was done using PAS, this study did not fully access all the special stains methods e.g. silver stains and this would have affected the outcome of the results.

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**APPENDICIES**

**Appendix I: Questionnaire.**

**CYTOLOGICAL FINDINGS OF SKIN LESIONS AMONG HIV INFECTED PATIENTS ATTENDING COMPREHENSIVE CARE CLINIC AT KENYATTA NATIONAL HOSPITAL, NAIROBI.**

This form is to be filled by the participants before sample collection.

Name…….Phone number……..

Study identity number…….……Date…….……...

Sex…….Age……

1. Residence ……(county)
2. What is the level of education you have attained?

None ☐

Primary ☐

Secondary ☐

Tertiary ☐

3. What is your occupation?

Small business enterprise ☐

Employed ☐

Unemployed ☐

Others (specify)……

4. Do you have any skin disease/condition?

Yes……No…….

5. Which part of the body has the lesions? Specify please……

6. For how long has the lesion been there? .....

(Write the specific time in terms of weeks, months or even years)

7. Are you on any medication?

(NO) ☐

(YES) ☐

8. Are you on HAART? .....

If yes, for how long? ……

(Write the specific time in terms of weeks, months or even years)

**For Investigator ONLY**

1. Clinical history:

a) Where is the lesion located? …….

b) What is its appearance (is it ulcerated, dry, cystic, solid etc.)

c) What is the size? ……

d) For how long has the lesion been there? .....

e) Clinical diagnosis…….

2. Specimen Adequacy: Satisfactory ☐ Unsatisfactory ☐

3. Cytological features:

a) Infection……. (Specify ) viral, bacterial, fungal,

b) Non infection…….. (Specify)

Reactive……. (Give a clinical correlation)

Neoplasm…… (Classify if benign, if malignant specify whether AIDS or Non AIDS defining and recommend for histology confirmation)

Signature (PI/Research assistance)…….

**Appendix II: Pathological request form**

Study identity number…….

---

**Clinical diagnosis/Impressions……**

**Cytological report**

Sample adequacy

Macroscopic appearance

Purulent ☐ ☐ Blood Stained ☐

Microscopic appearance

Viral cytopathic changes (specify)

……..……..

Fungal infection (specify) ……..…….

Bacterial infection (specify) …..……

Benign skin lesion ……...

Malignant skin lesion ……

Conclusion

Comment

Pathologist name…… ……Sign…….Date…….

Cytologist name……. .....Sign…….Date…….

**Appendix III: Adult Consent form**

**Title:** Cytological findings of skin lesions among HIV infected patients attending comprehensive care clinic at Kenyatta National Hospital, Nairobi.

**Consent explanation:** My name is Christine Nyambura Mwaniki (0724519131) a postgraduate student at the University of Nairobi. PO BOX 20732 Nairobi, Kenya; Phone: +254-2-7263000 Ext 43769.

I kindly request you to participate in the above study at your will. The purpose of this consent form is to give you information about the study. This will help you in deciding whether want to be in the study or not. Please feel free to ask any questions about the study. You are entitled to have a copy of this consent for your records.

**Objectives of the study**: The purpose of this research study is to investigate skin lesions among HIV infected patients.

**Benefits:** You will benefit from free screening of any lesions on your skin. The findings of this study will be important in planning skin care for HIV infected patients.In case of any abnormal findings the results will be sent to your file ( clinician) where you will receive care and appropriate management.

**Risk/discomfort**: The procedure is relatively safe but little pain will be experienced during FNA. Personal information will not be disclosed to anyone.

**Compensation mechanism:** There will be no compensation awarded to the participants during the study.

**Alternative treatments: There** will be no other extra treatment accorded to the participants.

**Procedure:** If you accept to participate in this study you will be asked to give your demographic parameters through a questionnaire. The specimens will be obtained from the skin using scrapings (for dry skin lesions), touch preparation (for wet skin lesions) and skin biopsies will be obtained in case of intradermal lesions forming masses.

**Voluntarism:** You are at liberty to be included in the study or not without any coercion. You may skip any questions that you don't want to answer and may terminate the interview at any time without consequence.

**Confidentiality:** All records will be identified by serial numbers only, to maintain your confidentiality and we will not record your names or the personal identifiers.

**Follow up:** Participants found with abnormal skin finding will be contacted for review and referral management facility through CCC KNH.

**Storage of specimen:** In case of further analysis (with the permission form the KNH/UON/ERC) all the specimens and data will be kept safely for five years in the Human Pathology Department, University of Nairobi at the end of the study after which it will be destroyed.

**Subject's rights:** If you have questions about your rights as a study participant, or are dissatisfied at any time with any aspect of the study, you may contact – anonymously, if you wish – KNH/UoN ERC ( Secretary of the Scientific Steering Committee, PO BOX 20732 Nairobi, Kenya**,** Phone:02-726300 Ext 44102)

I have read this form or had it read to me in a language that I Understand. I have discussed the information with study staff. My questions have been answered. My decision whether or not to

take part in the study is voluntary. If I decide to join the study I may withdraw at any time. By signing this form I do not give up any rights that I have as a research participants.

Participant Name……Participant's signature/thumbprint……

Date…….

Study staff conducting…….Study staff

signature…….Date…….

**Appendix IV Children assent form**

**Title:** Cytological findings of skin lesions among HIV infected patients attending comprehensive care clinic at Kenyatta National Hospital, Nairobi.

**Consent explanation:** My name is Christine Nyambura Mwaniki (0724519131) a postgraduate student at the University of Nairobi. PO BOX 20732 Nairobi, Kenya; Phone: +254-2-7263000 Ext 43769.

I kindly request you to allow your child to participate in the above study at your will. The purpose of this consent form is to give you information about the study. This will help you in deciding whether you want your child to participate in the study or not. Please feel free to ask any questions about the study. You are entitled to have a copy of this consent on behalf of your child, for your records.

**Objectives of the study**: The purpose of this research study is to investigate skin lesions among HIV infected patients.

**Benefits:** Your child will benefit from free screening of any lesions on the skin. The findings of this study will be important in planning skin care for HIV infected patients.In case of any abnormal findings the results will be sent to the child's file where the child will receive care and early management.

**Risk, Stress or discomfort**: The procedure is relatively safe but little pain will be experienced during FNA. Personal information will not be disclosed to anyone.

**Compensation mechanism:** There will be no compensation awarded to the participants during the study.

**Alternative treatments: There** will be no other extra treatment accorded to the participants.

**Procedure:** If you accept to let your child participate in this study you will be asked to give the child's demographic parameters through a questionnaire. The specimens will be obtained from the skin using scrapings (for dry skin lesions), touch preparation (for wet skin lesions) and skin biopsies will be obtained in case of intradermal lesions forming masses.

**Voluntarism:** You child is at liberty to be included in the study or not without any coercion. You may skip any questions that you don't want to answer and may terminate the interview at any time without consequence.

**Confidentiality:** All records will be identified by serial numbers only, to maintain your child confidentiality and we will not record your child's names or the personal identifiers.

**Follow up:** Participants found with abnormal skin finding will be contacted for review and referral management facility through CCC KNH.

**Storage of specimen:** In case of further analysis (with the permission form the KNH/UON/ERC) all the specimens and data will be kept safely for five years in the Human

Pathology Department, University of Nairobi at the end of the study after which it will be destroyed.

**Subject's rights:** If you have questions about your child's rights as a study participant, or are dissatisfied at any time with any aspect of the study, you may, on behalf of your child contact – anonymously, if you wish – KNH/UoN ERC ( Secretary of the Scientific Steering Committee, PO BOX 20732 Nairobi, Kenya**,** Phone:02-726300 Ext 44102)

I have read this form or had it read to me in a language that I Understand. I have discussed the information with study staff. My questions have been answered. My decision whether or not to let my child take part in the study is voluntary. If I decide to let my child join the study I may withdraw at any time. By signing this form I do not give up any rights that my child have as a research participants.

Participant parent Name……..Participant's parent signature/thumbprint……..

Date……..

Study staff conducting…… Study staff signature ……..

Date……..

## Appendix IV: Pap stain

**Principle:** Heamatoxylin stains the nuclei blue by dye-lake formation. The Eosin Azure stains the cytoplasm, Eosin has affinity stains young Endocervical cells. Orange G stains cytoplasm of the oldest superficial cell.

### Pap staining procedure

1. The smears will be fixed in 95% alcohol.

2. The smear hydrates in descending grades of alcohol 80%, 70% and 50% alcohol.

3. The smears will be stained in Harris Heamatoxylin for 4 minutes.

4. The smears will be rinsed in tap water.

5. The smears will be differentiated in 0.05% tap water 10 dips

6. The smears will be rinsed in Scott's tap water 10dips

7. The smears will be rinsed in ethanol 10 dips.

8. The smears will be stained in O.G for 1.5 minutes.

9. The smears will be rinsed in 95% ethanol for 10dips

10. The smears will be stained E.A 5O for 1.5 minutes.

11. The smears will be dehydrated in two changes of absolute alcohol.

12. The smears will be cleared in three changes of Xylene 10dips each

13. Mount in DPX.

## Appendix V: Harris heamatoxylin and eosin stain

**Principl**e: The oxidation product of heamatoxylin is haematin, and is the active ingredient in the staining solution. Heamatoxylin is not classified as a dye since the molecule possesses no chromophore. The *in situ* oxidation of heamatoxylin is effected by the addition of a strong oxidant to the stain, in this case sodium iodate.

### Staining technique

1. The smears will be fixed in 95% alcohol

2. Stained in Harris heamatoxylin for 5 minutes.

3. Rinsed in tap water.

4. Differentiated in 1% acid alcohol, 3dips.

5. Rinsed in tap water.

6. Blued in Scotts tap water for 30 seconds or in running tap water for 10 minutes.

7. Counter stained in Eosin for 5 minutes.

8. Rinsed in tap water to remove excess eosin followed by 70% ethanol to obtain the desired shades of red and pink.

9. Dehydrated in the 3 changes of absolute alcohol.

10. Cleared in 3 changes of Xylene.

11. Mounted with D.P.X.

**Appendix VI: Periodic acid Schiff**

**PRINCIPLE**:

Used for the demonstration of glycogen. Tissue sections are first oxidized by periodic acid. The oxidative process results in the formation of aldehyde groupings through carbon-to-carbon bond cleavage. Free hydroxyl groups should be present for oxidation to take place. Oxidation is completed when it reaches the aldehyde stage. The aldehyde groups are detected by the Schiff reagent. A colourless, unstable dialdehyde compound is formed and then transformed to the colored final product by restoration of the quinoid chromophoric grouping.(27)

**Procedure for PAS staining**

1. The smear will be fixed in 95% alcohol

2. The smear will be oxidized in 0.5 % periodic acid solution for 5min

3. The smears will be placed in Schiff reagent for 10minutes.

4. The smears will be rinsed in tap water.

5. The smears will be counterstained in Harris heamatoxylin for 4minutes

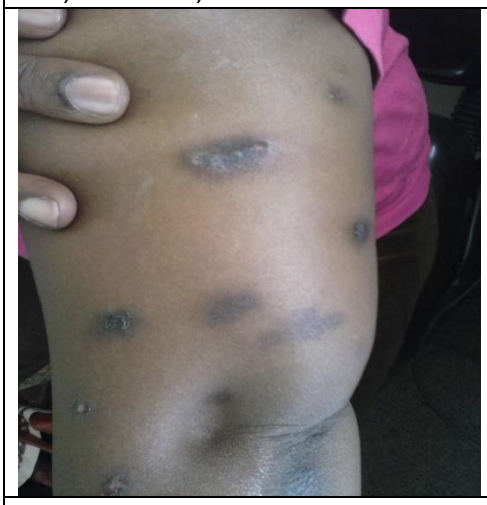
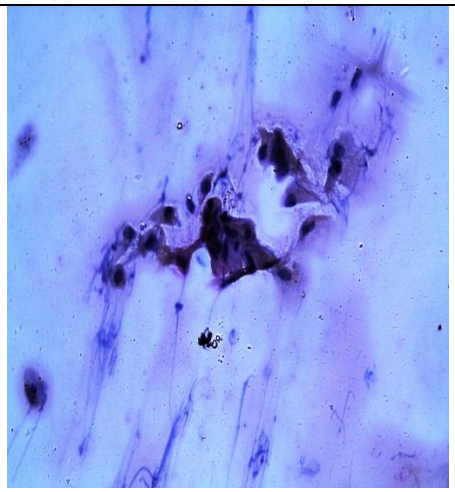
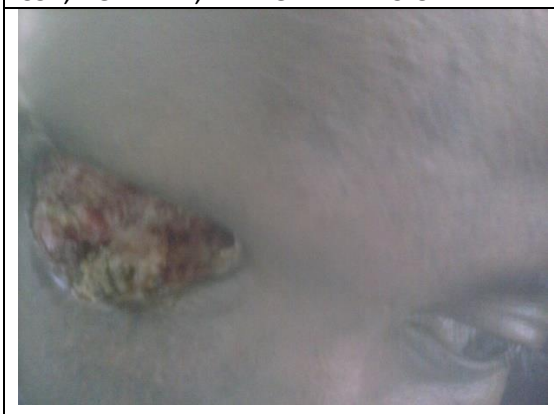
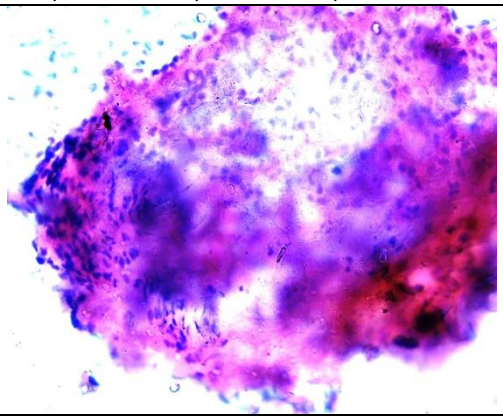
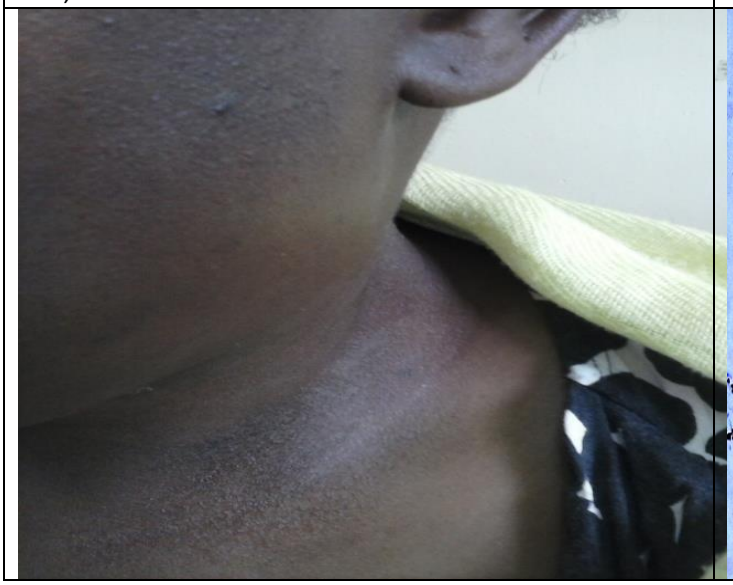
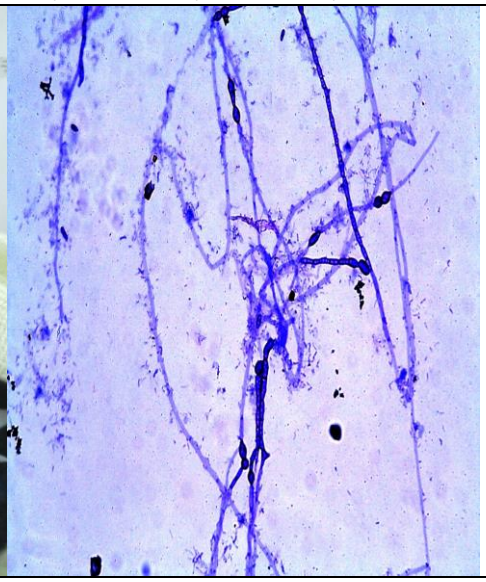
6. The smears will be rinsed in tap water.

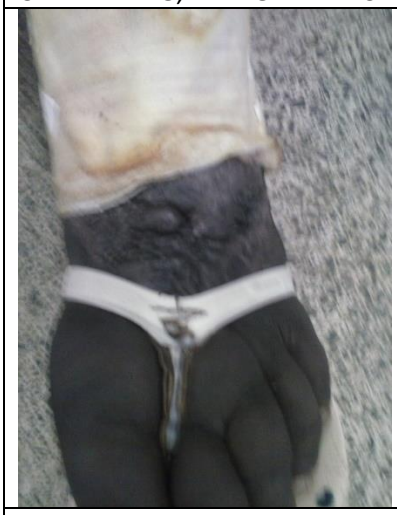
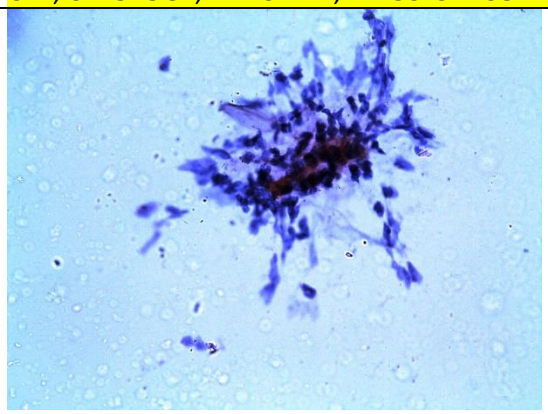
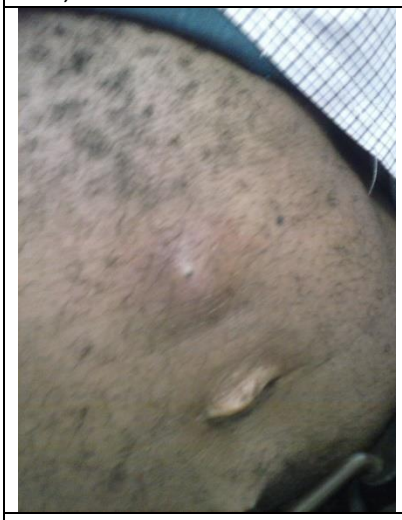
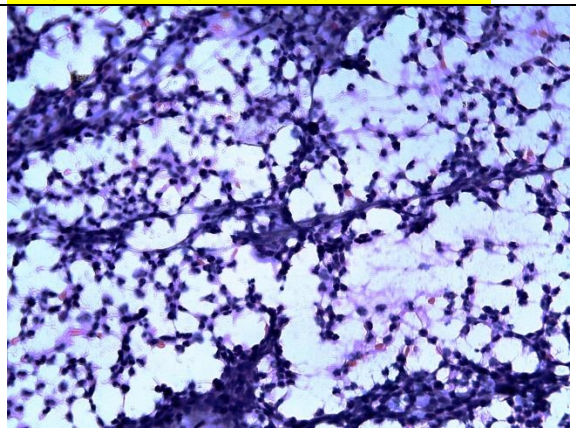
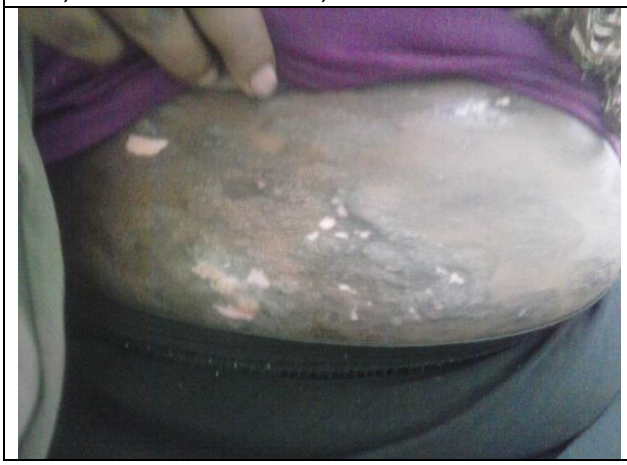
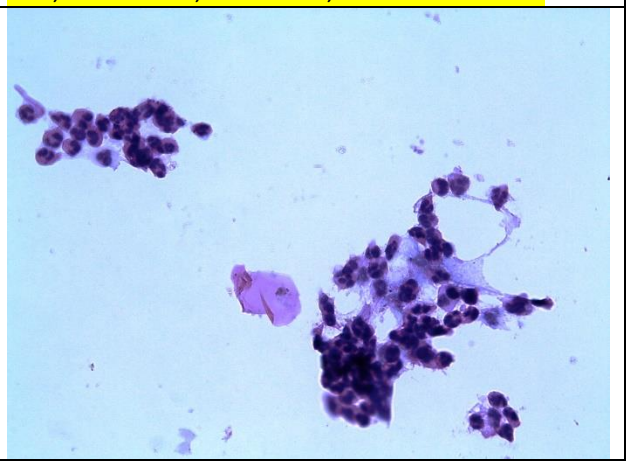
7. The smears will be dehydrated in the 3 changes of absolute alcohol.

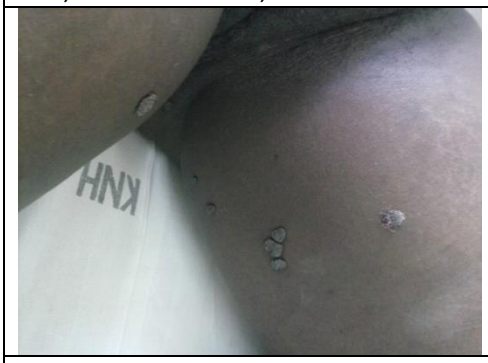
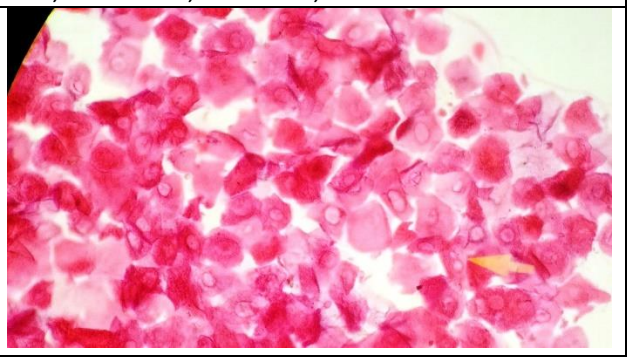
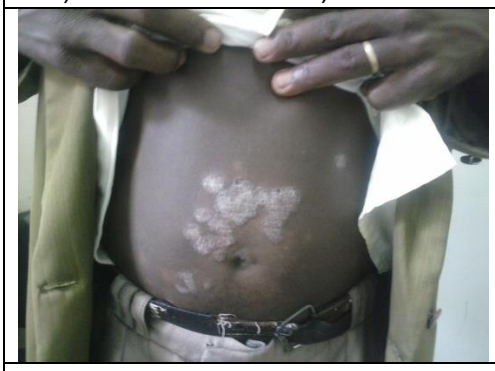
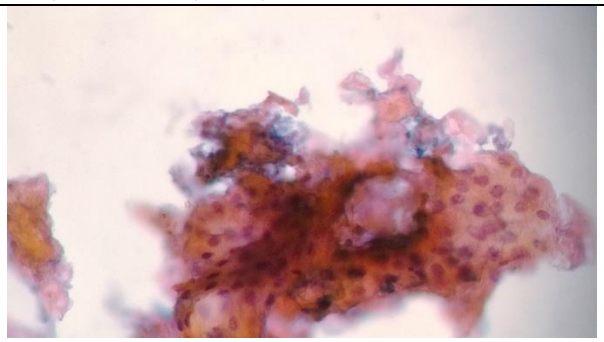
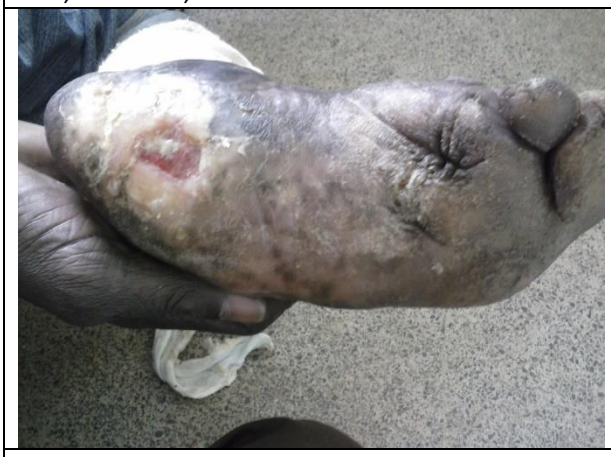
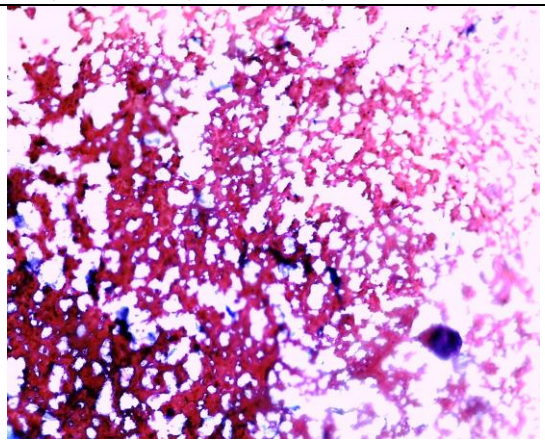
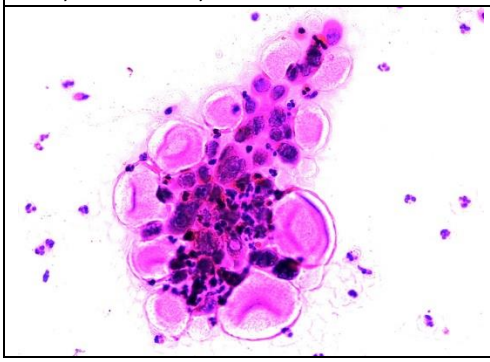
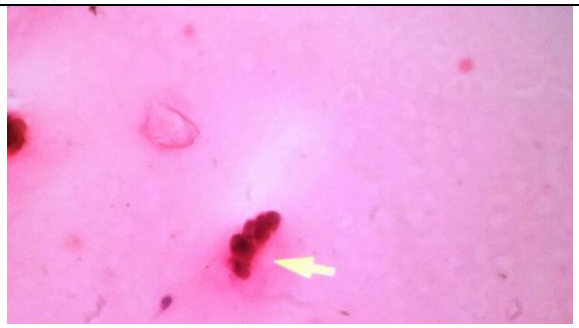
10. The smears will cleared in 3 changes of Xylene.

11. The smears will be mounted with D.P.X

## Appendix VI: Photographs and photomicrographs

| | |
|---|---|
| C21, LEFT ARM, PPE. | C21, CYTOLOGY, H&E, KAPOSI SARCOMA. |
|  |  |
| C37,RIGHT EYE, MALIGNANT LESION. | C37, CYTOLOGY, PAP STAIN,SCC. |
|  |  |
| C76, SKIN RASH | C76, CYTOLOGY, PAS STAIN,FUNGAL |
|  |  |

| | |
|---|---|
| C41 LEFT LEG, MALIGNANT TUMOUR | C41, CYTOLOGY, PAP STAIN,KAPOSI SARCOMA |
|  |  |
| C44, ABDOMINAL MASS | C44,CYTOLOGY,PAPSTAIN, GRANULOMA |
|  |  |
| C65, ABDOMINAL LESION, LYMPHOMA. | C65, CYTOLOGY, PAPSTAIN, INFLAMMATION. |
|  |  |

| C77, THIGH LESIONS, WARTS. | C77,CYTOLOGY,PAPSTAIN, WARTS. |
|---|---|
|  |  |
| C81, ABDOMINAL LESION, PSORIASIS. | C81, CYTOLOGY, H&E, PSORIASIS. |
|  |  |
| C85, LEFT LEG, CHRONIC ULCER. | C85,CYTOLOGY, PAPSTAIN,SCC. |
|  |  |
| C94,CYTOLOGY,HERPES SIMPLEX. | C46,CYTOLOGY,HERPES SIMPLEX. |
|  |  |