FINE NEEDLE ASPIRATION CYTOLOGICAL FINDINGS IN HIV POSITIVE PATIENTS PRESENTING WITH HEAD AND NECK MASSES AT KENYATTA NATIONAL HOSPITAL

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DECLARATION FORM

I hereby declare that this dissertation is my original work under the guidance of the supervisors listed below and has not been previously submitted to the University of Nairobi or any other higher learning institution.

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DEDICATION

To my parents, my brothers, my fiancé Eric and my best friend Laurie and her family, thank you for your financial and emotional support, God bless you all.

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LIST OF ABBREVIATIONS

FNA:	Fine needle aspiration
TB:	Tuberculosis
HIV:	Human Immunodeficiency virus
AFB:	Acid Alcohol Fast Bacilli
SCC:	Squamous Cell Carcinoma
HPV:	Human Papilloma virus
AIDs:	Acquired Immune Deficiency Syndrome
RNA:	Ribo Nucleic Acid
SsRNA:	Single-Stranded RNA
DNA:	Deoxyribonucleic Acid
H&E:	Hematoxylin and Eosin
ZN:	Ziehl-Neelsen
USG:	Ultrasound guided
HL:	Hodgkin Lymphoma
RS:	Reed-Sternberg
PAP:	Papanicolaou
PI:	Principal Investigator
KNH:	Kenyatta National Hospital
UON:	University of Nairobi
CCC:	Comprehensive Care Centre
SOP:	Standard Operating Procedure

WORKING DEFINITIONS

Congenital cysts: A cyst present at birth and results from abnormal development, such as a dermoid cyst or non-closure of embryonic clefts, ducts, and tubules as cervical cysts. Cervical congenital cystic masses constitute an uncommon group of lesions usually diagnosed in infancy and childhood. The diagnosis is easily established from the presence of a cystic lesion in the anterior midline portion of the neck.

Granulomatous: Tumor composed of granulation tissue produced in response to chronic infection, inflammation, a foreign body or unknown causes. A disorder of the immune system that is characterized by recurrent, serious infection that results from the inability of phagoctosis by white cells to destroy certain bacteria and fungi.

Hematoma: A collection/ accumulation of blood in a tissue or organ. It is caused by a break in vessel.

Lymphadenopathy: Is a disease of the lymph nodes in which they are abnormal in size, number or consistency. The most common is an inflammatory type called lymphadenitis.

Metastatic malignancy: Cancer cells break away from where they first formed, travel through the blood or lymph system, and form new tumors in other parts of the body.

ABSTRACT

Background: Head and neck masses commonly occur within the thyroid, salivary glands and lymph nodes as well as other soft tissues of the lesions. Lymphadenopathy is a common signs of Human Immunodeficiency Virus (HIV) infection or Acquired Immunodeficiency Syndrome (AIDS). In addition, persistent generalized lymphadenopathy is one of the earliest signs HIV infection. When malignancy is suspected, tissue sampling should be performed.

Objective: To describe the fine needle aspiration cytological findings of accessible head and neck masses in Human Immunodeficiency Virus (HIV) positive patients attending selected outpatient clinics in Kenyatta National Hospital (KNH).

Study design: This was a cross- sectional descriptive study.

Study Population: The study was done among **HIV** patients attending ENT clinic, FNA clinic and CCC clinic at KNH presenting with head and neck masses.

Materials and Method:

The study included HIV patients undergoing fine needle aspiration cytology for various head and neck swellings. A total of 84 patients with lesions in the head and neck regions from the Ear, Nose and Throat (ENT) clinic, Comprehensive Care Centre clinic (CCC) and at the Fine needle aspiration (FNA) clinic were processed at the Cytology Laboratory at Kenyatta National Hospital. Using an aseptic technique, aspiration was done using gauge needle 23 or 25 with 10ml syringe in the usual manner.

The material obtained was placed on the microscopic slide using a pick and smear technique. 4 thin slide smears, were made and 2 fixed in 95% alcohol immediately. The other 2 smears were air dried for staining using Romanowsky and Ziehl- Nielsen (ZN) stains. The 2 alcohol fixed smears were stained using Papanicolaou and Hematoxylin and Eosin stains. The air dried smears were stained with Ziehl Nielsen (ZN) and Giemsa stains for analysis of cases suspicious of TB or lymphoproliferative disorders.

Univariate analyses were used to describe distribution in demographics and cytological findings. Bivariate analyses were used to compare distribution between variables; used Fisher's exact for bivariate analyses: -used for categorical variable (presence/absence data); and on data with <5 observations per variable /category.

Results:

A total of 84 HIV positive patients were recruited. Patient age range was 0- 80 years with a median age of 27 and (IQR* 22-43 years). Granulomatous inflammation was the most identified lesion 27 (32%) followed by Colloid goiter 15 (18%).Anaplastic carcinoma, pleomorphic adenoma and round blue cell tumor were the least observed lesions at 1(1.2%) each. Seven of the 27 granulomas were positive for TB. Comparing adults with children the risk of reactive lymphadenitis significantly differed between the two groups. Reactive lymphadenitis was 90% (1-0.1*100) less likely to occur in adults compared to children. Out of 84 patients only 8 (6.72%) had HIV clinical staging 76 did not have. Enlarged lymph nodes were a common finding among adult female patients (n=69, 34.8%) and in male children (n=15, 46.7%). Thyroid lesions were more common in female adults (n=69, 20.3%) and less so in children (n=25, 13.3%).

Conclusion and Recommendations:

Commonest benign head and neck lesions in HIV positive patients attending selected clinics in KNH included tuberculous lymphadenitis and colloid goiter in adult females while in children the most common lesion was reactive lymphadenitis. Commonest neoplastic condition in adults was Squamous cell carcinoma while in children only one round blue cell tumor was found. Head and neck swelling in HIV positive patients can readily be screened for malignancy using FNA. Most of the common pathologic processes and swellings in head and neck region can be readily diagnosed on Cytomorphology

FNA should be performed on HIV positive patients with head and neck masses to classify the lesions for better and timely management of patients and to prevent unnecessary surgical intervention on head and neck masses. Granulomatous lymphadenitis should have ZN done in order to confirm or exclude TB. Fine Needle Aspiration Cytology should be the first screening procedure in clinics for the diagnosis of the cause of lymphadenopathy in HIV/AIDS patient. The second commonest benign lesion was colloid goiter, and hormonal profile including TSH/-FT3/-FT4 for thyroid lesions would be useful to complete the clinicopathological work-up.

1. INTRODUCTION

Head and neck region has anatomically complicated structures due to its richness in diverse structures and all these may become involved in disease, often resulting in a clinically apparent mass which may be inflammatory, infective or neoplastic. [(1)] The masses involving salivary glands, thyroid and scalp are commonly encountered visible lesions and easily accessible for FNA.

Human immunodeficiency virus (HIV) infects and destroys CD4+ lymphocytes among other cells and during the process of progression of the disease there is deterioration of the immune system of the individual. The infection of lymphocytes result in enlargement of lymph nodes (LNs), which is one of the most common early and consistent signs/symptoms of HIV infection. Lymphadenopathy can present in every stage of HIV infection, but also occurs in HIV negative individuals due to other disease conditions. [(2)]

The earliest data related to the human immunodeficiency virus (HIV) describing development of pneumocystis pneumonia and Kaposi sarcoma in male homosexuals were published in 1981. [(3)] In 2002 new HIV infections decreased from 3.3 million to 2.3 million by 2012. In 1990 there were an estimated 300,000 deaths from AIDS. Global AIDS-related deaths peaked at 2.3 million in 2005, and decreased to 1.6 million by 2012. In 2010, the 1.5 million estimated deaths from AIDS represented 2.8% of the 52.8 million deaths and AIDS was the sixth leading cause of years of useful life lost (YLL) worldwide. [(42, 43)] According to estimates by WHO and UNAIDS, 36.7million people were living with HIV globally at the end of 2016. That same year, some 1.8 million people become newly infected, and one million died of HIV- related causes.

There are two genetic types of HIV: HIV-1 which is present in Europe, Asia, America and Central Africa and HIV-2 which is only found in the Western Africa. [(4, 44)] The HIV is an enveloped RNA virus and is transmitted in single stranded, ssRNA form. After transmission the reverse transcriptase, which is also present in the virus, transforms the single-stranded RNA to a double-stranded DNA and then the virus DNA is integrated and transcribed with help from the cellular system of the host. After that there are two possibilities: either the virus becomes latent and the infected cells continue their function without any disturbance to the host cells or the virus becomes active and starts to replicate itself. In the latent stage the HIV can be ‘sleeping’ for several years. When a huge number of viruses are formed a cell lysis

occurs resulting in the spread of the virus to other cells and the extracellular compartment. [5, 6]

The process of the selection of target cells that are infected by HIV is based on the recognition of CD4 receptors on the host cells' surface by the virus. The cells that have such CD4 receptors on their cell surface are T-helper lymphocytes, monocytes, dendritic cells and microglia. T-lymphocytes have one of a crucial function of the immune system in the recognition of infectious agents; they are called CD4 'T-helper' cells. The activation of CD4 'T-helper' cells takes place as a response of the immune system to various microbial agents. When these cells are in the naïve state no replication of HIV takes place, only when the T-lymphocytes are activated then the HIV proceed with replication. [7] The main function of the subsets of the CD4+ lymphocyte population is to determine the host response to infection, the subset known as TH1 and TH2.[8]An abnormal activation of the immune system has been shown to be a major factor in disease progression. A pool of activated CD4 T-cells is formed that can be targeted by HIV and this may lead to the exhaustion of the immune system. [5]

The spread of HIV in Kenya has been uneven. Although much of Kenya has a low rate of infection, certain places have been more affected than others. In particular, new HIV infections increased by more than 50% in 2013 to 2015. Epidemics are more severe in Coastal and Nyanza regions and in urban centers. As a result, HIV prevalence ranges from 0.1 % in Wajir County in North Eastern Kenya, to 25.4% in Homa Bay County in Nyanza.
The earliest manifestations for opportunistic infections in HIV infected patients are lymphadenopathy and hematological alterations. [9] Studies have reported various cytological findings in head and neck lesions in HIV positive patients. In a study done in India on 32 HIV- positive cases presenting with lymphadenopathy the commonest cytological diagnosis was tuberculous (TB) lymphadenitis in 15 cases followed by reactive lymphadenitis in 10 cases. The others were acute suppurative lymphadenitis, 5 cases, and suspected malignancy, 2 cases. **[10]**

It is estimated that head and neck cancers constitute about (5-8%) of all malignancies worldwide and the trend appears to be increasing. **[11, 12]** Head and neck cancers are relatively uncommon in the West, constituting about 4% of all malignancies, while in the Asian continent and Indian subcontinent, they form 40 to 50% of all malignancies.**[13]** A study done by Gonzalez et al, at a hospital in Spain showed a prevalence of 40.3% whereas a study

done by Tatomirovic et al, at the Institute of Pathology in Serbia demonstrated a prevalence of 36.1%.[14, 15] In another study done in Rwanda, Fine Needle Aspiration (FNA) was found to be a useful tool in the diagnosis of tuberculous lymphadenitis (TL). FNA can reduce the number of surgical excisions and provide definite guidelines about further management. In the Rwandan study, a total number of 138 specimens from suspected TL patients were analyzed, of which 14 (10.1%) were ZN positive while cytology revealed 25 (18.1%) cases of tuberculous lymphadenitis. [17]

A study done in India by Rajesh et al, in 2014 on 62 HIV-positive individuals demonstrated that the most common lesion was TB 27 (42.19%) cases, followed by reactive lymphadenitis with 18 (28.12%) cases, acute suppurative lymphadenitis 7 (10.94%), fungal infection 5 cases, and malignancy 3 cases. A study done in pediatric patients by Lucumay et al in Western Tanzania showed 43.9% had inflammatory lesions, 38.5% had congenital lesions, while 14.9% had neoplastic. **[16]**

Patients with FNAs showing SCC with no clinical evidence of a head and neck primary might benefit from HPV testing. Patients with HPV-related tumors show a greater response to radiation and overall improved survival compared to patients with non-HPV associated tumors. Most common HPV attributed carcinomas are poorly differentiated non-keratinizing tumors. They may less frequently demonstrate cystic changes with keratinization. Many cases may share morphology with nasopharyngeal carcinoma. Epstein - Barr virus (EBV) In Situ Hybridization(ISH) testing on a cell block may be of value. [18]

Based on the paucity of reliable cytological information on head and neck masses in KNH, this study was important to describe the FNA cytological findings of accessible head and neck masses in HIV patients attending ENT and FNA clinics.

2. LITERATUREREVIEW

2.1. Fine needle aspiration

2.1.1. Definition

Fine needle aspiration is the procedure of obtaining a sample of cells from a tissue for examination by applying suction through a fine needle attached to a syringe. Fine needle aspirations are often performed when a suspicious mass, bump or lump is found.

2.1.2. History of use of FNA for head and neck masses

An Arabic physician, Abuleasim (1013-1107AD), described the use of needle puncture of the thyroid to diagnose different types of goiters in medieval times. Fine needle aspiration was first recorded by Kun in 1847, the same year when there was a formal statement of the cell theory by Schleiden and Schwann. However, it did not gain popularity in the field of medicine at the time. The interest in cytological techniques continued to flourish in the late 1920s. In 1930 Memorial Sloan Kettering re-discovered the utility of needle biopsy of head and neck masses. However, they utilized large bore (18gauge) needle which led to frequent complications such as seeding of tumor along biopsy tract. [19] These complications led to widespread resistance to the acceptance of this technique in other areas of America. Another generation passed before there was renaissance of interest in the technique in the United States. In the 1950s physicians in Sweden led to the resurgence of FNA where it was commonly used for the diagnosis of metastatic neoplasms in the head and neck region with excellent results.

Fine needle aspiration (FNA) is a useful cost-effective technique for preoperatively assessing lesions including head and neck region. [20] It is a simple and sensitive complementary test to open surgical biopsy. [21] Though it is a relatively new technique in many developing countries, there is a growing body of evidence that it can be easily adapted to address their particular healthcare challenges. [17] Fine needle aspiration is a type of biopsy procedure where a thin needle is inserted into a mass. As with other types of biopsies, the sample collected can help make a diagnosis or rule out conditions such as cancer, benign neoplasms, and infectious diseases. [10]

The technique uses a needle gauge of 22-25 G that is inserted intoa mass and a certain amount of material is withdrawn. The cells after being extracted and stained are studied under the microscope by a cytologist and/or a pathologist. [22]

Its advantages include minimal invasion and high sensitivity, specificity, and accuracy. [23] However, it also has disadvantages or side effects that include excessive bleeding at site of biopsy, occasionally a small hematoma forms and pain but these can be minimized by patient selection and good aspiration technique. Poor technique can result in unsatisfactory specimen and improper cytological interpretation. [24]

Fine needle aspirations smears can be processed rapidly by staining using diff-quick for 1 minute. This enables the use of FNA biopsy to be evaluated immediately for adequacy of the samples, rapid on site evaluation (ROSE).

2.1.3. Accurate procedure

Fine needle aspiration is capable of providing specific and conclusive results before definitive surgery, chemotherapy or radiation is commenced. FNA has the highest accuracy in the diagnosis of metastases, recurrence and staging of disease. [25]

2.2. Indications for head and neck FNA

2.2.1. Thyroid

The major indication for FNA on the thyroid is the presence of thyroid nodule that can be multinodular or diffuse goiter. It is done to detect thyroid neoplasms for surgical resection and to identify non-neoplastic lesions that may be managed conservatively. [26]

2.2.2. Lymph nodes

Enlarged lymph nodes are the primary target for FNA. Indication for nodes aspirations includes confirmation of clinical examination of reactive hyperplasia to diagnose a suspected infection, malignancy and to document metastasis. In adults, enlarged lymph nodes are more likely to be malignant than in children. [27]

Patients with HIV are at risk for infections or a lymphoma associated with HIV. A solitary enlarged lymph node is more likely to be malignant, while TB presents with matted lymph nodes. [30]

2.2.3. Soft tissue lesions

Patients present with tissue swelling /mass (head and neck) are eligible for FNA.

2.2.4. Congenital cervical cysts

For patients presenting with painless neck masses which can be accompanied by a combination of stridor, hoarseness of voice, and/or dysphasia which are the major indications for FNA of congenital cervical cyst. [29, 30]

2.3. Differential diagnosis of head and neck masses in HIV positive

Patient's age, gender, lifestyle, socio-economic status and duration, behavior of lesion, associated symptoms and presenting locations can assist significantly to narrow the differential diagnoses.

The spectrum of disease ranges from inflammatory, congenital and developmental, and neoplastic masses. Head and neck diseases that are spread through the lymphatics have characteristic patterns, thus the location of the mass in the cervical lymphatic nodal chain is key for the identification and differential diagnosis of the primary disease site.

The neck masses are evaluated by a detailed history, clinical examination and investigation like Fine Needle Aspiration Cytology (FNAC), Ultrasound (US) neck, Computed Tomography (CT) scan and excisional biopsy. Biopsy is useful in this region because there are many entities that can be considered in the differential diagnosis.

In most cases, FNAs of the neck are performed to investigate clinically suspicious masses. The primary differential diagnoses in most cases are reactive/infectious lymphadenopathy (LAD) and lymphomas. Diagnostic accuracy of FNA for metastatic disease ranges from 83-97%. Cervical lymphadenopathy (LAD) is the most common presenting sign of malignant disease elsewhere in the head and neck or distant sites. Squamous cell carcinoma (SCC) 90% after age 40, and nasopharyngeal carcinoma, salivary gland tumor metastases, thyroid carcinoma, melanoma, carcinomas from visceral organs, while 75% of bronchial cysts occur in patient age 20-40. [31]

2.3.1. Thyroid lesions

2.3.1.1. Acute and chronic thyroiditis

Patients presents with enlarged and tender glands caused by invasion of the thyroid by bacteria, mycobacterium, fungi or protozoa. Bacterial infection e.g. *Staphylococcus aureus* and streptococcus species and *Pneumocystis carinii* are common in HIV positive patients and characterized by an abundance of neutrophils, granular cellular debris, histiocytes and granulation tissue. Colloid is absent in chronic granulomatous inflammatory reaction following a viral infection, FNA yields hypo cellular smear with clustered epithelioid cells (granuloma), scattered lymphocytes and a few multinucleated giant cells, containing up to one hundred nuclei, engulfing colloid.

2.3.1.2. Chronic lymphocytic thyroiditis (Hashimoto Thyroiditis)

This is an autoimmune inflammatory disorder of the thyroid gland characterized by the presence of numerous benign lymphoid cells admixed with plasma cells and Hurthle cells.

It’s characterized by lymphohistiocytic aggregates or follicular cells with oncocytic features (Hurthle cells) and variable nuclear atypia.

2.3.1.3. Follicular lesions

These encompass goiters as well as follicular neoplasms. A goiter is an enlarged thyroid gland resulting from benign non-neoplastic hyperplasia and colloid storage. Goiters can be simple or multinodular. Follicular neoplasms comprise of follicular adenomas and follicular carcinoma.

2.3.1.4. Benign Colloid Nodule.

This group includes solitary benign colloid nodules and prominent benign colloid nodules in a multinodular colloid goiter.

A benign colloid nodule on cytology yields in abundant, thick colloid material with cracking or bubble pattern and sheets of benign follicular epithelial cells in “honeycomb” arrangement.

2.3.1.5. Papillary thyroid carcinoma

HIV infection and especially the antiretroviral therapy predispose patients to the development of metabolic disorders of thyroid gland. Cytology shows presence of papillary structures, Orphan-Annie nuclei, intranuclear cytoplasmic invaginations (INCI), grooved nuclei and psammoma bodies. [32]

2.3.1.6. Medullary Carcinoma

This is a neuroendocrine tumor arising from the parafollicular cells or C-cells. Cytoplasmic feature of this neoplasm is a combination of elevated serum calcitonin and a thyroid nodule. Cytologically it is recognized by the presence of plasmacytoid, spindling single cells, and amyloid, nuclei with salt and paper chromatin and metachromatic neurosecretory granules.

2.3.1.7. Anaplastic giant and spindle cell carcinoma

This is a neoplasm that is rare, but deadly. It is composed of malignant highly pleomorphic large, spindling cells, some with elongate and bizarre shapes. The nuclei are dark with coarse chromatin. Tumor diathesis and abnormal mitotic figures are usually present, admixed with a variable amount of necrotic debris.

2.3.2. Soft Tissue Lesions

2.3.2.1.Kaposi’s sarcoma

It’s common in HIV patients also called epidemic Kaposi sarcoma. Clinically the disease patterns show circumscribed, cutaneous and subcutaneous nodules on the face, inside the mouth and throat, on the outside of the eye and on inner parts of the eyelids.

Cytomorphology smears have scanty cellularity with a bloody background. Scattered tissue fragments comprised of loosely cohesive clusters of spindle cells resembling granulomas, with stroma. The spindle cells are bland with large, oval nuclei with smooth contours, evenly dispersed chromatin, and non-prominent nucleoli. The neoplastic cells have mild pleomorphism, indistinct cell borders, and prominent nuclear streak artifact. Cytoplasm is moderate and delicate and typically forms tapering tails which blend with that of the adjacent cells. Hyalines globules are occasionally present. [33]

2.3.2.2. Hemangioma

Hemangioma is a benign cutaneous vessel proliferation that presents as a red papule that can mimic other vascular tumors appears usually in middle-aged adults. It is found in the skin and subcutaneous tissue, particularly of the head and neck producing a so-called strawberry mole.Arteriovenous hemangioma in a patient with HIV infection might present as a differential diagnosis of Kaposi's sarcoma. [34]

Microscopy is composed of endothelial cells in small sheets or single, which is variously round-to spindle-shaped. The cells have a moderate amount of cytoplasm that ranges from pale and delicate to dense and homogeneous with regular, bland, and typical nuclei with longitudinal fold or groove and hemosiderin-laden macrophages.

2.3.2.3. Angiosarcoma

Cancer of inner lining of blood vessels that arises in any part of the body especially the skin of the head and neck region. Cytomorphology shows round, oval, spindle, and epithelioid cells, single cells, pseudo-acinar and rosette-like formations, papillary structures, and may show well-formed small vessels. [35]

2.3.3. Lymph Nodes Lesions

Cytology of Lymph node was found to be a useful tool for selecting lymphadenopathy cases for further evaluation and for identification of opportunistic infections, malignant and nonmalignant lesions.

2.3.3.1. Acute lymphadenitis

This is due to inflammation of lymph nodes caused by bacteria draining into the lymph nodes. Patients present with tender, swollen and red node. Microscopically there is presence of inflammatory cells like neutrophils, few mature lymphocytes and tingible body macrophages with a background of granular cellular debris.

2.3.3.2. Chronic lymphadenitis

Also known as reactive hyperplasia is the inflammation for an extended time characterized by follicular hyperplasia, paracortical hyperplasia and sinus histiocytosis with an increase in size and number of follicles, immunoblasts, histiocytes polymorphous population.

2.3.3.3.Granulomatous Lymphadenitis

Granulomas are chronic inflammations caused by bacteria e.g. TB, actinomycosis, leprosy, syphilis, rhinoscleroma or brucellosis sarcoidosis and cat scratch disease. Fungal causes include actinomycosis and histoplasmosis. Cytomorphology shows epithelioid cells and multinucleated giant cells. [36]

2.3.4. Metastatic Malignancy

A metastatic malignancy/metastatic tumor is a type of cancer which has spread from the primary site of origin into different area of the body. The key to the diagnosis of a metastatic malignancy is the recognition of foreign cells in an aspirate.

2.3.4.1.Squamous cell carcinoma

People with HIV infection have increased susceptibility to opportunistic infections and viral-associated cancers. Larynx is the primary site for most metastatic Squamous cell carcinoma of the head and neck. SCCs are characterized by large clusters or singly dispersed pleomorphic bizarre cell shapes, spindle and malignant cells. The nuclei are enlarged, with irregular angular nuclear outlines, densely hyper chromatic nuclei and coarse chromatin. The cytoplasm may be non-keratinized or keratinized with necrotic background. [37]

2.3.4.2. Adenocarcinoma

Metastatic adenocarcinoma may be from lungs, gastrointestinal tract, breast, thyroid, pancreas and ovary**.** These present with medium sized to large cells with abundant delicate cytoplasm, acinic “ball like” pattern, rosettes, papillary architecture or round to oval, irregular nuclear outlines, hyperchromasia and prominent nucleoli. The cytoplasm is usually vacuolated. [38]

2.3.4.3. Small cell carcinoma

Small cell carcinoma is a highly aggressive malignant cancer that has distant metastasis. Most primary head and neck small cell carcinoma arise from the esophagus (secondary small cell carcinomas would be from the lung). The cells show nuclear molding with minimal amount of cytoplasm and stippled chromatin resulting in high nuclei/ cytoplasmic ratio. [39]

2.3.5. Lymphoma

Lymphomas are malignant neoplasms of lymphoid tissue. A common type of cancer diagnosed in both HIV positive and negative patients. There are two types of lymphomas which are Hodgkin lymphoma and non-Hodgkin lymphoma.

Hodgkin lymphoma (HL) is a tumor of lymphatic tissue cytologically it is characterized by poly-nuclear cells the Reed-Sternberg (RS). The cells are present in a particular cell milieu comprising, a mixture of tumor and non-tumor reactive cells (lymphocytes, eosinophils, neutrophils, plasma cells) sometimes surrounded by collagen fibers. [40]Highly aggressive non-Hodgkin lymphoma is classified based on size of the cells and the degree of membrane irregularity (cleaved vs. non-cleaved). Non-Hodgkin lymphoma is characterized by a diffuse proliferation of small non-cleaved cells of B-lymphocyte origin. [41]

3. RATIONALE OF THE STUDY

A number of opportunistic infections and malignancies of the head and neck are frequently encountered in HIV infection. Early diagnosis to identify and differentiate infections either benign or malignant pathology provides the best chance of successful treatment. Fine Needle Aspiration (FNA) technique is a simple, quick and cost effective method to sample superficial masses found in the head and neck region. FNA can be both diagnostic and therapeutic in cystic swellings. Tuberculosis, Kaposi's sarcoma, nonspecific reactive lymphadenitis, Hodgkin's and non-Hodgkin lymphoma, toxoplasmosis and metastatic carcinoma are some of the common findings in FNA diagnosis.

This study describes the fine needle aspiration cytological findings of accessible head and neck masses in HIV positive patients attending ENT, CCC clinic and FNA clinics in KNH. The results form a baseline to enable determination of differentiation of FNAfindings in HIV positive patients presenting with head and neck masses and in formulation of head and neck mass management policies. The findings from this studywillenable narrow down the differential diagnoses of head and neck masses in HIV positive patients at KNH.

3.1. Research Question

What are the fine needle aspiration cytological findings of accessible head and neck masses in HIV positive patients attending ENT, Comprehensive Care Centre and FNA clinics in KNH?

3.2. Objectives

3.2.1. Broad Objective

To identify the fine needle aspiration cytological features of accessible head and neck masses in HIV positive patient's attending ENT, Comprehensive Care Centre and FNA clinics in KNH.

3.2.2. Specific objectives

1. To determine the cytomorphological patterns of the head and neck masses in HIV positive patients attending ENT, Comprehensive Care Centre clinic and FNA clinics in KNH.
2. To determine the proportion of various pathological conditions detected by FNA in HIV positive patients presenting with head and neck masses.

3.2.3. Secondary objective

To correlate Cytomorphological findings in HIV positive patients with age, gender and clinical stage of HIV disease

4. STUDY DESIGN AND METHODOLOGY

4.1. Type of Study

A cross-sectional descriptive study

4.2. Independent Variables:

- Age
- Gender
- Type of the mass (lesion)

4.3. Dependent Variables:

- FNA diagnosis (cytomorphological pattern)

4.4. Study Area Description

1. **ENT clinic**- recruitment of HIV positive patients with head and neck lesions referred from other hospitals or clinics within KNH with head and neck lesions.
2. **Comprehensive care Centre clinic**- recruitment of clients with head and neck lesions booked for FNA procedure.
3. **FNA clinic**-this is where FNA procedure was performed for majority of patients referred from other clinics and health facilities within Nairobi and KNH outpatient clinics Clinic days are on Monday and Thursday morning.
4. **Histology laboratory in KNH**-this is where sample processing, examination and reporting was done.

4.5. Study Population

Study populations were HIV patients attending ENT clinic, Comprehensive Care Centre clinic and FNA clinics in KNH who presented with head and neck masses.

4.6. Study Eligibility Criteria

4.6.1. Inclusion Criteria

HIV positive patients presenting with head and neck masses (children and adults).

4.6.2. Exclusion criteria

- HIV negative individuals
- HIV positive with masses other than head and neck
- Patients previously treated for head and neck malignancies

4.7. Sample Size Determination

The sample size was calculated using the Fishers formula (similarly used by Ayugi et al 2006) at prevalence rate of malignancy at 32%

$n= z^2 p (1-p)/d^2$

Where

Z-level of confidence interval

P-prevalence rate

D-degree of precision set at + or – 10%.

$n = [1.96^2(0.32) (1-0.32)]/0.1^2$

= 84

4.7.1. Sampling Method

Consecutive sampling technique was used to achieve desired sample size.

4.8. Recruitment and Consenting/Assenting processes

HIV-positive patients were enrolled into the study after the HIV status was confirmed from the clinical notes or referral letters. Two nurses working at the ENT and CCC respectively were recruited as research assistants.

Potential participants were identified by the research assistants who introduce themselves. The benefits and rationale of the study were explained to all the participants by PI and the research assistants using informed consent and explanation form (Appendix 1)

For patients under the age of 18, consent was sought from the parents or guardian accompanying them. Assent was also sought from the minors. This was done by first explaining what was to be done and what was expected of them in a language they understood. They were asked if they agree to participate in the study. Those that agreed were

signed the assent form (Appendix 4). The clinical summary of the patient was recorded. The data was entered into the questionnaire (Appendix5). After that sample collection procedures were performed.

4.9. Training of Research Assistants

This was done by the principal investigator prior to the start of data collection. Content of the training included knowledge of clinical research terminologies, research protocols, research site management and professional ethics and the skills of data and specimen collection. This was to ensure they meet the standards required for the research project.

4.10. Specimen Collection for Laboratory Analysis

After obtaining a signed consent/assent from the patient the senior pathology registrar/ pathologist explained the procedure, assured and placed the patient in a comfortable and convenient position for sample collection. The pathologist palpated the lesion and they made several passes within the lesion using a 22 or 25-gauge needle. FNA sample was obtained and the material was placed on the microscopic slide by PI. Another slide was used to spread the material thus obtaining a thin conventional smear in 2 slides, which were fixed with 95% alcohol immediately by the PI and 2 air dried smears were prepared for special stains (Ziehl-Nielsen and Giemsa). On arrival in the laboratory the PI stained the smears using both Papanicolaou staining and Hematoxylin and eosin (H&E) methods for cytomorphological diagnosis. Ziehl-Nielsen stain was used in cases suggestive for tuberculosis and Giemsa stain was used in clinically suspicious cases for lymphoproliferative disorders. Screening of the slides was done by the PI then signed out with the supervisors.

4.11. Quality Assurance

The study was conducted by trained, qualified and competent personnel. Pre-analytical phase was used based on standard operating procedures on Specimen collection, preservation and transportation of specimen.

Analytical phase involved the preparation of reagents according to the manufacturer's instructions and SOPs was followed during all procedures. This involved checking reagents for expiration dates, turbidity, odor and precipitation. The storage condition for all reagents

was observed. Post analytical processed involves the interpretation of results and checking of clerical errors.

4.12. Ethical Considerations

The study protocol was submitted for approval by KNH-UON ERC. Inclusion in the study and collection of biological specimens was carried out only after obtaining a written informed assent/consent from each participant. The parents gave consent for patients below18years. Then those above 18 years gave consent for themselves. Participation was voluntary and no payment or incentives was offered to the study participants. The patient information will be held in total confidentiality and will only be revealed to the participant through the attending/referring clinician.

4.13. Data Management and Statistical Analysis

The age, study site, laboratory number, cytological morphological changes was tallied into data collection sheets after review by the PI (principal investigator) and the supervisor(s). The data sheets were filed and kept in lockable cabined then saved as soft copy and password protected.

Data was entered in Microsoft excel (MS2007), pass word protected and analyzed SPSS version15.0 for windows (SPSS.inc). Data was analyzed and presented in form of frequency, proportions/percentage, p-chart. Univariate analyses were used to describe distribution in demographics and cytological findings, bivariate analyses were used to compare distribution between variables. Fisher's exact for bivariate analyses: Used for categorical variable (presence/absence data) and on data with <5 observations per variable /category. A p-value<0.05was regarded statistically significant.

5. RESULTS

5.1. Demographics, Cytological Sites and Findings in HIV Patients with Head and Neck Mass

Between December 2018 and April 2019, 84 HIV positive patients with head and neck masses were enrolled. The majority of patients in this study were found in 12-24 years age group 22(26.2%) followed by 25-34 years age group 21(25%).Median age was 27 years (Interquatile range; 27 to 43 years). Female were 51(60.7%).

Majority of the lesions occurred on lymph nodes 46 (54.8%) and thyroid gland 22 (26. 2%).Granuloma was the most identified lesion 27 (32.1%) followed by Colloid goiter 15 (17.9%). Anaplastic carcinoma, Pleomorphic Adenoma and Round blue cell tumor were the least observed lesions at (1.2%) each (Table 1).

Table 1: Demographics, Cytological Sites and Findings in HIV Patients with Head and Neck Mass Visiting Kenyatta National Hospital for Fine Needle Aspiration

Characteristics	**cases (n=84)**	**%**
Age groups		
Under 5 years	5	6.0
5-11 years	6	7.1
12-24years	22	26.2
25-34years	21	25.0
35-49years	14	16.7
50 plus	16	19.1
Median age	27 years (IQR* 22-43 years)	
Gender		
Females	51	60.7
Anatomical site of the lesion		
Lymph nodes	46	54.8
Thyroid gland	22	26.2
Salivary gland	4	4.8
Submandibular	4	4.8
Others	8	9.0
Cytological features		
Granuloma	27	32.1
Colloid goiter	15	17.9
Epidermoid cyst	12	14.3
Reactive lymphadenitis	12	14.3
Lymphoma	5	6.0
Nasopharyngeal carcinoma	3	3.6
Squamous cell carcinoma	3	3.6
Hurthle cell neoplasm	2	2.4
pillar cyst	2	2.4
Anaplastic carcinoma	1	1.2
pleomorphic adenoma	1	1.2
Round blue cell tumor	1	1.2

5.2. Distribution of Lesion Sites by Age and Gender among Patients with Head and Neck

A commonest sites of lesion among adults and children patients with head and neck mass were lymph nodes and thyroid gland. Among adult patients (n=69) with lesions on lymph nodes (34.8%) were females and (17.4%) were males. Lymph nodes lesions were observed in n=15, (20%) female and (46.7%) male children. Thyroid lesions occurred in n=69, (20.3%) adult females and (13.3%) males. only female children n=15, (13.3%) had lesions on thyroid gland (table 2)

Table 2: Distribution of Lesion Sites by Age and Gender among HIV Positive Patients with Head and Neck Mass Attending Fine Needle Aspiration Clinic at Kenyatta National Hospital

	Adults (n=69)				Children (n=15)			
Site	**Female**	**%**	**male**	**%**	**female**	**%**	**Male**	**%**
Lymph nodes	24	34.8	12	17.4	3	20.0	7	46.7
Thyroid	14	20.3	6	8.7	2	13.3	0	0.0
Others	4	5.8	3	4.3	0	0.0	1	6.7
Salivary gland	3	4.3	1	1.4	0	0.0	0	0.0
Submandibular	1	1.4	1	1.4	0	0.0	2	13.3

5.3. Distribution of Lesions by Gender in HIV Positive Patients

Granuloma were common in adult patients (female (n= 51, 31.4%) and male (n=33, 27.3%)) and did not vary by gender. Colloid goiter occurred commonly among adult female patient (n=51, 25. 5%).All malignancies occurred among adult patients except for the round blue cell tumor, and did not vary by gender

Table 3: Distribution of cytological findings by gender and age among patients with head and neck mass attending FNA clinic at Kenyatta National Hospital

	Female (n=51)				Male (n=33)			
Cytological features	**Adult**	**%**	**Child**	**%**	**Adult**	**%**	**Child**	**%**
Granuloma	16	31.4	1	2.0	9	27.3	1	3.0
Colloid goiter	13	25.5	1	2.0	1	3.0	0	0.0
Epidermoid cyst	5	9.8	2	3.9	3	9.1	2	6.1
Lymphoma	3	5.9	0	0.0	2	6.1	0	0.0
Reactive lymphadenitis	3	5.9	1	2.0	2	6.1	6	18.2
Pilar cyst	2	3.9	0	0.0	0	0.0	0	0.0
Squamous cell carcinoma	2	3.9	0	0.0	1	3.0	0	0.0
Anaplastic carcinoma	1	2.0	0	0.0	0	0.0	0	0.0
Nasopharyngeal carcinoma	1	2.0	0	0.0	2	6.1	0	0.0
Hurthle cell neoplasm	0	0.0	0	0.0	2	6.1	0	0.0
Pleomorphic adenoma	0	0.0	0	0.0	1	3.0	0	0.0
Round blue cell tumour	0	0.0	0	0.0	0	0.0	1	3.0

5.4. Comparison of Cytological Finding in HIV Patients between Children and Adults

In comparing adults to children, most of the identified cytological lesions did not significantly differ between the two age groups except for reactive lymphadenitis which was less likely to occur in adults when compared to children (Odds ratio 0.10 [0.02-0.43]). The risk of reactive lymphadenitis was 90% (1-0.1*100) less likely to occur in adults compared to children.

Table 4: Comparison of cytological finding in HIV patients between children < 18 years old and adults 18> years old with head and neck mass attending FNA clinic at Kenyatta National Hospital

Final diagnosis	Adult (>=18 years)	%	Child (<18 years)	%	fisher's exact p value	odds ratio
Anaplastic carcinoma	1	1.5	0	0.0		
Colloid goiter	14	20.3	1	6.7	0.2871	
Epidermoid cyst	8	11.6	4	26.7	1.0000	
Granuloma	25	36.2	2	13.3	0.2137	
Hurthle cell neoplasm	2	2.9	0	0.0		
Lymphoma	5	7.3	0	0.0		
Nasopharyngeal carcinoma	3	4.4	0	0.0		
Pillar cyst	2	2.9	0	0.0		
Pleomorphic adenoma	1	1.5	0	0.0		
Reactive lymphadenitis	**5**	**7.3**	**7**	**46.7**	**0.0007**	**0.10 (0.02-0.43)**
Round blue cell tumor	0	0.0	1	6.7		
Squamous cell carcinoma	3	4.4	0	0.0		

5.5. Comparison of Cytomorphological Features between Male and Female Patients

Colloid goiter was less likely to occur in males compared to females OR 0.08 (0.01-0.61). The risk was 92% less likely in males compared to females. Risk of reactive lymphadenitis was three times higher (3.76 -1) in males compared to females.

Table 5 :Comparison of cytomorphological features between males and female patients with head and neck mass attending Fine Needle Aspiration clinic at Kenyatta National Hospital

Final diagnosis	Males	%	Females	%	Fisher's exact P Value	Odds ratio
Anaplastic carcinoma	0	0.0	1	2.0		
Colloid goiter	**1**	**3.0**	**14**	**27.5**	**0.004**	**0.08(0.01-0.61)**
Epidermoid cyst	5	15.2	7	13.7	1.000	
Granuloma	10	30.3	17	33.3	0.815	
Hurthle cell neoplasm	2	6.1	0	0.0		
Lymphoma	2	6.1	3	5.9	1.000	
Nasopharyngeal carcinoma	2	6.1	1	2.0	0.558	
Pilar cyst	0	0.0	2	3.9		
Pleomorphic adenoma	1	3.0	0	0.0		
Reactive lymphadenitis	**8**	**24.2**	**4**	**7.8**	**0.054**	**3.76 (0.89-18.48)**
Round blue cell tumor	1	3.0	0	0.0		
Squamous cell carcinoma	1	3.0	2	3.9	1.000	

5.6. Cytology of Head and Neck in HIV Positive

In HIV positive patients the most common lesion was granuloma (suppurative and necrotizing) 27 (32. 14%). A ZN test was performed for 27 granulomas which were suspicious for TB. Of these 7(25.9%) were positive for TB. Benign, colloid goiter was the second common lesion 15(17.86%) followed by epidermoid cyst 12(14.29%), reactive lymphadenitis 12(14.29%), suspicious lymphoma 5(5.95%), nasopharyngeal carcinoma 3(3.57%), Hurthle cell neoplasm 2 (2.38%), pillar cyst2 (2.38), Anaplastic carcinoma (1.19%), pleomorphic adenoma 1(1.19%) and round blue cell tumour 1 (1.19%).

Table 6 : Common head and neck diagnosed in HIV positive

Final diagnosis	number (n=84)	%
Granuloma	27	32.14
Colloid goiter	15	17.86
Epidermoid cyst	12	14.29
Reactive lymphadenitis	12	14.29
Lymphoma	5	5.95
Nasopharyngeal carcinoma	3	3.57
Squamous cell carcinoma	3	3.57
Hurthle cell neoplasm	2	2.38
pillar cyst	2	2.38
Anaplastic carcinoma	1	1.19
Pleomorphic adenoma	1	1.19
Round blue cell tumour	1	1.19

5.7 Distribution of Lesions by Anatomical Sites

The anatomic distribution of lesions is presented by the pie chats below.

5.7.1 Lymph Nodes

Granuloma (suppurative and necrotizing lymphadenitis) was the most common lesion 22(48%) found in lymph nodes, followed by reactive lymphadenitis11 (24%), suspicious lymphoma was 5(11%), nasopharyngeal carcinoma 3(7%), epidermoid cyst 2 (4%), squamous cell carcinoma 2(4%), and round blue cell tumor 1(2.17%) spectively.

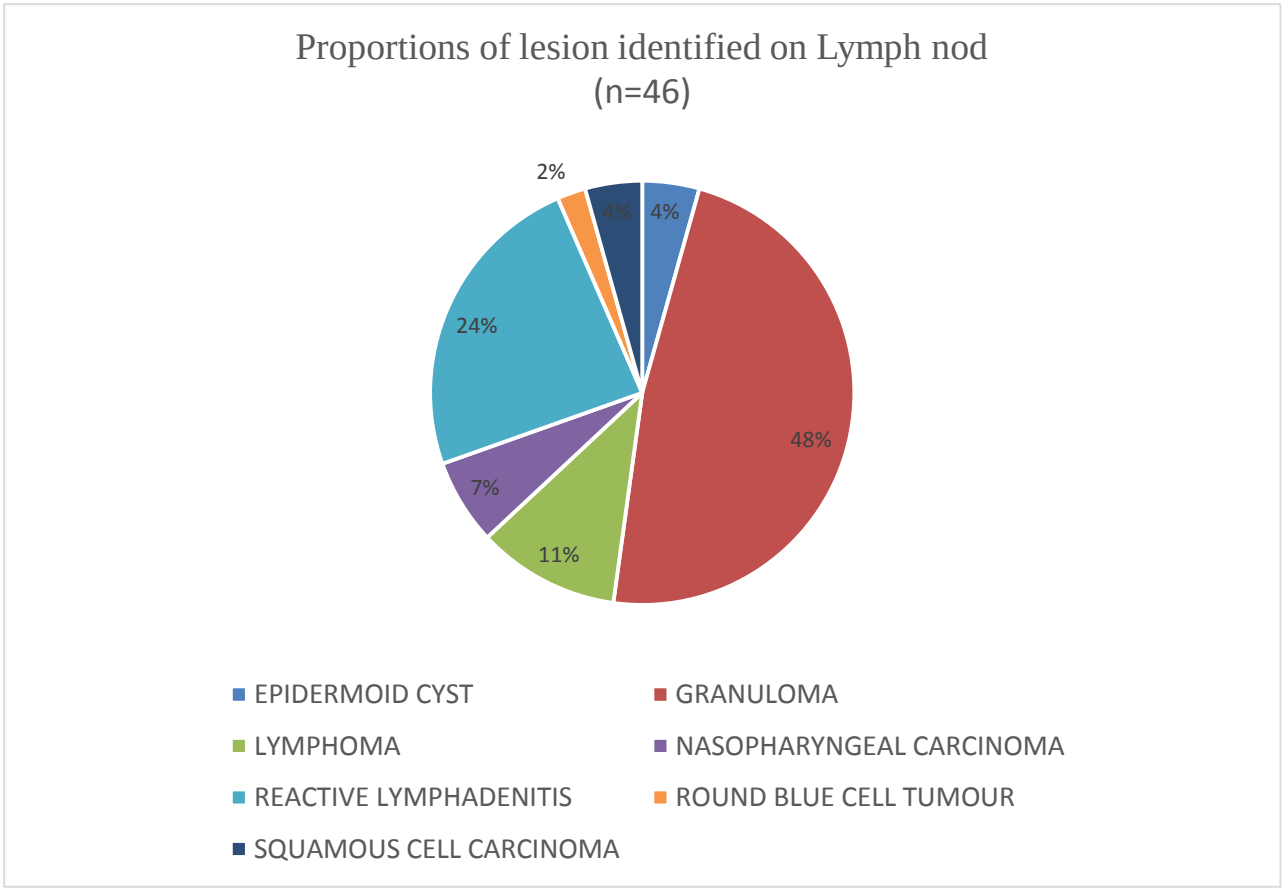

Figure 1: Proportions of Lesion Identified on Lymph Node

5.7.2 Thyroid gland

Colloid goiter was the most common lesion in thyroid gland 15(68%) and was followed by granulomatous thyroiditis 2 (9%), Hurthle cell neoplasm 2(9%), Anaplastic carcinoma 1(4%), squamous cell carcinoma 1(4%).

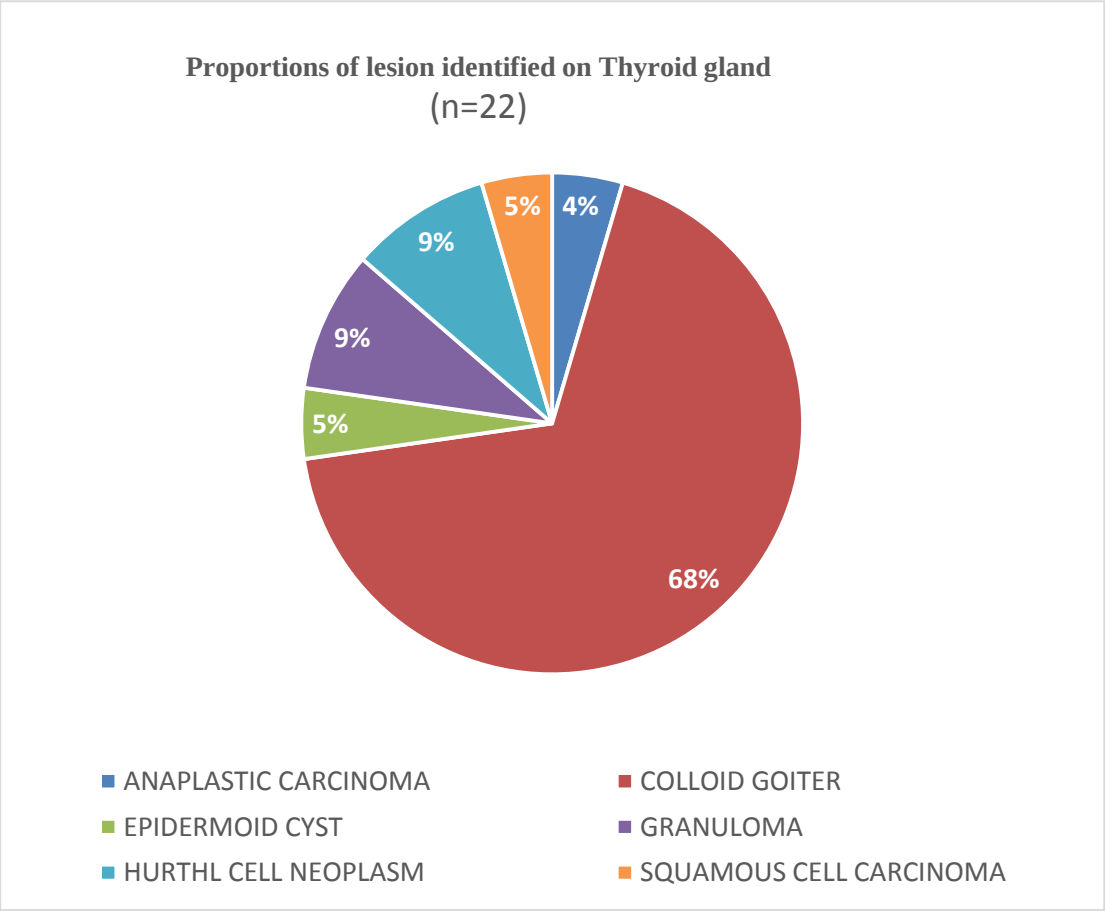

Figure 2: Proportion of Lesion On Thyroid Gland

5.7.3 Salivary glands

About half of salivary gland lesion were epidermoid cyst 2(50%) and were followed by granulomatous 1(25%) and pleomorphic adenoma 1(25%).

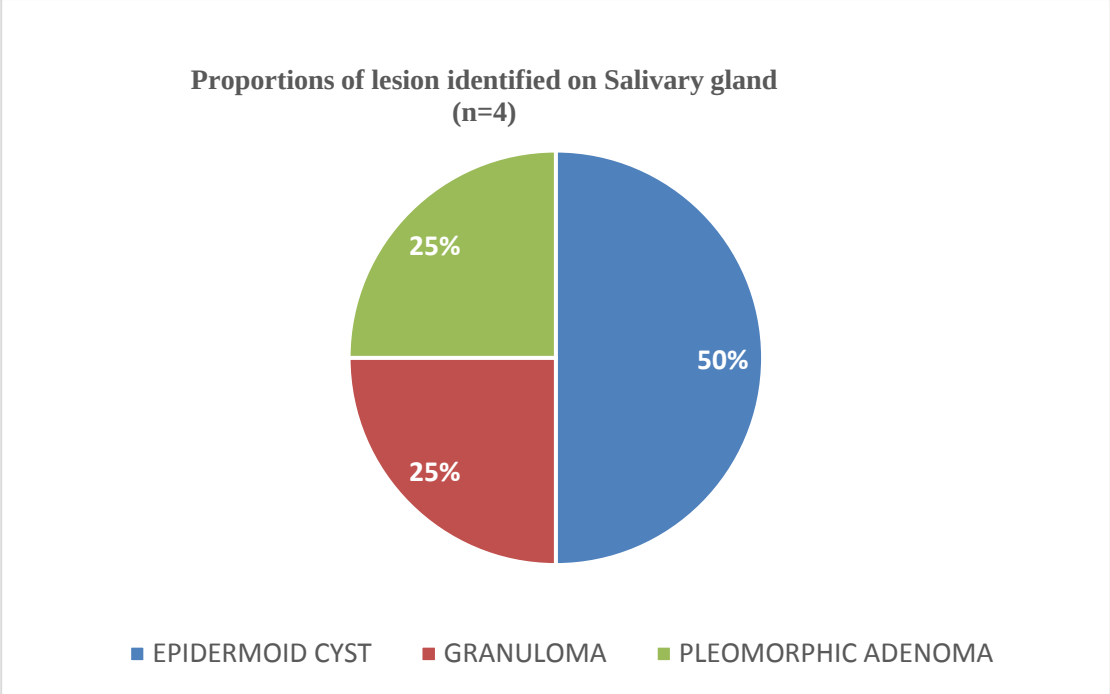

Figure 3:Proportions of Lesions Identified on Salivary Gland

5.7.4 Submandibular swelling

The two Submandibular lesions were epidermoid cyst 2(50%) and granuloma 2(50%).

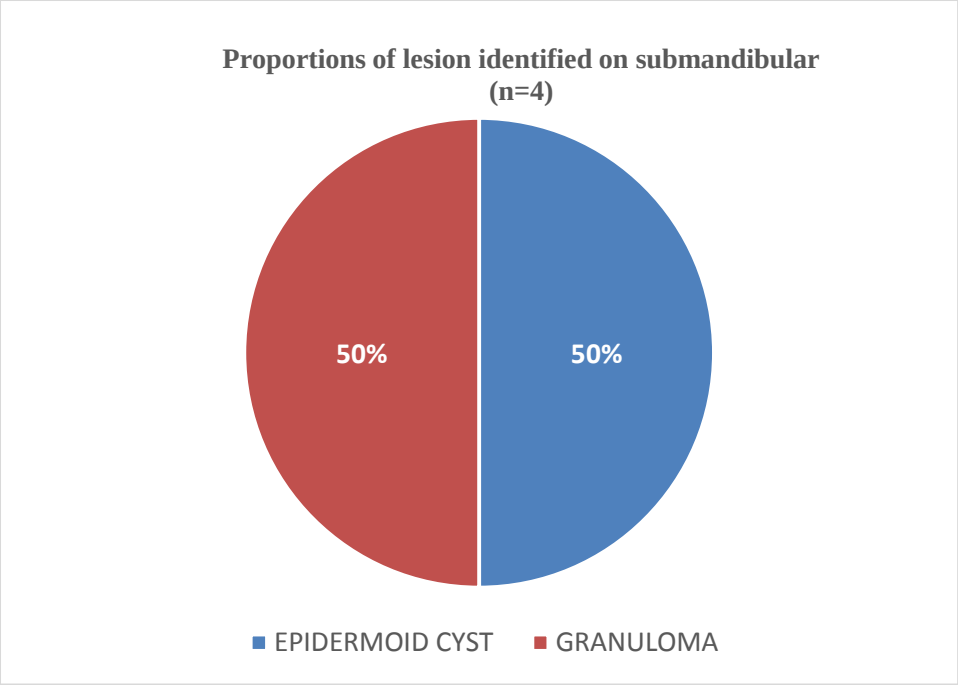

Figure 4:Proportions of Lesion Identified on Submandibular

5.7.5 Others

These comprised occipital and posterior neck masses. The commonest diagnosis in these sites were epidermoid cyst 5(62.5%), Pilar cyst 2(25%) and reactive lymphadenitis1 (12.5%).

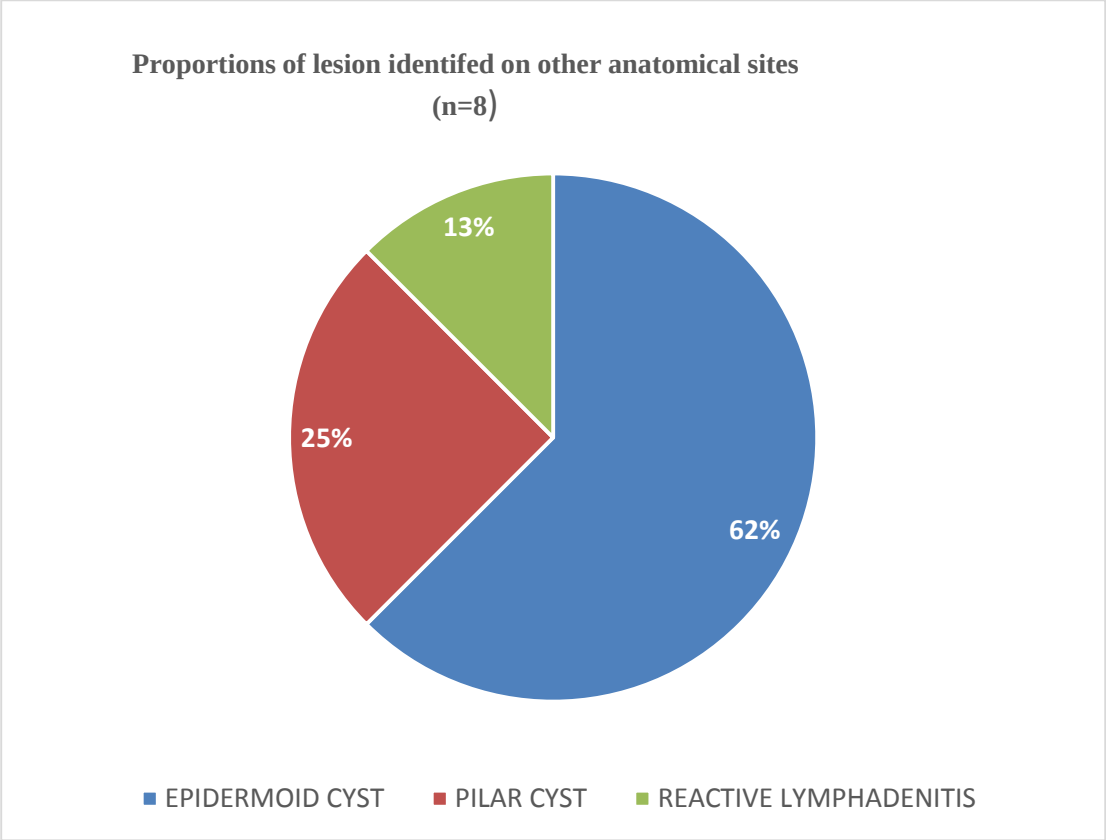

Figure 5:Proportions of Lesion Identified on other Anatomical Sites

5.8 Photomicrographs of the Lesions

Below are photomicrographs and description of selected lesions

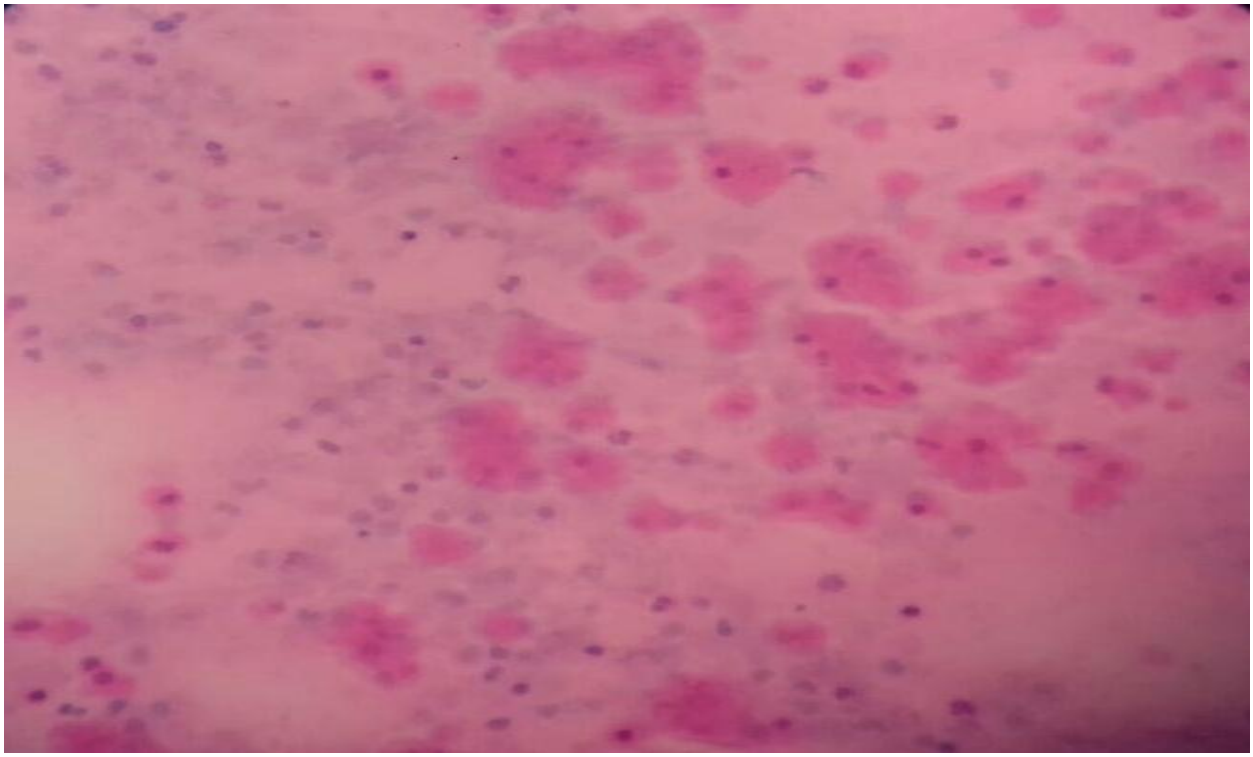

Figure 6: Case 1, Inflamed epidermoid cyst: Squamous cells, anucleate squames and inflammatory cells (Leica) (X40, H&E stain)

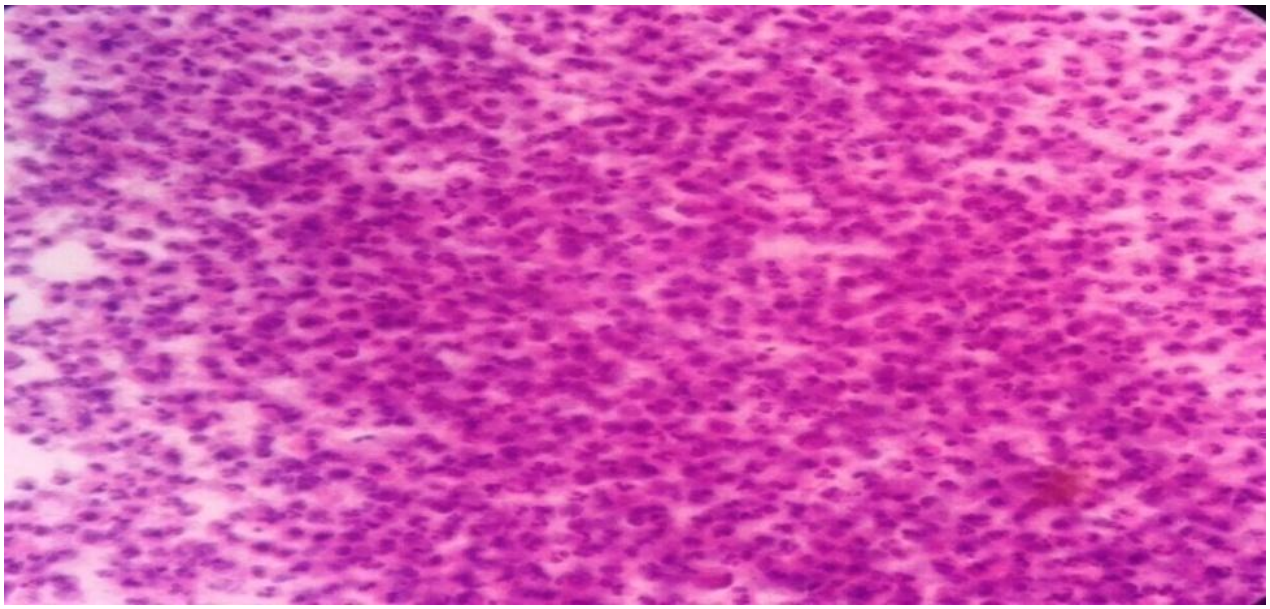

Figure 7: Case 68, Suppurative thyroiditis: acute inflammatory cells (pus cells)(Leica) (X40, H&E stain).

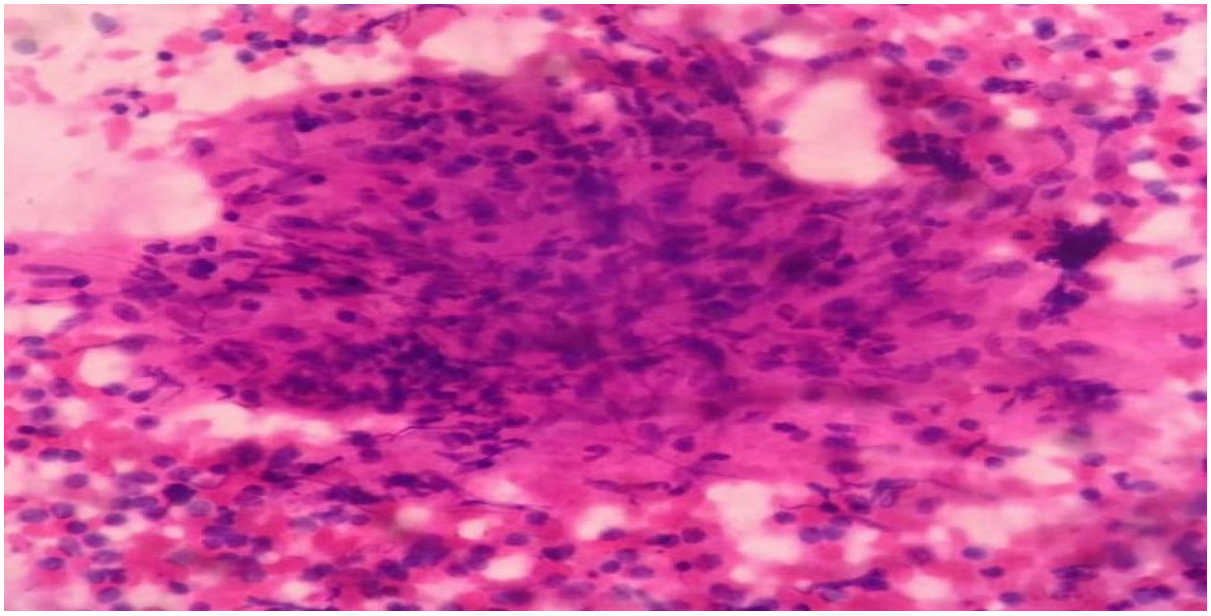

Figure 8:Case23, Cytomorphological features of chronic granulomatous lymphadenitis. The granulomas were composed of syncytial aggregates of oval or fusiform epithelioid histiocytes sometimes bent or curved nuclei, Langhan type giant cells were very occasionally seen lymphocytes were present in the back ground. (Leica) (X40, H&E stain).

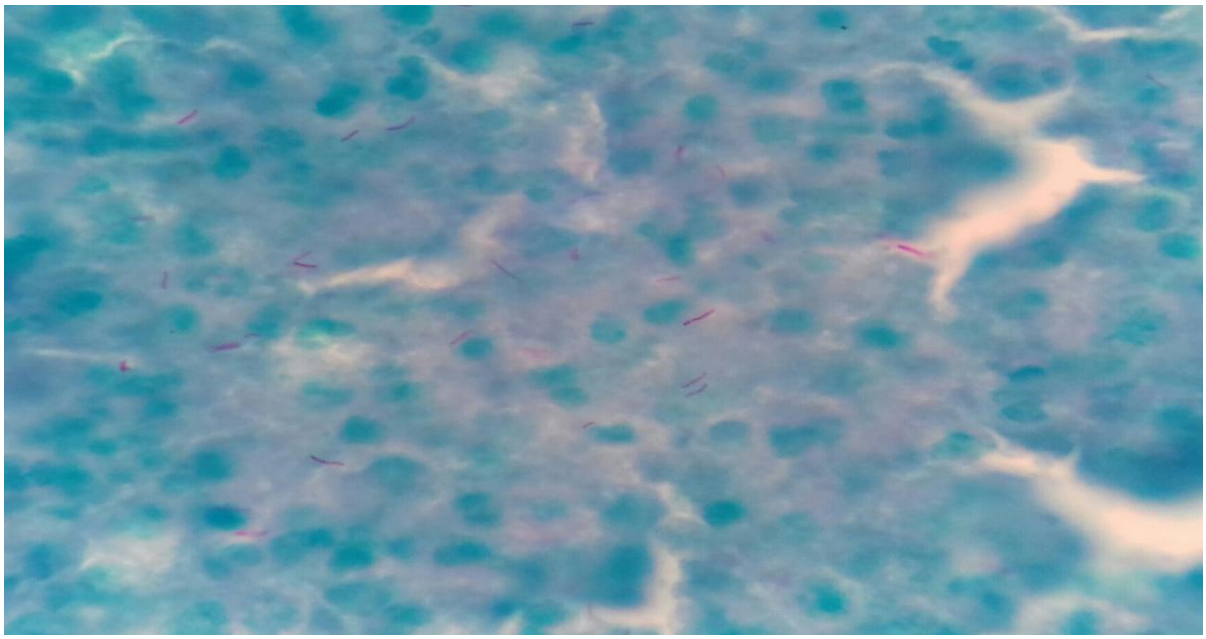

Figure 9: Case3, positive for tuberculosis: Acid fast bacilli stained red (Leica) (X100, ZN stain).

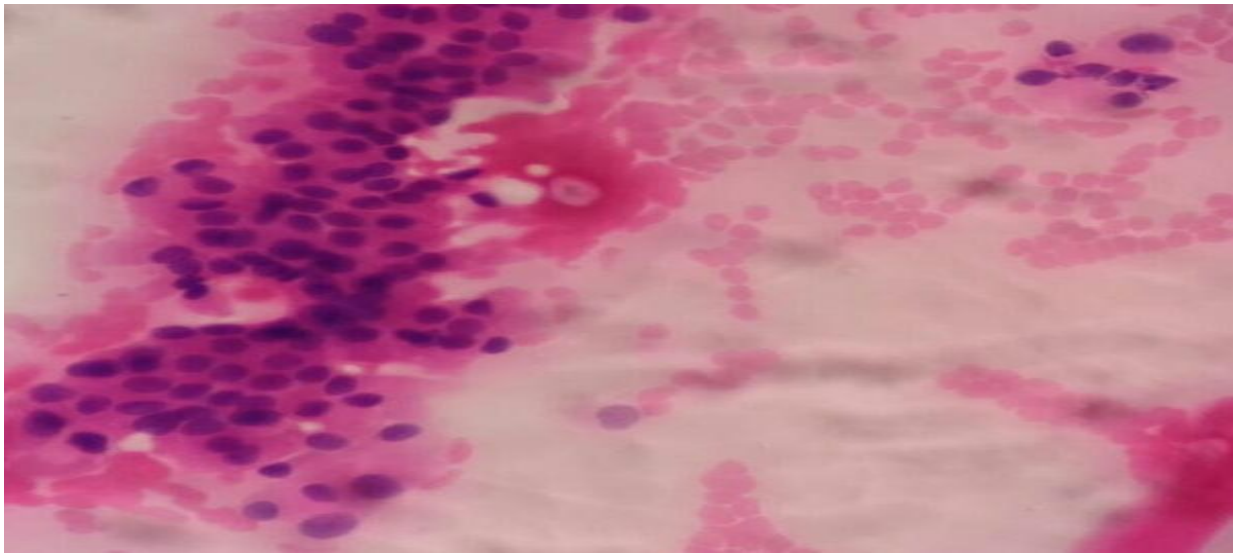

Figure 10: Case 22, Hurthle cell neoplasms: Macrofollicle, microfollicle Hurthle cells with scant colloid in the back ground (Leica) (X100, H&E stain).

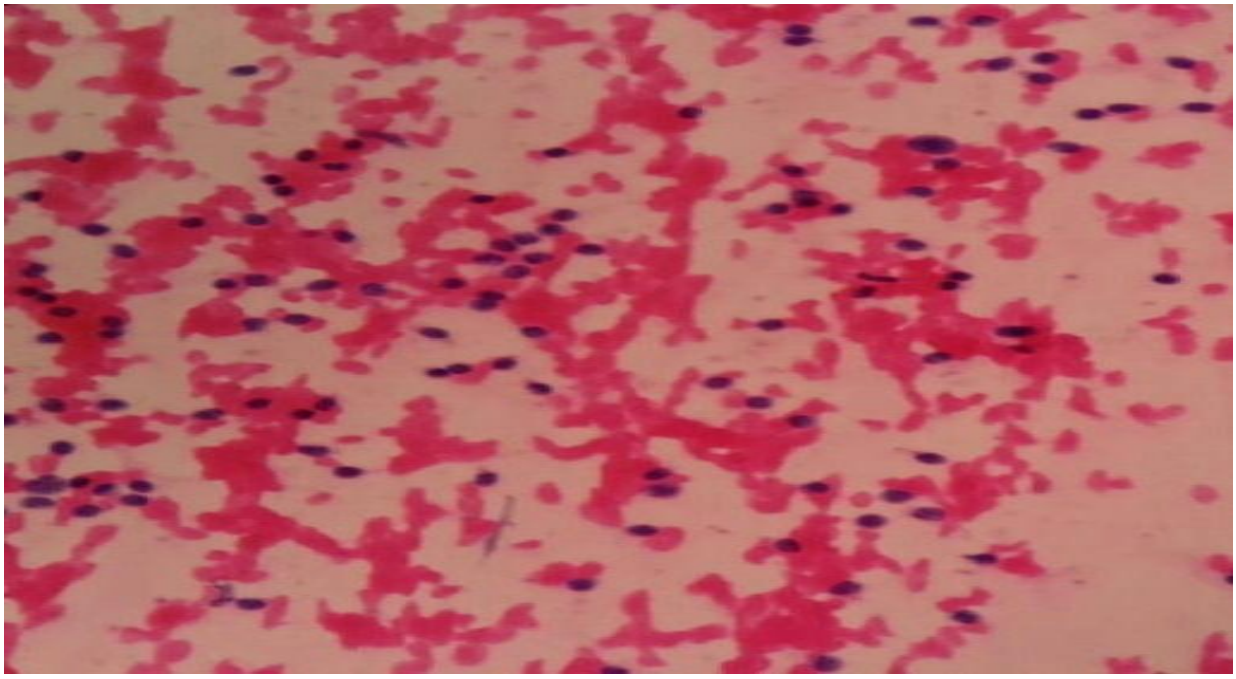

Figure 11: Case 41, Cytomorphological features of reactive lymphadenitis. The diagnosis of reactive lymphadenitis was based on finding a heterogeneous population of cells, which included a spectrum of small and large immature lymphocytes (Leica) (X40, H&E)

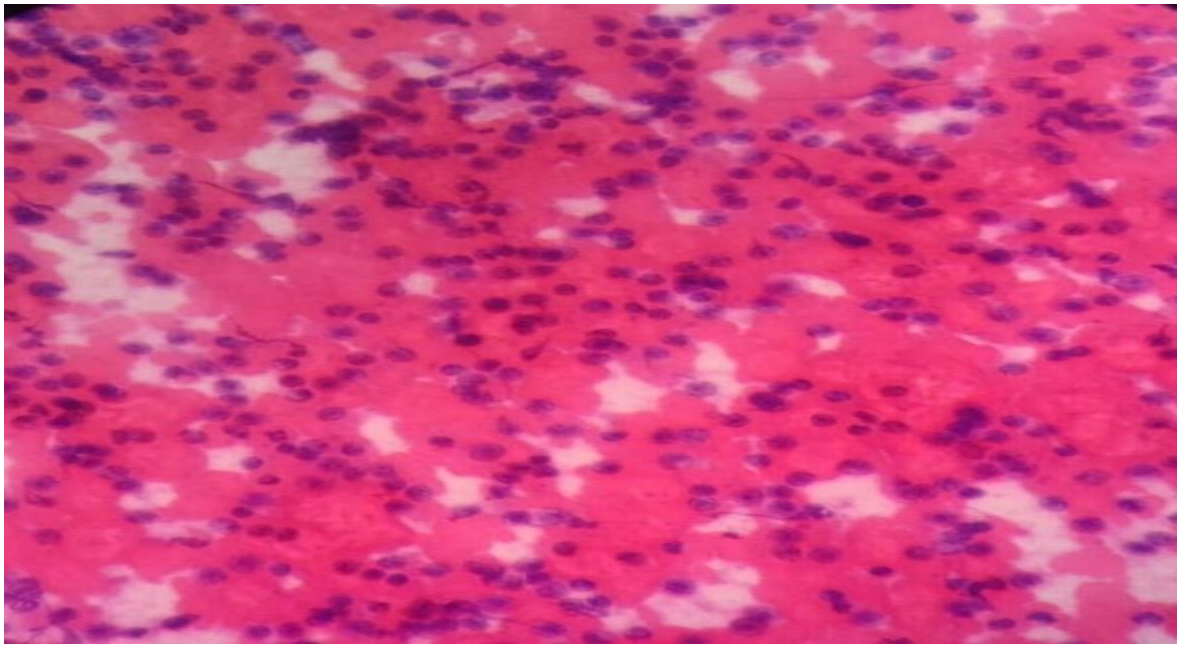

Figure 12: Case 44, suspicious for lymphoma: monotonous population of lymphoid cells (Leica) (X40, H&E). Immunocytochemistry and biopsy were recommended.

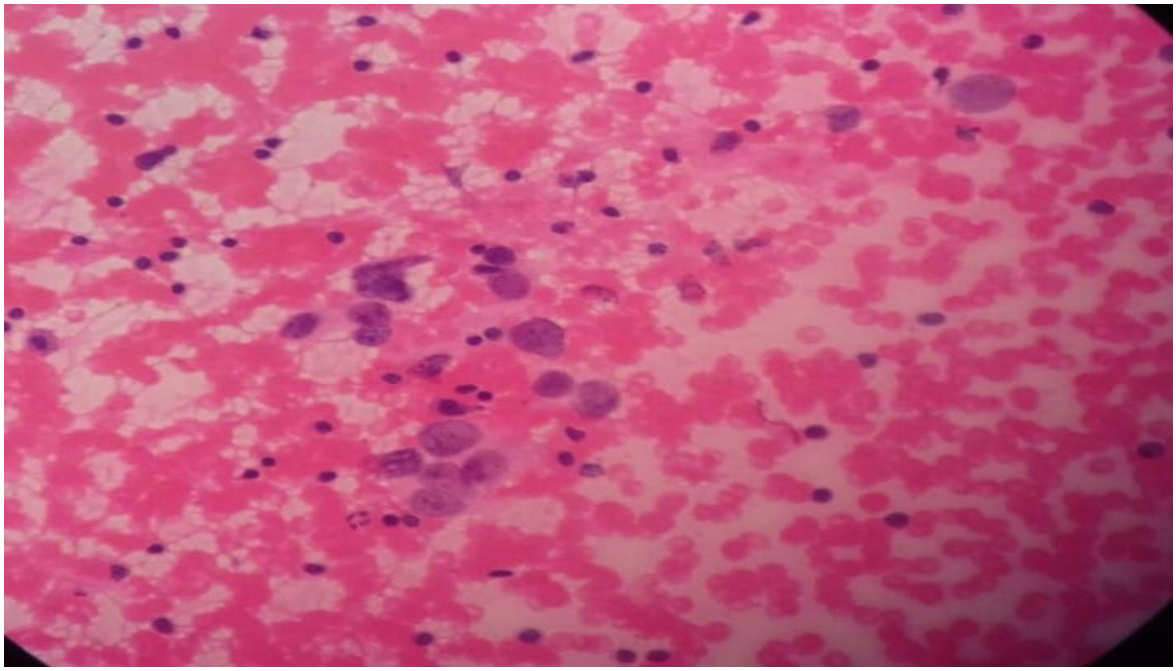

Figure 13:Case43, Positive for malignancy, large cells which are loosely cohesive they are pleomorphic, round to oval irregular nuclear membrane coarse chromatin. DDX: Nasopharyngeal carcinoma: Immunocytology and biopsy were recommended (keratin, EMA, EBV) (Leica)

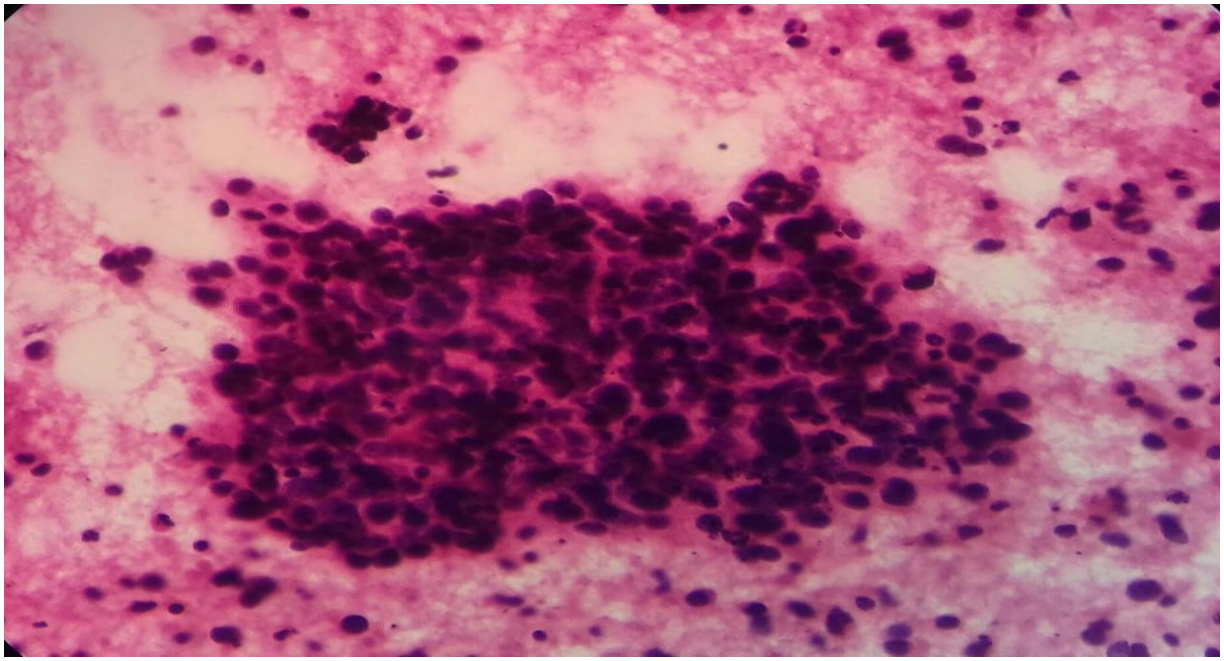

Figure 9: Case 55, Positive for malignancy round blue cell; cluster of malignant cells which are cohesive overlapping the cells are pleomorphic hyper chromatic increased N: C ratio nuclear membrane is irregular mitotic Fugger and apoptotic bodies were present. Tumor DDX: Neuroblastoma, immunocytochemistry and biopsy were recommended (EMA, CD56) (Leica) (X40, H&E).

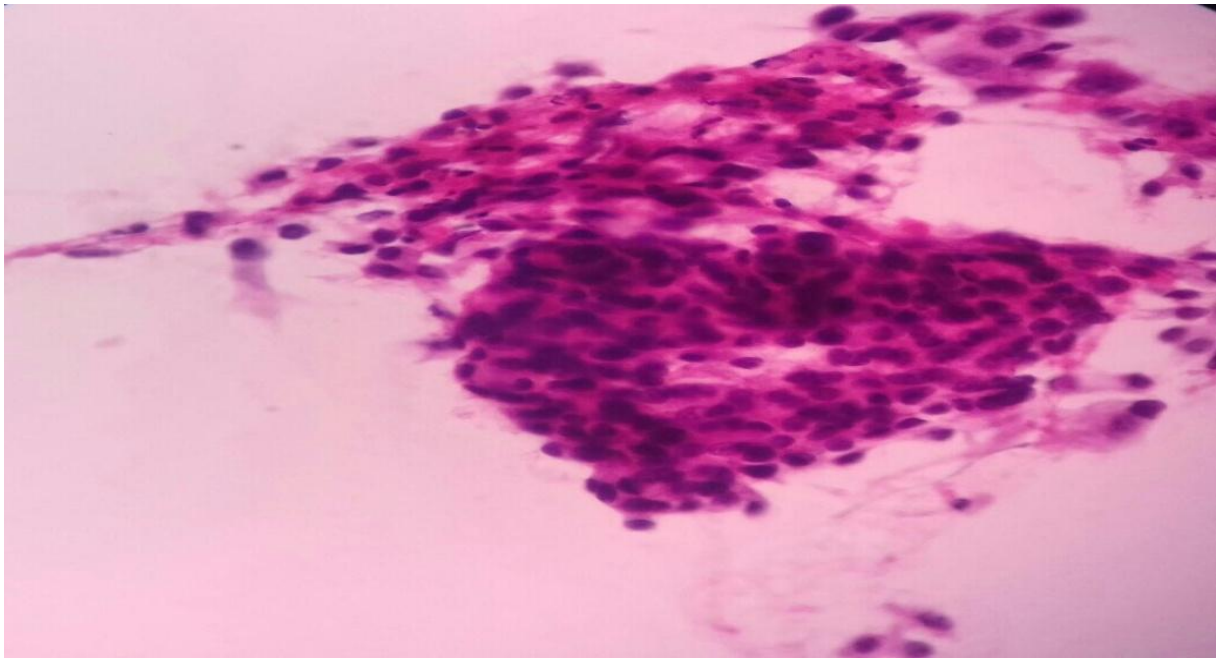

Figure 10: Case62, Positive for Squamous cells carcinoma of the Thyroid FNA showing pleomorphic cells with abundant eosinophilic cytoplasm and keratin formation along small, pyknotic nuclei and low nuclear to cytoplasmic ratios. DDX: Anaplastic carcinoma of Thy (Immunos and histology were recommended). (Lexica) (X40, H&E stain).

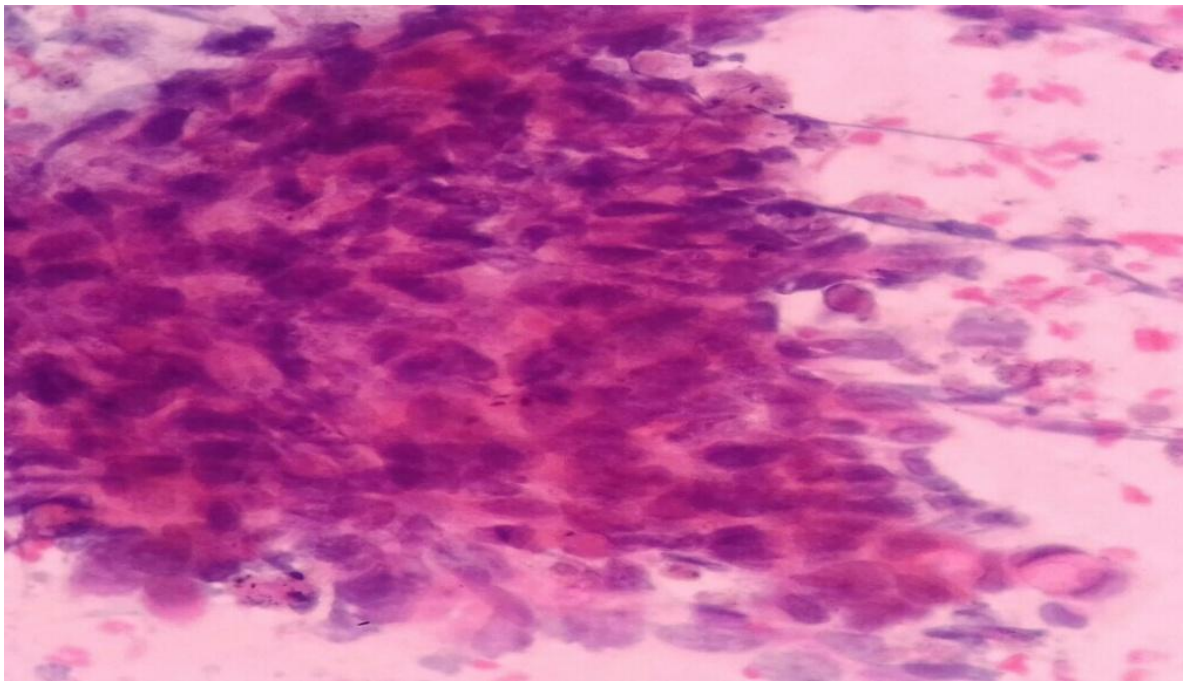

Figure 11: Case 45, Cytomorphological features of metastatic squamous cell carcinoma. A cluster of squamous cells with abundant dense cytoplasm. Squamous cells with round to oval, small, pyknotic nuclei and low nuclear to cytoplasmic ratios with tumor diathesis in the background. (Lexica) (X40, H&E stain).

6 DISCUSSION

Between December 2018 and April 2019, a total of 84 HIV positive patients with head and neck mass were enrolled in the study. Adults 20 to 29 years old were the majority followed by adults 40 to 49 years old in which maximum numbers of cases are in sexually active age group. Out of 84 participants 51(60.7%) were female, 33(39.3%) were male, children were 15(17. 86%). We have more Females who are HIV infected in the population and has more HIV related conditions [(45)]. This is in total contrast to a study done by El Hag et al in Saudi Arabia which showed an even gender distribution of 50/50[(52)].

FNAC has proven to be an easy, quick, reliable, and cost-effective tool for head and neck lesions. It is suitable for an initial rapid diagnosis in HIV positive patients with lymphadenopathy and others masses, among the peripheral lymph nodes involved, cervical lymphadenopathy was the common. The majority of cases were found in the age group 20-29 years, followed by 40-49 years. In a study done by Shenoy *et al*, the age group commonly affected was between 25 - 30 years with cervical group of nodes being the most common site [(46)].

In Kenya the most common opportunistic infection in AIDS patients is tuberculosis. In our study, majority of the lesions occurred in lymph nodes 46 (55%); granulomas were the most common finding identified, 27(32.14%) and seven of this were positive for tuberculosis (25.92%). They were: 1) Caseating granuloma showing classical epithelioid granuloma, giant cells, and caseation in a milieu of lymphoid cells; 2) Granulomatous lymphadenitis showing only granulomas with or without giant cells; 3) Necrotising lymphadenitis which showed degenerating epithelioid cells in a necrotic background and 4) Suppurative lymphadenitis where smears showed degenerating and viable neutrophils. In various studies elsewhere different distributions of granulomatous lesions in HIV positive patients with head and masses have been described .In Mangalore, Shenoy at al found 50%[(46)] ,in Malaysia Jayaram et al found 53.84%[(47)]. Other studies in India have shown 57.67% and 58.3% distribution of the granulomas in HIV positive with head and neck masses [(48; 49)].

The FNA material obtained from lymph nodes with tuberculous lymphadenitis showed caseation necrosis or epithelioid granulomas identical in appearance with those seen in HIV negative patients. A definite Cytologic diagnosis of tuberculous lymphadenitis can be offered in smears with Caseating granulomas with or without giant cells, while the necrotizing suppurative smears would be dismissed as acute suppurative lymphadenitis in the absence of

Ziehl-Nielsen stain. Thus Ziehl-Nielsen staining should be performed on all aspirates from cases of suspected tuberculosis. Tuberculous lymphadenitis may be more common in HIV patients with superficial lymphadenopathy than is generally believed. Greater use of lymph node aspiration or biopsy may improve the diagnosis of suspected tuberculosis.

Reactive lymphadenitis was the fourth common finding in the present study 12(14. 29%).Similar observations were also made by Vanisri *et al*[49] (36.1%). Reactive lymphadenitis was more common in children than in adults 7/12(58.33 %,), 5/12(41. 66%).In comparing adults to children the risk of reactive lymphadenitis significantly differed between the two groups where the risk of reactive lymphadenitis was 90% less likely to occur in adults compared to children. Lymph node FNAC is avaluable investigation in HIV patients where most opportunistic diseases (bacterial and malignancy) can be correctly identified and high-grade lymphoma can be diagnosed [47].

The second most common site of aspiration was from the thyroid, where thyroid lesions were common in both adult 20.3% and children 13.3% female patients. Benign colloid goiter was the most common lesion diagnosed in thyroid in this study 17.86%. Two malignant which are Anaplastic carcinoma and squamous cell carcinoma 4.55% each, the study reported two Hurthle cell neoplasm 9.09% which is again similar to those reported in other studies by Caruso D et al 20% [50] Granulomatous thyroiditis 9.09%, Epidermoid cyst 4.55%. Management guidelines recommend follow up in benign conditions, treatment for inflammatory conditions, and lobotomy in neoplastic lesions and thyroidectomy in malignant conditions.

Three types of malignancies were found accounting for 3.6% of the total and included nasopharyngeal carcinoma representing 3.57%, squamous cells carcinoma where two of these were metastases and one was primary in the thyroid, one anaplastic carcinoma, and one round blue cell tumor. Similar observations were made by Rajesh et al in 2014 of a low rate of malignancies, which included one metastatic adenocarcinoma, the other two being lymphomas.

Among the cases suspicious for malignancy, five were suspicious for lymphomas, 2.4% were hurtle cell neoplasms same observations made by Nayak et al in 2003. The role of FNA in the cyto-diagnosis of lymphoma is controversial. In our study where five cases were suspicious for lymphoma, a surgical biopsy was recommended for a definitive diagnosis. Thus, the role

of FNA was limited to the identification of cases for referral for further management as promptly as possible, making it a screening test. The limitation of cytomorphological diagnosis and classification of lymphoma can be overcome by the use of various ancillary techniques such as Immunocytochemistry which can be done on cell blocks and aspirates respectively [51]. Based on management guidelines, surgery can be avoided in inflammatory and metastatic lesions. Therefore, FNA of lymph nodes in HIV positive patients makes surgery for diagnosis in many cases.

Salivary gland aspirates (n=5) consisted of two epidermoid cysts, one granulomatous inflammation and one pleomorphic adenoma. The procedure was valuable and reliable in distinguishing inflammatory and simple cystic lesions from neoplastic ones, which are treated differently. Our findings confirm that FNA is helpful for the diagnosis and treatment planning of salivary gland lesions in HIV patients, for instance, a pleomorphic adenoma requires surgery with wide margins to avoid recurrence. Based on management guidelines, surgery can be avoided in inflammatory conditions while benign cystic lesions require surgical excision.

FNAC of head and neck in HIV/AIDS patients with clinical correlation can provide most useful information to physicians to determine the further mode of management. However, to obtain maximum benefit from the procedure, co-operation between patients, trained cytopathologist and an experienced clinician is essential. With today's increasing cost of medical practice, any technique which speeds up the process of diagnosis is of tremendous value.

6.7 Conclusion

1. Commonest benign findings were TB adenitis and colloid goiter in adult females while in children the most common lesion was reactive lymphadenitis.

2. Commonest neoplastic lesion in adults was lymphoma followed by Squamous cell carcinoma while in children it was only one round blue cell tumor.

3. Head and neck swellings in HIV positive can readily be screened for malignancy using FNA.
4. In HIV positive patient with head and neck swellings the pathological processes can readily be diagnosed by Cytomorphology.

Limitations

Could not establish clinical stage of HIV disease because the enrolled patients were referrals and did not have clinical information on the stage of the disease. Out of 84 patients only 8 (6.72%) had HIV clinical staging 76 did not have.

6.8 Recommendation

1. FNA should be performed on HIV positive patients with head and neck masses to classify the lesions for better management of the lesions and prevention of unnecessary surgical interventions.

2. We recommend that Fine Needle Aspiration Cytology should be the first diagnostic procedure in clinics for the diagnosis of the cause of lymphadenopathy in HIV/AIDS patient

3. Lesions diagnosed as Granulomas should have special stains such as Ziehl Neelsen done even as PCR is done.

4. Further studies on FNA of head and neck for pediatric age groups should be undertaken to determine utility in diagnoses and management of these age group.

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APPENDICES

Appendix I: Informed Consent Explanation (Adults)

My name is SERAPHINEMUKANGAMIJE, a post graduate student doing a Master of Science degree in Clinical Cytology in a Human Pathology Department, University of Nairobi.

Research title:

Fine needle aspiration cytological findings in HIV positive patients presenting with head and neck masses at KENYATTA NATIONAL HOSPITAL

The objective of the study

Broad Objective

To determine the fine needle aspiration cytological findings of accessible head and neck masses in HIV positive patients attending ENT, Comprehensive Care Centre and FNA clinics in KNH

Benefits and Risks of the study to patients:

Benefits: Patients will benefit from this study because there will be additional treatment for those who will be positive for malignancy or for infectious diseases, also the special stains will be done in case it's needed at no cost to the patient.

Risks: The aspiration procedure is negligibly traumatic; however, this is tolerable. Sometime hematoma formation and infection may occur at the site of the procedure, but it's manageable so the procedure will be done by a trained expert.

Procedure

After the patient agrees to participate in the study a needle will be inserted into the mass. Material aspirated will be evaluated under the microscope to evaluate/determine the pathology behind the mass.

Confidentiality

Study numbers will be used instead of names. The questionnaires will be kept under lock and only the principal investigator will access it. Also those questionnaires will be kept for one

year after which they will be destroyed. Any information given to us will remain confidential and will be for client benefit.

Withdrawal from study

Participation in this study will be voluntary and patient will be allowed to withdraw any time he/she wants and will be managed routinely.

Date ----------

Signature of Patient / Relative----------

Signature of questionnaire administrator ----------

Contact information

In case of any question regarding this study you can contact me **SERAPHINEMUKANGAMIJE** mobile number +250784072596/+254795717953 or my supervisors **Professor L.Muchiri** and**Dr. W.Waweru** at theUniversity of Nairobi, P.O BOX 1976-00202 Nairobi or telephone number Tel:726300 Ex 43774. And if you have any ethical issue regarding the conduct of this study, please contact Prof M.L. CHINDIA, The Secretary, KNH/UON Ethical Research committee, Tel: 726300-9Ext44102.

Appendix II: Consent Form

I…….after reading and being explained the study purpose and what it entails I do hereby give informed consent to participate in the diagnostic study fully aware of the benefits.

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

Participants signature /Thumb print……..date …….

Doctor/Nurse signature……..date……

Principle investigator……date……

Appendix III: Assent Form

My name is **Seraphine Mukangamije** and I am doing a study to determine what could have caused your condition.

If you agree to be in my study, I request your permission to use the specimen which will be removed from your lesion so that I use it in my study. There are no risks involved as I will just use part of the tissue that will be removed for the initial diagnosis of the disease.

You can ask questions about this study at any time. If you decide at any time not to finish, you can ask us to stop.

If you sign this paper, it means that you have read this and that you want to be in the study. If you don't want to be in the study, don't sign this paper. Being in the study is up to you, and no one will be upset if you don't sign this paper or if you change your mind later. Your parent / guardian have to consent for you. Remember there is no monetary benefits you are going to receive.

I have read this information (or had the information read to me) I have had my questions answered and know that I can ask questions later if I have them.

I agree to take part in the research.

Only if child assents:

Name of child _____ AGE_____

Date: _____

Day/month/year

If illiterate*:*

I have witnessed the accurate reading of the assent form to the child, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Name of witness (not a parent) _____ AND Thumb print of participant

Signature of witness _____

Date _____

Day/month/year

I have accurately read or witnessed the accurate reading of the assent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given assent freely.

Name of researcher_____

Signature of researcher_____

Date_____

Day/month/year

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the child has understood.

I confirm that the child was given an opportunity to ask questions about the study, and all the questions asked by him/her have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Print Name of assistant Researcher_____

Signature of principle investigator _____Date _____

Parent/Guardian has signed an informed consent ___Yes ___No _____ (initialed by researcher/assistant)

Appendix IV: ChetiCha Kukubali

Mimi SERAPHINE MUKANGAMIJE, mwanafunzi washahada la udhamili katika sayansi ya chembechembe za mwili, kitengo cha magonjwa ya binadamu, katika chuo kikuu cha Nairobi. Ninaji shughulisha na utafiti wakuonyesha matumishiya ‘fine needle aspiration cytology’kwauvimbewakichwanashingokwawatotonawatuwazimakatikahospitalirufaaya Kenyatta.

Kichwa cha utafiti

Matokeo ya chembechembe ya ‘fine needle aspiration’wangojwawenyeuvimbewakichwanashingokatikahospitaliyarufaaya Kenyatta.

Lengo kuu la utafitihuu ni

Kuonyesha matumizi ya ‘fine needle aspiration’ katika uvimbe wa shingo na kichwa kwa watoto na watu wakubwa katika hospitali ya rufaa ya Kenyatta.

Faida na hatari ya utafiti huu

<u>Faida kuu</u>

Wagonjwa watafaidika katika utafiti huu; kwa uchunguzi Zaidi utafanywa kwa mahabara.

<u>Hatarikubwa</u>

Madhara ya kudungwa na sindano siokubwamnolakinihilininafuukwasababulaweza kuvumiliwa, la ziada, madhara ya uvimbena maaambukizi yaweza tokea kwa mahali palipo dungwa sindano, ijapokuwa hili laweza shughulikiwa.

Utaratibu

Baada ya kukubali kuhusika katika utafiti, muhusika atadungwa shindano katika uvimbe na chembechembe za mwili kunyonywa, hizo chembechembe zitafanyiwa utafiti kwa kutumia chombo cha darubini ilikuthibithisha uwepo wamagonjwa ambayo husababisha uvimbe.

Usiri

Matumizi ya majina haya tatumiwa katika utafiti huu.Nambariz ausajili itatumika iIi kuihifadhi usiri wa wahusika.

Hatiyamaswaliyatawekwamahalisalamapaliponausirikwamudawamwakammojakablayakuondolewambali. Habari yote itakayo tolewa itakuwa siri ila tu kwa mhusika ambaye atapata matokeo yake wakati atakaporudi hospitali ni mara ya pili.

Kujitoa Kwa utafiti

Wahusikakatikautafitiwamejitoleanaunawezakujiondoawakatiwowoteambapo unahita jibi la kupoteza haki yoyote ya matibabu katika hospitali kuu.

Mawasiliano:

Ukiwa na swali lolote kuhusu utafiti huu tafadhali nipigie mimi SERAPHINE MUKANGAMIJE nambari ya simu +254795717953, pia waweza kuwapigia wahadhiri wakuu wangu Professor L. Muchirina Dr. Waweru chuo kikuu cha Nairobi, P.O BOX 19676-00202 Nairobi, nambari ya simu 726300 Ext. 43774. Ukiwa na swali lingine linalo husu maadili ya utafiti, tafadhali mpigie Prof. M. L. CHINDIA, karani, KNH/UON kitengo cha maadiliya utafiti, nambari ya simu: 726300-9 Ext 44102. 55

Appendix V: Fine Needle Aspiration Procedure

1. The skin above the area to be aspirated is swabbed with an antiseptic solution
2. A10 ml disposable syringe is attached to a 23 or25-gauge needle
3. The needle is then inserted just below the skin and negative suction is applied to the syringe by pulling the plunger back.
4. The mass is then pierced with the needle followed by multiple passes without exiting the skin surface. The number of passes depends on the consistency of the masses.
5. If the aspirator encounters a cyst, it should be completely evacuated and the fluid sent for cytological examination.
6. After evaluation, it is important and ideal to re-aspirate any solid residual portions ofthe mass.
7. The vacuum of the syringe is then released and the skin is exited.
8. Should pus be encountered during the aspiration procedure, it is encouraged to remove as much as possible to bring relief to the patient and obtain some for culture and other ancillary tests.

Appendix VI: Papanicolaou, H&E and ZN Staining Procedure

The smears will be stained using PAP, H&E and ZN then it will be examined by SERAPHINE MUKANGAMIJE as PI microscopically and then reported by two pathologists.

A. Papanicolaou staining method

Principle

Hematoxylin basic dye stains the nuclei blue. Orange G an acidic counter stain solution stains the cytoplasm of mature cells and keratin to orange or pale yellow.

Eosin azure solution being acidic stains the Cytoplasm-Eosin (EA) stains the mature cells while light green stains the young cells to light green or pale blue.

Staining technique

1. Fix the smear in 95%ethanol for 15minutes

2. Hydrate smears through ethanol grades of 90%, 80%, and then 70%

3. Rinse in distilled water 10 dips

4. Stain in Harris hematoxylin for 10 minutes

5. Rinse in tap water

6. Differentiate in 0.05%acid water 10 dips

7. Rinse in tap water and blue in Scott's tap water 10 dips

8. Rinse in 95% ethanol

9. Stain in O.G 6 for 2 minutes

10. Rinse in 95% ethanol 10 dips

11. Stain in E.A 50 for 4 minutes

12. Rinse in 95% ethanol 10 dips

13. Dehydrate in changes of absolute ethanol 10 dips each

14. Clear in 3 changes of Xylene 10 dips

15. Mount in D.P.X

B. **Hematoxylin –Eosin staining methods**

Principle

Alum acts as mordant and hematoxylin containing alum stains the nucleus light blue. This turns red in presence of acid, as differentiation is achieved bytreating the tissue with acid solution. Bluing step converts the initial soluble red color within the nucleus to an insoluble blue color. The counter staining is done by using eosin which imparts pink color to the cytoplasm.

Staining technique

1. Absolute ethyl alcohol

2. Hydrate smears through ethanol grades of 90%, 80%, and then 70%

3. Stain in Harris hematoxylin for 3-5 minutes

4. Rinse in tap water for 10 dip

5. Differentiate in 1%acid alcohol for 5 minute

6. Rinse in tap water and blue in Scott's tap water 10 dips

7. Stain in 1% Eosin Y for 10 minutes

8. Rinse in tap water 1-5 minutes

9. Dehydrate in increasing concentration of alcohols and clear in xylene

10. Mount in D.P.X

11. Observe under microscope

C. Ziehl Nielsen's staining methods (ZN)

Principle

My colic acid in the bacilli enables the uptake of carbo fuchsine and resists decolourisation with acid and alcohol, phenol acts as an acentuator and facilitates the penetration of the dye.

Staining technique

1. Hydrate the smears

2. Cover the smears with a filter paper _ avoid floatens, air drying of the smears.

3. Flood the smear with filtered paper cabol fuchsine for 15 minute.

4. Remove the filter paper and wash with tap water.

5. Decolorize with 20% sulphuric acid for 3 min and rinse in water.

6. See if the smears are pale pint

7. Counter stain with methylene blue for 2-3 min or malachite green.

8. Wash in water.

9. Dehydrate in 3 changes of ethanol, air- dry.

10. Clear in 3 changes of xylene

11. Mount in DOX.

Appendix VII: Data Collection Sheet and Questionnaire

1. Study identification number……DATE ……..…

2. Demographic and socio-economic data:

A. Age ……Years…

B. Gender …….M (), OR F ( )

C. Occupation ----------

D. Alcohol consumption--------- Y ( ) or N ( )

E. HIV positive……Y ( )

3. Clinical history and examination

a. Location of the lesion:

-Thyroid lesions …….Y ( ) OR N ( )

-Soft tissue lesions……..Y ( ) OR N ( )

-Lymph node lesions……Y ( ) OR N ( )

-Glandular lesions……Y ( ) OR N ( )

-Others……

b. Duration of the lesion: ……

c. Tenderness: …….

d. Mass consistency……

e. Clinical diagnosis: ……..

4. Microscope examination

A. **specimen adequacy:** 1) satisfactory…..Y ( ) or N ( )

2) Unsatisfactory….Y()or N ( )

B**. microscopic description ……**

……
……
……

C.**Interpretation:**

- Negative for malignancy: -Normal……Y ( ) or N ( )
  -Reactive …….Y ( ) or N ( )
  -Inflammation……Y ( ) or N ( )
  -Granulomatous……Y ( ) or N ( )
  -Goiter……Y ( ) or N ( )
  -Sialadenatis…….Y (….) or N ( )
  - Hachimotos ……Y (….) or ( )
  -Chronic lymphocytic thyroiditis…..Y ( ) or N ( )

- Suspicious for malignancy :-Benign mixed tumor…..Y ( ) or N ( )

- Positive for malignancy:-Lymphoma……Y( ) or N ( )
  -Malignant mixed tumors….Y ( ) or N ( )
  -metastatic carcinoma…….Y ( ) or N ( )
  -Adenoma……..Y ( ) or N ( )
  -Others……

Signatures

Cytology Pathologist 1 Pathologist 2

……. ……..…… ……..

Appendix VIII: Sample correction procedure -Flow chart