A Histological Autopsy Study of the Thyroid in HIV Infected Adults at the University Teaching Hospital in Lusaka, Zambia in the Period 2010 to 2012.

BY

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LIST OF ABBREVIATIONS
ABC- Abacavir
AIDS- Acquired immunodeficiency syndrome
ART- Anti retroviral therapy
CMV- Cytomegalovirus
D4T- Stavudine
EVF- Efavirenz
HAART- Highly active antiretroviral therapy
H&E- Hematoxylin and eosin
HIV- Human immunodeficiency virus
KS- Kaposi sarcoma
NVP- Niverapine
PAS- Periodic acid Schiff
SOP- Standard operating procedure
T3- Triiodothyronine
T4- Thyroxine
TDF- Tenofovir
UNZA- University of Zambia
UNZABREC- University of Zambia Biomedical Research Ethics Committee
UTH- University Teaching Hospital
ZN- Ziehl Neelsen
CHAPTER ONE

1.0 INTRODUCTION

The thyroid gland is a butterfly-shaped endocrine organ located below and anterior to the larynx, at the base of the neck. It consists of two bulky lateral lobes connected by a thin isthmus. The thyroid gland produces, stores and releases hormones that control metabolism. These hormones include Triiodothyronine (T3) and Thyroxine (T4). T3 and T4 hormones regulate vital body functions including: breathing, heart rate, muscle strength, body temperature and body weight.

Functional abnormalities with specific endocrine glands have been reported by several investigators in association with Human immunodeficiency virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS).7, 10, 21 The most common thyroid abnormalities in these patients include euthyroid sick syndrome and hypothyroidism.22 However, there is very scanty literature regarding the histology appearance of the thyroid gland in patients with HIV and AIDS, especially in Sub Saharan Africa. It is therefore postulated that the thyroid gland would be involved in AIDS patients as evidenced by functional abnormalities.

This study is the first retrospective report to describe the appearance of the thyroid gland in HIV infected patients in Zambia. It is a nested sub study within the Zambia HIV Neuro-AIDS study (Sub type C Neuro-AIDS and pathogenesis in Zambia) that was set up to investigate the effects of HIV on the brain at autopsy [see appendix J].
1.1 STATEMENT OF THE PROBLEM
Despite studies available showing that thyroid function is deranged in HIV infection, the spectrum of thyroid lesions in patients with AIDS has not been described in the Zambian population.

So far, literature on histological appearances of the thyroid gland in HIV infected adults may not be representative of the histological appearances in Sub-Saharan Africa in that most studies were done outside sub-Saharan region were the disease burden is low, epidemiology of opportunistic infections are different and the studies were mostly done on Caucasians.

Prompt recognition and treatment of thyroid disease improves the management and response of infected individuals to antiretroviral therapy.

1.2 JUSTIFICATION OF THE STUDY

Thyroid gland lesions can clinically be detected by physical examination which involves palpating the thyroid gland and also by laboratory examination of thyroid function tests in blood which involves T3, T4 and TSH levels.

Histological examination of the thyroid gland is usually indicated in symptomatic lesions.

In this study, autopsy histological examination of the thyroid gland will identify subclinical thyroid pathology in HIV, which does not present with indications for thyroid biopsy.

Knowledge of the histological lesions of the thyroid gland in HIV infection is important for operative management in patients infected with HIV.

The findings of this study will provide for evidence based recommendations on the management of thyroid diseases in the HIV infected in Zambia.
1.3 RESEARCH QUESTION
What are the histological lesions of the adult thyroid gland in patients who died of HIV related diseases at UTH?

1.4 AIM:
To study the histological appearances of adult thyroid glands in patients who died of HIV related diseases at UTH in the period 2010 to 2012.

1.5 SPECIFIC OBJECTIVES

1.5.1 To determine the types of infections in the thyroid gland at autopsy in HIV infected patients.

1.5.2 To determine the types of neoplasms in the thyroid gland at autopsy in HIV infected patients.

1.5.3 To determine the types of non-specific and specific structural changes in the thyroid gland at autopsy in HIV infected patients.

1.5.4 To evaluate some of the determinants of thyroid gland pathology in HIV infected adults at autopsy.
CHAPTER TWO

2.1 LITERATURE REVIEW

In the early years of the AIDS epidemic, the diverse endocrine manifestations of HIV infection were more often a consequence of opportunistic infections (OIs), neoplasms, or concomitant systemic illness. Infection by a diverse array of organisms, as well as HIV-associated malignancies has been detected in the thyroid gland. Such occurrences were far more common prior to the widespread introduction of HAART, although they may still be observed in patients not receiving ART or who have antiretroviral drug resistant infection.\textsuperscript{24}

The appearance of primary HIV infection ranges from an asymptomatic presentation to a symptomatic illness resembling infectious mononucleosis. Severe unusual presentations include acute myopericarditis, renal failure, and opportunistic infections such as esophageal candidiasis, cytomegalovirus infection, and Pneumocystis jiroveci pneumonia. Multiple organ failure in HIV has been described in many studies. It has been reported that multiple organ failure ensues in individuals who have primary HIV infection. This condition rapidly improves after the initiation of antiretroviral therapy.\textsuperscript{25, 30, 31}

HIV infection weakens the immune system, making infected individuals highly susceptible to numerous opportunistic infections and certain types of cancers. The common infections include \textit{Mycobacterium tuberculosis}, \textit{Cryptococcus neoformans}, \textit{Cytomegalovirus}, \textit{Candidiasis albicans} and \textit{Toxoplasmosis gondii}. The most common neoplasms include Kaposi sarcoma, cervical cancer and lymphomas. HIV infection leads to a diverse range of opportunistic infections and cancers by suppressing the immune system. It does so by killing cells, particularly CD4+ T cells, responsible for guarding against development of infections and cancers.\textsuperscript{26, 27}

Advances in the understanding of cancer biology have established the importance of immune evasion as a critical feature of malignancies. Immune evasion is
critical for the success of all malignancies. Without immune evasion malignant cells could be recognized by innate and adaptive cells of the immune system and cleared or destroyed. 32, 33, 34

HIV endocrinopathy encompasses a broad spectrum of disorders. Almost all the endocrine organs are virtually affected by HIV infection. HIV can directly alter glandular function. More commonly secondary endocrine dysfunction occurs due to opportunistic infections and neoplasms in immunocompromised state. Antiretroviral therapy as well as other essential medications often results in adverse endocrinal consequences. 28, 29

The common endocrine abnormalities include thyroid gland dysfunction, adrenal insufficiency, hypogonadism, diabetes, AIDS wasting syndrome and HIV lipodystrophy. Functional abnormalities with specific endocrine glands have been reported by several investigators in association with Human immunodeficiency virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS). 7, 10, 21, 28, 29

Subtle alterations of thyroid function tests are more common in HIV infection and are sometimes already detectable in the early phase of the disease. A high rate of thyroid dysfunction in asymptomatic subjects has been described in HIV. Most well described are a reduction in T4, T3 and elevated TBG, rT3 and TSH levels. Thyroid hormone homeostasis is also influenced by cytokines such as IL-6 and TNF, which acutely decrease TSH and T3 and increase rT3 levels. The drugs used in HIV may also alter thyroid function. Potent anti-retroviral drugs may precipitate a resurgence of autoimmune disease whilst others may alter thyroid hormone clearance. Nutritional state also influences thyroid function and may contribute to the disturbances seen in HIV. 35, 36, 37

Despite the availability of studies describing thyroid function abnormalities in HIV infection, there is very little literature regarding the histology appearance of the thyroid gland in patients with HIV and AIDS. No study has been done in Sub Saharan Africa, to describe the histology of the thyroid in HIV infected.
In a study done in Brazil, *Mycobacterium tuberculosis* was recorded in 23% of the thyroid glands, *Cytomegalovirus* in 17%, *Cryptococcus* in 5%, *Mycobacterium avium* in 5%, *Pneumocystis* in 4%, and other bacteria or fungi in 7%. Kaposi's sarcoma was recorded in 2% of patients and occult papillary carcinoma in 4%. Four patients had dual infections of the thyroid. The mean weight of the thyroid was lower than normal.2

A retrospective and prospective study of thyroids obtained at autopsy in 93 patients with AIDS at a tertiary level hospital in Mumbai, India, was carried out and significant pathologic lesions were identified in 35% cases. Tuberculosis was the predominant finding in 6% of the cases. The other pathologies identified were *Cryptococcus* 5%, *Cytomegalovirus* 2%, Hashimoto’s thyroiditis 3%, fibrosis 6% and goiter 3%. *Pneumocystis jiroveci* thyroiditis and malignant neoplasms of the thyroid were not seen.20

A similar autopsy study in USA on African Americans was done. There were 102 patients. Thirteen patients with abnormal thyroid findings were identified. Interstitial fibrosis was the most common histological finding (4.9%), followed by thyroid hyperplasia (1.9%). Infectious disease affecting the thyroid gland was limited to 2.9% and consisted of *Mycobacterium tuberculosis*, *Cryptococcus neoformans*, and *Cytomegalovirus*. Kaposi sarcoma of the thyroid gland was present in only one case (0.9%).18

The above three studies were done in three different epidemiology set ups. The Brazil and India study were done using cases before the advent of HAART. These two studies showed that infectious diseases were more common than the USA study which was done more recently; after the introduction of HAART.2,18,20

All the studies identified *Mycobacterium tuberculosis* in the thyroid gland. However, the frequency in the Brazil and India studies was relatively higher compared to USA study. This could have been due to the same reason that the studies involving cases before the advent of HAART showed more infectious diseases.2,18,20
Both the USA and Indian studies did not show any case of *Pneumocystis jiroveci* thyroiditis.\(^2,20\)

The Brazil and USA studies both found Kaposi sarcoma. The Brazil study also showed that occult papillary carcinoma was present in 4% of cases as opposed to the other two studies.\(^2,20\)

**Table 2.1: Comparison between the Brazil and Indian researches on thyroid pathology in HIV**

<table>
<thead>
<tr>
<th>SN</th>
<th>Brazil (%)</th>
<th>India (%)</th>
<th>USA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tuberculosis</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>Cryptococcosis</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>CMV</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Mycobacteria avium</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Hashimoto’s thyroiditis</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>Fibrosis</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>7</td>
<td>Goiter</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>PCP</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>Papillary carcinoma</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>Kaposi’s sarcoma</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>Other bacterial and fungal</td>
<td>7</td>
<td>0</td>
</tr>
</tbody>
</table>
CHAPTER THREE

3.0 METHODOLOGY

3.1 RESEARCH DESIGN

This study was a nested study within the Zambia Neuro-AIDS study (Sub type C Neuro-AIDS and pathogenesis in Zambia) of autopsy thyroid samples that were collected in the period 2010 and 2012. This research was a descriptive retrospective study of adult thyroid glands collected at autopsy during the stated period.

The principal investigator performed and assisted in 25 autopsies out of the 500 autopsies done between 2011 and 2016.

3.2 RESEARCH SETTING

This study was done in Lusaka, Zambia. It took place at UTH, in the Department of Pathology and Microbiology. UTH is a tertiary referral hospital with a bed capacity of 1800. All general hospitals in the country refer to this hospital.

3.3 TARGET POPULATION

Patients infected with HIV, admitted to UTH and died in hospital.

3.4 INCLUSION CRITERIA

Paraffin blocked thyroid gland samples from the deceased who were HIV infected and aged above 19 years in the Zambia Neuro-AIDS study. This included adult patients admitted to UTH and died in hospital.
3.5 EXCLUSION CRITERIA

Paraffin blocks were excluded from the study if:

1. They did not have corresponding clinical files
2. They contained autolyzed tissue
3. They did not contain any tissue at all.

3.6 SAMPLE SIZE

All paraffin blocks which were collected in the period 2010 to 2012 were considered. All the blocks that met the inclusion criteria were enrolled in the study. A total of 200 thyroid gland samples from 200 patients were enrolled.

3.7 SAMPLE SELECTION

Convenient sampling was used. All specimens collected between August, 2010 and July, 2012 and met the inclusion criteria were selected.

3.8 TISSUE PROCESSING

Paraffin blocks were processed according to the standard operating procedures (SOP) in the histopathology laboratory at UTH. Lusaka. Paraffin blocked kidney samples were frozen and then sectioned on a microtome set at 3 µm and placed on a glass slide that was labelled appropriately. This was done by a histopathology laboratory technician.

The slides were stained with routine Hematoxylin and Eosin stains and special stains Periodic Acid Schiff (PAS), Ziehl Neelsen stain (ZN) and Mucicarmine stain as per standard operating procedure used at The UTH histopathology laboratory.[See appendix F].
The slides were then examined using an Olympus BX 50 binocular biological microscope with the following eye piece objective lens; x 20, x 40, x 100, x 200 and x400.

**Flow chart of activities in the study**

**Station 1:** Sectioning at 3 microns.

**Station 2:** Staining using Hand E. [See appendix F]

**Station 3:** Special staining was done as per microscopy results using ZN and PAS [See Appendix G]

**Station 4:** Microscopy.

**3.9 DATA COLLECTION TOOLS AND VARIABLES.**

Data was collected from patients’ clinical files and after microscopic examination entered onto standardized data collection tools (See appendix C and D).

**3.9.1 VARIABLES.**

**3.9.1.1 INDEPENDENT VARIABLES.**

These included age, sex, drug history (HAART combinations), CD4 count results, clinical diagnosis and cause of death as documented at autopsy.

This information was obtained from patients’ clinical files.

The information obtained was entered in standardized templates (See appendix C)
Table of independent variables

<table>
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<tr>
<th>SN</th>
<th>INDEPENDENT VARIABLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Age</td>
</tr>
<tr>
<td>2</td>
<td>Sex</td>
</tr>
<tr>
<td>3</td>
<td>Drug History</td>
</tr>
<tr>
<td>4</td>
<td>CD4 Count results</td>
</tr>
<tr>
<td>5</td>
<td>Thyroid function tests</td>
</tr>
<tr>
<td>6</td>
<td>Clinical Diagnosis</td>
</tr>
<tr>
<td>7</td>
<td>Autopsy Diagnosis</td>
</tr>
</tbody>
</table>

3.9.1.2 DEPENDENT VARIABLES

These included thyroid lesions seen under microscopy examination. These were grouped into non-specific thyroid lesions, Specific thyroid infections and neoplasms. The findings were entered in standardized templates (See Appendix D).

Table of dependent variables

<table>
<thead>
<tr>
<th>SN</th>
<th>DEPENDENT VARIABLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Non-specific thyroid lesions</td>
</tr>
<tr>
<td>2</td>
<td>Specific infections</td>
</tr>
<tr>
<td>3</td>
<td>Neoplasms</td>
</tr>
</tbody>
</table>
3.10 DATA COLLECTION TECHNIQUE

3.10.1 CLINICAL DETAILS

Clinical details were obtained from clinical files and the information filled in table a standardized table (See appendix C)

3.10.2 LIGHT MICROSCOPY

H&E slides prepared were viewed under the microscope. Diagnosis made was entered in a standardized table (See Appendix D)

Lesions suggestive of infections were to be subjected to special stains to accurately make a diagnosis.

Neoplastic lesions were to be subjected to immunohistochemistry stains.

3.10.3 CLINICO-PATHOLOGIC CORRELATION

Clinical details and light microscopic examination results will be used to make a diagnosis where possible.
3.11 STATISTICAL ANALYSIS

DATA ANALYSIS:

Data was examined using SPSS version 20.

Frequencies, modes and mean were used to analyze the type of infections and neoplasms on histological evaluation of the thyroid gland samples.

Cross table analysis and Chi square test were used to analyze associations between categorical variables. The Fisher’s exact test was used when the sample size values were less than 5. The association between variables was regarded as statistically significant if the P value was less than 0.05.

3.12 EXPECTED OUTCOME

Subclinical thyroid disease in HIV infected will be identified on autopsy examination.

The histological appearances will be neoplasms and opportunistic infections associated with immune suppression.

The most common neoplasms will be papillary carcinoma and Kaposi’s sarcoma.

The most common opportunistic infections will either be TB or fungal infections.

3.13 ETHICAL ISSUES

Informed and voluntary consent from next of kin was sought by the Zambia HIV Neuro study which is the parent study. This parent study was approved by UNZAREC on 16th July, 2007: The Reference number is 018-06-07.
This study used blocked autopsy thyroid gland material from the Zambia Neuro-AIDS study. Clearance to proceed was sought from ERES Converge IRB (see appendix B).

Data collected was delinked from name and other identifier details of the participant. Only research numbers were used on standardized data collection tools.

Permission to perform the study was granted by the office of the Senior Medical Superintendent of the University Teaching Hospital (see appendix A).

3.13.1. POSSIBLE RISKS

This study involved already prepared Paraffin blocks. They possessed no risks of infections to the investigators and laboratory staff handling the specimens.

3.13.2. POSSIBLE BENEFITS

The findings of this study in Zambia will provide evidence based recommendations on the management of thyroid diseases in the HIV infected in Zambia.

3.13.3. CONFIDENTIALITY

The deceased name was not included in the study. The information collected was only accessed by the investigators. The autopsy report was only revealed to the next of kin. This ensured that confidentiality was strictly maintained.

3.13.4. CONSENT

This was a nested sub study from the parent study which is the Zambia Neuro-AIDS study. The parent study obtained consent from the next of kin. This was
done after a thorough explanation of what the Zambia Neuro-AIDS study was all about. It was explained to the next of kin that the specimens collected would be used in the study without getting consent from the next of kin. There is no consent to be obtained for this nested sub study.

The next of kin did not suffer any consequences if they decided not to participate in the study. Participation in this study was strictly voluntary. The next of kin was allowed to withdraw from the study without consequences.

Clarifications and questions by the next of kin were addressed to Dr. Victor Mudenda, at The UTH, Department of Pathology and Microbiology.
CHAPTER FOUR

4.0 RESULTS

PATIENT DEMOGRAPHICS AND CHARACTERISTICS

This study involved 200 autopsy cases. There were 123 males (62.5%) and 77 females (37.5%). The age range was 19 to 72 years with a mean of 34 years. The median was 33 years.

CD4 count was documented in 31 (15.5%) of the case files. Cases with CD4 count of less than 50 cells/𝜇L were 13 (42%). The number of cases with CD4 count of less than 200 cells/𝜇L was 25, representing 81% of patients. None of the case files had viral load.

Eighty-six (43%) patients were pre-HAART while 114 (57%) patients were on HAART. Out of the patients who were on HAART, 50% were on Atripla-Tenofovir/Emtricitabine /Efavirenz (TDF/FTC/EFV) and the rest were on TDF/FTC/NVP (12%), D4T/3TC/NVP (9%) and ABC/3TC/EVF (29%).

None of the patient clinical records had thyroid function test results documented.

PATHOLOGIC FINDINGS

Pathologic findings included interstitial fibrosis (52%) and non-specific chronic inflammation (21.5%), infections by Mycobacterium tuberculosis (1%) and Cryptococcus neoformans (0.5%). Interstitial fibrosis was the most common histological finding accounting for 104 cases which represents (52%). There was no neoplastic lesion.

Table 4.1 (A) shows summary of demographic characteristics and pathologic findings of the cases that were sampled.
Table 4.1 (A): Demographic and Baseline Characteristics of the Participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Frequency N(%)</th>
<th>Clinal File</th>
<th>Thyroid Lesions N (%)</th>
<th>Infectious Lesions</th>
<th>Tumor</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fibrosis N(%)</td>
<td>Chronic Thyroiditis N(%)</td>
<td>Granulomatous Thyroiditis N(%)</td>
<td>Crypto-coccus neoformans N(%)</td>
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<tr>
<td>Age</td>
<td>16-30</td>
<td>43 (21)</td>
<td>19 (18)</td>
<td>11 (24)</td>
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<td>31-45</td>
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<td>72 (69)</td>
<td>28 (61)</td>
<td>2 (100)</td>
<td>1 (100)</td>
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<td>11 (5)</td>
<td>9 (9)</td>
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<td>61-75</td>
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<td></td>
<td>&gt;75</td>
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</tr>
<tr>
<td>Total</td>
<td></td>
<td>200 (100)</td>
<td>104 (100)</td>
<td>46 (100)</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>123 (62)</td>
<td>57 (55)</td>
<td>31 (67)</td>
<td>1 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>77 (38)</td>
<td>47 (45)</td>
<td>15 (33)</td>
<td>1 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>200 (100)</td>
<td>104 (100)</td>
<td>46 (100)</td>
<td>2 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Drug History</td>
<td>HAART-naive</td>
<td>114 (57)</td>
<td>54 (51)</td>
<td>28 (61)</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td></td>
<td>HAART</td>
<td>86 (43)</td>
<td>50 (49)</td>
<td>18 (39)</td>
<td>(0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>200 (100)</td>
<td>104 (100)</td>
<td>46 (100)</td>
<td>2 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Drug Combinations</td>
<td>TDF/FTC/NVP</td>
<td>10 (12)</td>
<td>4 (8)</td>
<td>2 (10)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>TDF/FTC/EVF</td>
<td>43 (50)</td>
<td>28 (56)</td>
<td>10 (50)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>D4T/3TC/NVP</td>
<td>8 (9)</td>
<td>6 (12)</td>
<td>4 (20)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>ABC/3TC/EVF</td>
<td>25 (29)</td>
<td>12 (24)</td>
<td>2 (10)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>86 (100)</td>
<td>50 (100)</td>
<td>18 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>CD4 count</td>
<td>Done</td>
<td>31 (15)</td>
<td>24 (29)</td>
<td>17 (37)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Not done</td>
<td>169 (85)</td>
<td>80 (71)</td>
<td>29 (63)</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>200 (100)</td>
<td>104 (100)</td>
<td>46 (100)</td>
<td>2 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td>CD4 Follow-up</td>
<td>&lt;50</td>
<td>13 (42)</td>
<td>11 (46)</td>
<td>8 (47)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>51-100</td>
<td>8 (26)</td>
<td>6 (25)</td>
<td>3 (17)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>101-200</td>
<td>4 (13)</td>
<td>3 (13)</td>
<td>2 (12)</td>
<td>0 (0)</td>
<td>0 (0)</td>
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<tr>
<td></td>
<td>201-300</td>
<td>2 (6)</td>
<td>2 (8)</td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>301-400</td>
<td>1 (3)</td>
<td>1 (4)</td>
<td>2 (12)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>401-500</td>
<td>3 (10)</td>
<td>1 (4)</td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>&gt;500</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>31 (100)</td>
<td>24 (100)</td>
<td>17 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Thyroid Function: T3</td>
<td>Done</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Not done</td>
<td>200 (100)</td>
<td>104 (100)</td>
<td>46 (100)</td>
<td>2 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>31 (100)</td>
<td>104 (100)</td>
<td>46 (100)</td>
<td>2 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Thyroid Function: T4</td>
<td>Done</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Not done</td>
<td>200 (100)</td>
<td>104 (100)</td>
<td>46 (100)</td>
<td>2 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>31 (100)</td>
<td>104 (100)</td>
<td>46 (100)</td>
<td>2 (100)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
**CLINICAL DIAGNOSIS AND AUTOPSY DIAGNOSES**

Pulmonary tuberculosis, disseminated tuberculosis and meningitis were the commonest clinical and autopsy causes of death. The total number of cases which disagreed between clinical diagnosis and autopsy diagnosis was 110 representing 55% of the total cases.

Table 4.1 (B) Shows a summary of clinical and autopsy causes of death.

<table>
<thead>
<tr>
<th>SN</th>
<th>Diagnosis</th>
<th>Clinical Diagnosis N (%)</th>
<th>Autopsy Diagnosis N (%)</th>
<th>Difference N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Anemia</td>
<td>6 (3)</td>
<td>0 (0)</td>
<td>6 (3)</td>
</tr>
<tr>
<td>2</td>
<td>Congestive Cardiac Failure</td>
<td>8 (4)</td>
<td>6 (3)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>3</td>
<td>Chronic Renal Failure</td>
<td>0 (0)</td>
<td>1 (0.5)</td>
<td>1(0.5)</td>
</tr>
<tr>
<td>4</td>
<td>Chronic Liver Disease</td>
<td>0 (0)</td>
<td>1 (0.5)</td>
<td>1(0.5)</td>
</tr>
<tr>
<td>5</td>
<td>Chronic Obstructive Pulmonary Disease</td>
<td>1 (0.5)</td>
<td>0 (0)</td>
<td>1(0.5)</td>
</tr>
<tr>
<td>6</td>
<td>Cardio Vascular Accident</td>
<td>4 (2)</td>
<td>1 (0.5)</td>
<td>3(1.5)</td>
</tr>
<tr>
<td>7</td>
<td>Dilated Cardiomyopathy</td>
<td>2 (1)</td>
<td>0 (0)</td>
<td>2(1)</td>
</tr>
<tr>
<td>8</td>
<td>Disseminated KS</td>
<td>3 (1.5)</td>
<td>4 (2)</td>
<td>1(0.5)</td>
</tr>
<tr>
<td>9</td>
<td>Disseminated TB</td>
<td>59 (29.5)</td>
<td>71 (35)</td>
<td>12(6)</td>
</tr>
<tr>
<td>10</td>
<td>Gastroenteritis</td>
<td>6 (3)</td>
<td>0 (0)</td>
<td>6(3)</td>
</tr>
<tr>
<td>11</td>
<td>Hepatitis</td>
<td>12 (6)</td>
<td>0 (0)</td>
<td>12(6)</td>
</tr>
<tr>
<td>12</td>
<td>Hepatocellular Carcinoma</td>
<td>1 (0.5)</td>
<td>1 (0.5)</td>
<td>0(0)</td>
</tr>
<tr>
<td>13</td>
<td>Hypertensive heart disease</td>
<td>3 (1.5)</td>
<td>0 (0)</td>
<td>3(1.5)</td>
</tr>
<tr>
<td>14</td>
<td>Kaposi’s Sarcoma</td>
<td>3 (1.5)</td>
<td>0 (0)</td>
<td>3(1.5)</td>
</tr>
<tr>
<td>15</td>
<td>Liver cirrhosis</td>
<td>7 (3.5)</td>
<td>2 (1)</td>
<td>5(2.5)</td>
</tr>
<tr>
<td>16</td>
<td>Malaria</td>
<td>0 (0)</td>
<td>1 (0.5)</td>
<td>1(0.5)</td>
</tr>
<tr>
<td>17</td>
<td>Meningitis</td>
<td>3 (1.5)</td>
<td>20 (10)</td>
<td>17(8.5)</td>
</tr>
<tr>
<td>18</td>
<td>Miliary TB</td>
<td>4 (2)</td>
<td>0 (0)</td>
<td>4(2)</td>
</tr>
<tr>
<td>19</td>
<td>Pneumonia</td>
<td>14(7)</td>
<td>6 (3)</td>
<td>8(4)</td>
</tr>
<tr>
<td>20</td>
<td>Pulmonary Tuberculosis</td>
<td>61 (30.5)</td>
<td>70 (35)</td>
<td>9 (4.5)</td>
</tr>
<tr>
<td>21</td>
<td>Pulmonary Embolism</td>
<td>0 (0)</td>
<td>2 (1)</td>
<td>2(1)</td>
</tr>
<tr>
<td>22</td>
<td>Sepsis</td>
<td>2 (1)</td>
<td>3 (1.5)</td>
<td>1(0.5)</td>
</tr>
<tr>
<td>23</td>
<td>TB spine</td>
<td>1(0.5)</td>
<td>1 (0.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>24</td>
<td>Undetermined</td>
<td>0 (0)</td>
<td>10 (5)</td>
<td>10 (5)</td>
</tr>
<tr>
<td></td>
<td><strong>Total</strong></td>
<td><strong>200 (100)</strong></td>
<td><strong>200 (100)</strong></td>
<td><strong>110 (55)</strong></td>
</tr>
</tbody>
</table>
4.2 AGE
Table 4.2 (A): Shows that the mean age was 34 years. The median age was 33 years and the mode was also 33 years.

Table 4.2 (A): Age; Statistics

<table>
<thead>
<tr>
<th>N</th>
<th>Valid</th>
<th>200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missed</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (years)</td>
<td>34</td>
</tr>
<tr>
<td>Median(years)</td>
<td>33</td>
</tr>
<tr>
<td>Mode (years)</td>
<td>33</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>7.35</td>
</tr>
</tbody>
</table>

AGE DISTRIBUTION

Figure 4.2 (B) shows age distribution. As shown below, most of the cases were in the age range 31-45 years old. There was no case with age above 75 years.

Figure 4.2 (B): Age distribution.
4.3 SEX

Figure 4.3 shows gender distributions of the cases. As shown below, males were more than females. The number of males was 123 representing 61.5% and that of females was 77 representing 38.5%.

![Gender Distribution Chart]

**Figure 4.3 Sex**
4.4 DRUG HISTORY

Figure 4.4 (A) shows drug history. As shown below there were more HAART-naive cases than HAART cases. The number of HAART-naive cases was 114 representing 57% and that of HAART cases was 86 representing 43%.

Figure 4.4 (A): Drug History
HAART COMBINATIONS

Figure 4.3 (B) shows distribution of HAART drug combinations. As shown below, most cases were seen in drug combination TDF/FTC/EVF which had 43 cases representing 50% of cases who were on HAART. The lowest frequency was seen in drug combination D4T/3TC/NVP which had 8 cases representing 4% of cases who were on HAART.

Figure 4.4 (B): HAART Combinations
4.5 (A) CD4 COUNT

Figure 4.5 (A) show the number of cases which had CD4 count results and those which did not have. As shown below, the number of cases without CD4 count results was more that those with results. The number of cases without CD4 results was 169 representing 84.5% and those with results were 32 representing 15.5%.

![CD4 COUNT Graph](image)

Figure 4.5 (A): CD4 Count
4.5 (B): CD4 COUNT DISTRIBUTION

Figure 4.5 (B) show the distribution of cases at different levels of CD4 count results. As shown below, the highest number had CD4 count results that were less than 50. There were 13 cases with CD4 count results less than 50 representing 42% of cases with CD4 count results. There was no case with CD4 count results above 500.

Figure 4.5 (B): CD4 Count Distributions.
4.6 CLINICAL AND AUTOPSY CAUSES OF DEATH

Figure 4.7 shows clinical and autopsy causes of death. As shown on the chart, disseminated TB followed by pulmonary TB was the leading diagnosis both clinically and on autopsy. The chart also shows that meningitis is usually missed clinically but diagnosed on autopsy. Cases diagnosed as anaemia clinically were not found at autopsy.

![Clinical and Autopsy Causes of Death](image_url)

**Figure 4.7: Clinical and Autopsy causes of Death**
Abbreviations:

CCF - Congestive cardiac failure
CLD - Chronic liver disease
COPD - Chronic obstructive pulmonary disease
CVA - Cerebral vascular accident
DCM - Dilated cardiomyopathy
GE - Gastroenteritis
HEP - Hepatitis
HCC - Hepatocellular carcinoma
HTN - Hypertension
K.S - Kaposi sarcoma
LC - Lung cancer
T.B - Tuberculosis
P.E - Pulmonary embolism.

Clinical and Autopsy causes of Death

As shown in figure 4.7, most of the clinical diagnosis matched with the autopsy diagnosis.

At autopsy, meningitis was diagnosed in 20 cases (10%) compared to clinical diagnosis of meningitis 3 cases (1.5%). This shows that clinically meningitis is under diagnosed.

Clinically anaemia and gastroenteritis were seen in 6 cases each representing 3% of the total cases seen. At autopsy, none of the cases were diagnosed with either anaemia or gastroenteritis. This shows that both these disease conditions were over diagnosed.

As shown in the figure above, 10 cases representing 5% of the total cases were undetermined at autopsy.
4.8 PATHOLOGIC FINDINGS

Out of 200 cases, 112 cases (56%) had thyroid lesions compared to 88 cases (44%) which had no lesions.

**Distribution of thyroid gland lesions:**

Interstitial fibrosis was the most common histological finding accounting for 104 cases which represents (52%), followed by nonspecific chronic thyroiditis with 42 (21.5%). Infectious disease affecting the thyroid gland consisted of Mycobacterium tuberculosis 2 (1%) and Cryptococcus neoformans 1(0.5%).

The numbers do not add up to 100%, because the categories are not mutually exclusive. As shown in figure 4.8 (a)

![Histopathological findings of thyroid gland](image)

**Figure 4.8 (a): Summary of histopathological findings of thyroid gland**
Total thyroid gland lesions and gender distribution.
Female cases were significantly more associated with pathologic lesions than male cases ($P = 0.04$). Out of the 77 female cases, 50 cases (65%) had thyroid lesions compared to the 62 cases (50%) of the 123 male cases. As shown below in Figure 4.8(a).

Figure 4.8 (b): Pie charts showing thyroid gland lesions and Gender distribution.
A1. PICTOGRAPH SHOWING INTERSTITIAL FIBROSIS (H&E): AT LOW POWER

Dense fibrous band with compressed thyroid follicles

Pictograph 4.8 (A1): Pictograph showing interstitial fibrosis at X40

H&E: Low power. x40

Dense fibrous band (Black arrow).

Compressed thyroid follicles (White arrow)
A2. PICTOGRAPH SHOWING INTERSTITIAL FIBROSIS (H&E): AT HIGH POWER

Dense fibrous band with compressed thyroid follicles

Pictograph 4.8 (A2): Pictograph showing interstitial fibrosis at X100

H&E: Medium power. x100

- Dense fibrous band (Black arrow).
- Compressed thyroid follicles (White arrow)
B1. PICTOGRAPH SHOWING INTERSTITIAL FIBROSIS
(MASSON TRICROME): AT LOW POWER

Dense fibrous bands in blue compressing thyroid follicles

Pictograph 4.8 (B1): Pictograph showing interstitial fibrosis

- Masson Trichrome: Low power. x40
- Dense fibrous band (Yellow arrow).
- Compressed thyroid follicles (White arrow)
B2. PICTOGRAPH SHOWING INTERSTITIAL FIBROSIS
(MASSON TRICHOME): AT HIGH POWER

Dense fibrous bands in blue compressing thyroid follicles

Pictograph 4.8 (B1): Pictograph showing interstitial fibrosis

Masson Trichrome: Medium power. x100
Dense fibrous band (Yellow arrow).
Compressed thyroid follicles (White arrow)
C1. PICTOGRAPH SHOWING NON-SPECIFIC CHRONIC THYROIDITIS (H&E): AT LOW POWER

An infiltration of mixed inflammatory cells predominantly composed of Lymphocytes.

Pictograph 4.8 (C): Pictograph showing non-specific chronic thyroiditis with interstitial fibrosis

H&E: Low power. x40
Dense fibrous band (Black arrow).
Lymphocytes (White arrow)
C2. PICTOGRAPH SHOWING NON-SPECIFIC CHRONIC THYROIDITIS (H&E): AT HIGH POWER

H&E: Medium power. x100

Dense fibrous band (Black arrow).

Lymphocytes (White arrow)
D1. PICTOGRAPH SHOWING GRANULOMATOUS THYROIDITIS (H&E): LOW POWER
A thin rim of mononuclear cells surrounding an area of caseous necrosis

Pictograph 4.8 (D1): Pictograph showing granulomatous thyroiditis

H&E: Low power. x40

Thin rim of lymphocytes. (Black arrow).

Caseating necrosis (White arrow)
**D2. PICTOGRAPH SHOWING GRANULOMATOUS THYROIDITIS (H&E): HIGH POWER**

A thin rim of mononuclear cells surrounding an area of caseous necrosis

**Pictograph 4.8 (D2): Pictograph showing granulomatous thyroiditis**

H&E: Low power. x40

Thin rim of lymphocytes (Black arrow).

Caseating necrosis (White arrow)
E1. PICTOGRAPH SHOWING CRYPTOCOCCUS NEOFORMANS

(H&E): LOW POWER

The pictograph below shows Cryptococcus neoformans within thyroid follicles.

Pictograph 4.8 (E): Pictograph showing Cryptococcus neoformans

H&E: Low power. x40

Cryptococcus neoformans.
E2. PICTOGRAPH SHOWING CRYPTOCOCCUS NEOFORMANS

(H&E): HIGH POWER

H&E: Medium power. x100

Cryptococcus neoformans
F1. PICTOGRAPH SHOWING CRYPTOCOCCUS NEOFORMANS (MUCICARMINE): LOW POWER

The pictograph below shows Cryptococcus neoformans within thyroid follicles.

Pictograph 4.8 (F1): Pictograph showing Cryptococcus neoformans

Mucicarmine: Low power. x40
Cryptococcus neoformans.
F2. PICTOGRAPH SHOWING CRYPTOCOCCUS NEOFORMANS (MUCICARMINE): HIGH POWER

Mucicarmine: Medium power. x100
Cryptococcus neoformans
Description of Pathologic Findings:

**Interstitial Fibrosis**
Interstial fibrosis was described as thick fibrous bands compressing thyroid follicles without any inflammatory infiltrates. The thick fibrous bands consisted of hypercellular and hypocellular areas separated by collagen. The spindle cells stained dark blue and collagen stained pink on H&E stain. The fibrous bands stained blue with Masson trichrome. [See pictographs A1, A2, B1 and B2]

**Non-specific chronic thyroiditis**
An infiltrate of chronic inflammatory cells predominantly composed of lymphocytes with no evidence of specific infection, with or without fibrosis was used to describe nonspecific chronic thyroiditis. Chronic inflammatory cells consisted of mononuclear cells that stained dark blue on H&E stain. A few plasma cells and neutrophils were also observed. [See pictographs C1 and C2]

**Mycobacterium tuberculosis**
The presence of caseating granulomatous inflammation was used to describe features consistent with tuberculosis. The granulomas consisted of caseating necrotic areas which stained pink on H&E stain. A rim of lymphocytes consisted of mononuclear cell that stained dark blue on H&E. [See pictographs D1 and D2]

**Cryptococcus neoformans**
*Cryptococcus neoformans* infection was described as the presence of round to oval yeast spores, measuring 4-10 microns that are covered by a thick, mucinous capsule which stained bright red with mucicarmine. The yeast spores stained light dull grey on H&E stain but were highlighted to pink color on mucicarmine stain. [E1, E2, F1 and F2].
4.8.1 INTERSTITIAL FIBROSIS
Interstitial fibrosis was the most common histological finding accounting for 104 cases which represents 52%.

4.8.1 (A) INTERSTITIAL FIBROSIS AND GENDER DISTRIBUTION
Female cases were significantly more affected by interstitial fibrosis than male cases ($P = 0.04$). Out of the 77 female cases, 47 cases (61%) had interstitial fibrosis compared to 57 cases (46%) of the 123 male cases. As shown below on figure 4.8.1 (A).

![Figure 4.8.1 (A) Summary of Interstitial Fibrosis and Gender Distribution](image)

Figure 4.8.1 (A) Summary of Interstitial Fibrosis and Gender Distribution
4.8.1 (B) INTERSTITIAL FIBROSIS AT DIFFERENT AGE DISTRIBUTIONS

There was no significant association between interstitial fibrosis and age range categories (P = 0.1035). Out of the 142 cases in the (31-45) year category, 72 cases (51%) had interstitial fibrosis. Those in age categories (16-30), (46-60), (61-75) years had 19, 9 and 3 cases of interstitial fibrosis respectively. As shown below on figure 4.8.1 (B).

Figure 4.8.1 (B): Presence of interstitial fibrosis at different age categories.
4.8.1 (C) INTERSTITIAL FIBROSIS AND DRUG HISTORY

There was no significant association between interstitial fibrosis and drug history ($P = 0.13$). Out of a total of 114 pre-HAART cases, 54 cases (47%) presented with interstitial fibrosis. The number of cases with interstitial fibrosis who were on HAART was 50 (58%) out of 86 cases. As shown below on figure 4.8.1 (C).

![Figure 4.8.1 (C) Interstitial Fibrosis and drug history](image-url)
4.8.1 (D) INTERSTITIAL FIBROSIS AND DRUG COMBINATIONS

There was no significant association between interstitial fibrosis and different drug combinations ($P = 0.26$). Out of a total of 8 cases that were on D4T/3TC/NVP, 6 cases (75%) presented with interstitial fibrosis. Those on TDF/FTC/EVF, ABC/3TC/EVF, and TDF/FTC/NVP had 28 cases (65%), 12 cases (48%) and 4 cases (40%) of interstitial fibrosis respectively. As shown below on figure 4.8.1 (D)

**Figure 4.8.1 (D) Interstitial Fibrosis and Drug Combinations**
4.8.1 (E) INTERSTITIAL FIBROSIS AT DIFFERENT CD4 COUNT LEVELS

Interstitial fibrosis was not significantly associated with CD4 count categories ($P = 0.46$). As shown below, cases with CD4 count results less than 50 had 11 cases (84%). There was no case at CD4 count level that was more than 500. Figure 4.8.1 (F) show Interstitial Fibrosis at different CD4 Count Levels.

![Figure (E): Interstitial Fibrosis at different CD4 Count Levels](image-url)
4.8.2 NON-SPECIFIC CHRONIC THYROIDITIS
Out of a total the 200 cases, 23 cases (46%) had nonspecific chronic thyroiditis.

4.8.2 (A) NON-SPECIFIC CHRONIC THYROIDITIS AND GENDER DISTRIBUTION
Nonspecific chronic thyroiditis was not significantly associated with gender distribution (P = 0.35). Out of a total of 123 male cases, 31 cases (25%) had nonspecific chronic thyroiditis. In females, 15 cases (19%) out of 77 cases presented with interstitial fibrosis. Figure 4.8.2 (A) show gender distribution in cases with nonspecific chronic thyroiditis

![Chronic Thyroiditis in Males](image1)
![Chronic Thyroiditis in females](image2)

Figure 4.8.2 (A): Interstitial Fibrosis and Gender Distribution
4.8.2 (B) NONSPECIFIC CHRONIC THYROIDITIS AND AGE DISTRIBUTION

Nonspecific chronic thyroiditis was not significantly associated with age distribution (P = 0.09). Out of the 142 cases in the age category (31-45) years, 28 cases (20%) presented with nonspecific chronic thyroiditis. Those in age ranges (16-30), (46-60) and (61-75) years had 11 cases (26%), 5 cases (45%) and 2 cases (50%) respectively. As shown below on Figure 4.8.2 (B)

Figure 4.8.2 (B) Non-specific Chronic Thyroiditis with Age distribution
4.8.2 (C) NON-SPECIFIC CHRONIC THYROIDITIS AND DRUG HISTORY

Nonspecific chronic thyroiditis was not significantly associated with drug history ($P = 0.54$). Out of the 114 cases that were pre-HAART, 28 cases (24%) presented with nonspecific chronic thyroiditis. For the cases on HAART, 18 cases (21%) presented with nonspecific chronic thyroiditis. As shown below on Figure 4.8.2 (C).

![Figure 4.8.2 (C): Non-specific Chronic Thyroiditis and drug history](image)

**Figure 4.8.2 (C): Non-specific Chronic Thyroiditis and drug history**
4.8.2 (D) NON-SPECIFIC CHRONIC THYROIDITIS AND DRUG COMBINATIONS

There was a significant association between nonspecific chronic thyroiditis and specific drug combinations ($P = 0.007$). Cases that were on D4T/3TC/NVP were more affected by nonspecific chronic thyroiditis. Out of a total of 8 cases that were on D4T/3TC/NVP, 4 cases (50%) presented with nonspecific chronic thyroiditis compared to TDF/FTC/EVF 10 cases (23%), TDF/FTC/NVP 2 cases (20%) and ABC/3TC/EVF 2 cases (8%). As shown below on figure 4.8.2 (D).

![Figure 4.8.2: (D) Non-specific Chronic Thyroiditis and Drug Combinations](image-url)
4.8.2 (E) NON-SPECIFIC CHRONIC THYROIDITIS AT DIFFERENT CD4 COUNT LEVELS

There was no significant association between nonspecific chronic thyroiditis and CD4 count levels \((P = 0.54)\). Cases with CD4 count results less than 50 had 4 cases and those with levels in the category (51-100) had only one case. There was no case at CD4 count level that was above 100. As shown below on Figure 4.8.2 (F).

![Chronic Thyroiditis at different CD4 Count Levels](image)

**Figure 4.8.2(F): Chronic Thyroiditis at different CD4 Count Levels**
4.8.3 GRANULOMATOUS THYROIDITIS
Out of a total 200 cases, 2 cases (1%) presented with caseous granulomatous thyroiditis.

4.8.3 (A) GRANULOMATOUS THYROIDITIS AND GENDER DISTRIBUTION
Out of 122 male cases, 1 case (0.8%) was affected with granulomatous thyroiditis whereas 1 case (1.3%) out of 77 female cases showed granulomatous thyroiditis.

4.8.3 (B) GRANULOMATOUS THYROIDITIS AND AGE DISTRIBUTION
All cases of caseating granulomatous thyroiditis seen were in the age range 31 years to 45 years.

4.8.4 SPECIFIC INFECTIOUS CAUSES
Out of a total of 200 cases, only one case was found with Cryptococcus neoformans. This represented 0.5% of the cases. This case was a male patient aged 32 years and was HAART-naive. The patient did not have CD4 count results.

4.9 SUMMARY OF SIGNIFICANT DETERMINANTS OF PATHOLOGIC FINDINGS
Female cases were significantly more associated with pathologic lesions than male cases (P = 0.04). Out of the 77 female cases, 50 cases (65%) had thyroid lesions compared to the 62 cases (50%) of the 123 male cases. [See table 4.3]

Female cases were significantly more affected by interstitial fibrosis than male cases (P = 0.04). Out of the 77 female cases, 47 cases (61%) had interstitial fibrosis compared to 57 cases (46%) of the 123 male cases. [See table 4.3]

Nonspecific chronic thyroiditis was not significantly associated with gender distribution (P = 0.35). Out of a total of 123 male cases, 31 cases (25%) had
nonspecific chronic thyroiditis while in females, 15 cases (19%) out of 77 cases presented with interstitial fibrosis. [See table 4.3]

There was a significant association between nonspecific chronic thyroiditis and specific drug combinations (P = 0.007). Cases that were on D4T/3TC/NVP were more affected by nonspecific chronic thyroiditis. Out of a total of 8 cases that were on D4T/3TC/NVP, 4 cases (50%) presented with nonspecific chronic thyroiditis compared to TDF/FTC/EVF 10 cases (23%), TDF/FTC/NVP 2 cases (20%) and ABC/3TC/EVF 2 cases (8%). [See table 4.3]

Two cases presented with caseous granulomatous thyroiditis representing 1%. These were described in a 44 year old male and a 38 year old female. Both cases were not on cART and neither did they have CD4 count documentation.

Only one case of *Cryptococcus neoformans* (0.5%) was described. This case was described in a 32 years old male who was not on cART and had no CD4 count documentation.
Table 4.3: Determinants of Pathologic findings

<table>
<thead>
<tr>
<th>Thyroid Gland Lesion</th>
<th>Present N (%)</th>
<th>Absent N (%)</th>
<th>Total N (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>62 (50)</td>
<td>61 (50)</td>
<td>123 (100)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>50 (65)</td>
<td>27 (35)</td>
<td>77 (100)</td>
<td><strong>0.04</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interstitial Fibrosis and Gender Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial Fibrosis</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nonspecific Chronic Thyroiditis and Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nonspecific Chronic Thyroiditis And Specific Drug Combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDF/FTC/EVF</td>
</tr>
<tr>
<td>10 (23)</td>
</tr>
<tr>
<td>TDF/FTC/NVP</td>
</tr>
<tr>
<td>ABC/3TC/EVF</td>
</tr>
<tr>
<td>D4T/3TC/NVP</td>
</tr>
</tbody>
</table>
CHAPTER FIVE

5.0 DISCUSSION:

Although the prevalence of thyroid disease does not appear to be significantly increased in HIV-infected patients, compared with the general population, specific patterns of abnormal thyroid function test findings are more frequently identified among HIV-infected patients.¹⁰

Unlike all of the similar studies that have been done worldwide, this study represents the first detailed report of thyroid gland abnormalities in a HIV-infected Black African population in the African continent. It defines the relationship between clinical details and thyroid autopsy findings in Africa.

This study involved a total of 200 autopsy samples. Out of 200 cases, 112 cases representing 56% had thyroid lesions compared to 88 cases (44%) which had no lesions. The lesions found included interstitial fibrosis, non-specific chronic thyroiditis, Mycobacterium tuberculosis and Cryptococcus neoformans. No thyroid neoplasm was found.

5.1 REVIEW OF CLINICAL FILES:

A total of 200 clinical records corresponding to the 200 autopsy cases were reviewed. All the cases involved Zambian black Africans. This represents the majority ethnic group in Zambia. The youngest age was 19 years and the oldest was 72 years. The mean age was 34 years. Most of the cases seen were in the age range 31 to 45 years. The median and mode were both 33 years. These results are lower than the expected life expectancy at birth in the country which is 49.2 years in males and 53.4 years in females.³⁸

The male to female ratio in this study was significantly higher than the expected Zambian population male to female ratio (P =0.039). There were more males than females with a male to female ratio of 3:2. The total number of male cases was 123 representing 62.5% while that of females was 77 representing 38.5%. This did not match the male to female ratio in Zambia population studies of 49.3 % to 50.7%.³⁹
In most Southern African countries, proportionally more females are on HIV antiretroviral treatment than men, even when the higher HIV infection prevalence in females is accounted for. The difference in these HIV related mortalities could be due to the fact that women access HAART earlier than males as they are captured earlier during antenatal services and PMTCT. ⁴⁰

Among the cases which were on HAART, the drug combination which had the highest frequency was TDF/FTC/EVF, which had 43 cases representing 50% of cases on HAART. The lowest frequency was seen in drug combination D4T/3TC/NVP which had only 8 cases representing 4%. This might be explained by the fact that ART guidelines in Zambia were significantly changed by the World Health Organization to tenofovir based regimens in 2010. ⁴¹

The number of cases without CD4 results documentation was 169 representing 85% and those with documented results were 31 representing 15%. There was no case file which had viral load results documentation. Most of these cases without CD4 results could have been “Loss To Follow-Up” (LTFU) patients. Studies show that LTFU is common in antiretroviral therapy programmes. The rates of loss to follow-up in Zambia in the first 6 months of ART are 53.5 per 1,000 person-years. ⁴² The lack of CD4 count documentations and viral load results could also show that these tests were not routinely done at UTH during the period in question.

Lower levels of CD4 count had the highest number of cases compared to high CD4 count levels. The highest number of cases had CD4 count results that were less than 50. There were 13 cases with DC4 count results that were less than 50 representing 42% of cases with CD4 count results. There was no case with CD4 count results above 500. This agrees with studies which show that Low absolute CD4 count value is a strong risk for mortality in HIV infected individuals. ⁴³

Out of all the clinical files that were reviewed, none of the cases had thyroid function test results. This was expected as Thyroid Function Tests is not one of the routine investigations required for initiation of ART in Zambia.
5.2 PATHOLOGIC FINDINGS

The lesions found included the following: interstitial fibrosis 104 (52%) and non-specific chronic thyroiditis 46 (23%). Infections by *Mycobacterium tuberculosis* was seen in 2 cases (1%) and by *Cryptococcus neoformans* in 1 case (0.5%). None of the cases showed neoplasms.

These results deviated from the previous studies done. In a study done in Brazil, *Mycobacterium tuberculosis* was the most common lesion accounting for 23% of the patients compared to the 1% recorded in the Zambian study. *Cytomegalovirus* was a more common finding in the Brazil study accounting for 17%, in contrast to the Zambian study which did not record any case. The most common pathologic finding in the Zambian study was interstitial fibrosis which was not recorded in the Brazilian study. Both the Zambian and Brazil studies recorded cases of *Cryptococcus neoformans* representing 1% and 5% of the cases respectively. Neoplastic lesions such as Kaposi's sarcoma and occult papillary carcinoma were recorded in 2% and 4% of the patients, respectively, in the Brazilian study. Neoplastic lesions were not recorded in the Zambian study. ²

In another similar study done in India, significant pathological lesions were identified in 35% cases. Tuberculosis was the predominant finding, identified in 6% of the cases. The other pathologies identified were cryptococcosis 5%, cytomegalovirus 2%, Hashimoto’s thyroiditis 3%, fibrosis 6% and goiter 3%. *Pneumocystis jiroveci* thyroiditis and malignant neoplasms of the thyroid were not seen in agreement with the Zambian study. ²

Rabia C. et al did a similar autopsy study in USA on African Americans in 2013. There were 102 patients. Thirteen patients with abnormal thyroid findings were identified. Interstitial fibrosis was the most common histological finding (4.9%), followed by thyroid hyperplasia (1.9%). Infectious disease affecting the thyroid gland was limited to 2.9% and consisted of *Mycobacterium tuberculosis*,.
Cryptococcus neoformans, and Cytomegalovirus. Kaposi sarcoma of the thyroid gland was present in only one case (0.9%).

The Brazilian and Indian studies were done before advent of HAART. These two studies showed that infectious diseases were more common than the Zambian and USA studies which were done more recently. That is after the introduction of HAART.\textsuperscript{2, 18, 20}

All the studies identified Mycobacterium tuberculosis in the thyroid gland. However, the frequency was more in the Brazilian and Indian studies compared to the Zambian and USA studies. This could have been due to the same reason that the studies involved cases before the advent of HAART.\textsuperscript{2, 18, 20}

Unlike the Brazilian study which recorded Pneumocystis jiroveci thyroiditis in 4% of the cases, the Zambian, USA and Indian studies did not record any case. These results in the Zambian study could be explained by the effects of routine co-trimoxazole prophylaxis against PCP which is implemented as an integral component of the HIV care package.\textsuperscript{2, 18, 20}

The Zambian study recorded similar results with the Indian study in that there was no neoplastic lesion seen in the thyroid. However, the Brazilian and the USA studies recorded Kaposi sarcoma as one of the neoplastic thyroid gland lesions. The Brazilian study also recorded occult papillary carcinoma in 4% of cases as opposed to the other studies which did not record any case. These differences can partly be explained by differences in ethnicity. Ethnicity-related differences in organ systems involvement in HIV patients have been described previously. It has been recorded that there is a difference in multiple organ involvement in HIV infected between Black and Whites or Hispanic individuals. However the exact explanation for these discrepancies is not clear.\textsuperscript{2, 15, 18, 20}

5.2.1 INTERSTITIAL FIBROSIS

Interstitial fibrosis was the most common pathologic finding in this study. Previous studies have linked thyroid fibrosis to transforming growth factor beta
(TGF-beta) in a setting of selenium deficiency. Follicular cell necrosis occurs first followed by thyroid fibrosis. Interstitial fibrosis of the thyroid gland in HIV infected could represent the histologic sequel of previous inflammatory or infectious assaults coupled with impaired tissue repair due to the underlying immunosuppression. In addition, HIV infection itself is associated with increased levels of transforming growth factor beta (TGF-beta).

Female cases were significantly more affected by interstitial fibrosis than male cases (P = 0.04). Out of the 77 female cases, 47 cases (61%) had interstitial fibrosis compared to 57 cases (46%) of the 123 male cases. More studies need to be done to explain this trend.

5.2.2 NON-SPECIFIC CHRONIC THYROIDITIS

Nonspecific chronic thyroiditis was significantly high in drug combination D4T/3TC/NVP (P = 0.007). Out of a total of 8 cases that on D4T/3TC/NVP, 4 cases (50%) presented with nonspecific chronic thyroiditis compared to TDF/FTC/EVF 10 cases (23%), TDF/FTC/NVP 2 cases (20%) and ABC/3TC/EVF 2 cases (8%). These results can be partly explained by the efficacy of Tenofovir based regimens compared to Stavudine regimens. However, studies need to be done to establish the relationship of these different ART regimens to inflammation.

There was no significant association between nonspecific chronic thyroiditis and gender distribution (P = 0.35), age distribution (P = 0.09), drug history (P = 0.55) and (P = 0.54).

5.2.3 GRANULOMATOUS THYROIDITIS

Only 2 cases were found to have granulomatous thyroiditis. This represented 1% of the cases. Although ZN stain did not reveal any acid alcohol fast bacilli, the histological features were consistent with tuberculosis.
One case was male and the other was female. Both patients were HAART-naive. None of the cases had CD4 count results. One of the cases had autopsy diagnosis of disseminated tuberculosis whereas the other one had pulmonary tuberculosis.

5.2.4 INFECTIOUS CAUSES

One case of *Cryptococcus neoformans* representing 0.5% of the cases was recorded in a 32 year old male, in pre-HAART phase without documented CD4 count results.

In the Brazilian study, *Pneumocystis jiroveci* was a prominent cause of thyroiditis in HIV patients accounting for 4% of the cases. In the Zambian study, this microorganism was not identified. This may be due to the fact that most pre-HAART patients presenting to UTH are on presumptive co-trimoxazole prophylaxis against *Pneumocystis jiroveci* pneumonia.\(^2,18,20\)
CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION
In conclusion, subclinical thyroid lesions are significantly high in the HIV-infected Zambian population.

The subclinical histopathological lesions seen in the thyroid glands are interstitial fibrosis accounting for 52% of the cases followed by non-specific chronic thyroiditis representing 21.5%.

Infections by *Mycobacterium tuberculosis* and *Cryptococcus neoformans* are uncommon.

Nonspecific chronic thyroiditis is significantly high in drug combination D4T/3TC/NVP accounting for 50% of cases.

Females are significantly more affected by interstitial fibrosis of the thyroid gland than male in the HIV-infected Zambian population.

Neoplastic thyroid lesions are uncommon in the HIV-infected Zambian population according to this study’s findings.

6.2 STUDY LIMITATIONS
There were no documented thyroid function tests from the clinical files.

The study did not have controls.

6.2 RECOMMENDATION
Due to subclinical thyroid lesions being common in the HIV infected Zambian populations; thyroid function tests should be included in the routine investigations for initiation of HAART.

Systems should be put in place to ensure that all records on HIV infected are captured.
Prospective studies correlating histopathological findings with thyroid function tests need to be done for a better assessment of thyroid pathology in the HIV infected Zambian population.
REFERENCES


2. Basilio-De-Oliveira, C A; Infectious and neoplastic disorders of the thyroid in AIDS patients: an autopsy study. Department of Pathology, Gaffree and Guinle University Hospital, University of Rio de Janeiro (Uni-Rio).


20. Rio de Janeiro, RJ, Brazil. “Pathology of thyroid in AIDS: An Autopsy study”, Department of Pathology, Grant Medical College and Sir J.J. Hospital, Byculla, Mumbai 08, India.


APPENDIX

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APPENDIX B
## APPENDIX C: VARIABLES FROM CLINICAL FILES:

<table>
<thead>
<tr>
<th>SERIAL NUMBER</th>
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<tbody>
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</table>

### DRUG HISTORY

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<th>Yes</th>
</tr>
</thead>
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<th>2. Type of HAART combination</th>
<th>TDF/FTC/NVP</th>
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<td>TDF/FTC/EVF</td>
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<tr>
<td></td>
<td>D4T/3TC/NVP</td>
</tr>
<tr>
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<td>ABC/3TC/EVF</td>
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### LABORATORY FINDINGS

<table>
<thead>
<tr>
<th>1. CD4 Count Results</th>
<th>Present</th>
</tr>
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<tbody>
<tr>
<td></td>
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<tr>
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<tr>
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<td>51-100</td>
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<td>101-200</td>
</tr>
<tr>
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<td>201-300</td>
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<td>301-400</td>
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<tr>
<td></td>
<td>401-500</td>
</tr>
<tr>
<td></td>
<td>&gt;500</td>
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<th>3. Thyroid Function Tests</th>
<th>Present</th>
</tr>
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<tr>
<td></td>
<td>Absent</td>
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<tr>
<td></td>
<td>Normal levels</td>
</tr>
<tr>
<td></td>
<td>High levels</td>
</tr>
<tr>
<td></td>
<td>Low levels</td>
</tr>
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### CAUSE OF DEATH

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<th>1. Clinical cause of death</th>
<th>Diagnosis</th>
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<td>(Specify</td>
<td></td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>2. Post Mortem cause of death</th>
<th>Diagnosis</th>
</tr>
</thead>
</table>
## APPENDIX D: DEPENDED VARIABLES

| SERIAL NUMBER |  |  |
| AGE |  |  |
| SEX |  |  |

### NON SPECIFIC FINDINGS

| Tick |  |  |
| 3. Interstitial Fibrosis | Present |  |
| Absent |  |
| No data |  |
| 4. Non Specific Acute Inflammation | Present |  |
| Absent |  |
| No data |  |
| 5. Non Specific Chronic Inflammation | Present |  |
| Absent |  |
| No data |  |

### SPECIFIC INFECTIONS

| Tick |  |  |
| 4. Cryptococcus | Present |  |
| Absent |  |
| No data |  |
| 5. Cytomegalovirus | Present |  |
| Absent |  |
| No data |  |
| 6. Caseating granulomatous Inflammation consistent with TB | Present |  |
| Absent |  |
| No data |  |
| 7. Other specific infections | Specify |  |

### NEOPLASMS

| Tick |  |  |
| 3. Kaposi Sarcoma | Present |  |
| Absent |  |
| No data |  |
| 4. Papillary carcinoma | Present |  |
| Absent |  |
| No data |  |
| 5. Other Neoplasm | Specify |  |
APPENDIX E: FLOW CHART OF TISSUE PROCESSING

Flow chart of tissue processing

<table>
<thead>
<tr>
<th>Station 2</th>
<th>Container</th>
<th>Fluid</th>
<th>Purpose</th>
<th>Time (Hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10% Formalin</td>
<td>Fixation</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>10% Formalin</td>
<td>Fixation</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>70% Ethanol</td>
<td>Dehydration</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>95% Ethanol</td>
<td>Dehydration</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>100% Ethanol</td>
<td>Dehydration</td>
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</tr>
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<td>6</td>
<td>100% Ethanol</td>
<td>Dehydration</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>100% Ethanol</td>
<td>Dehydration</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Xylene</td>
<td>Clearing</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Xylene</td>
<td>Clearing</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Xylene</td>
<td>Clearing</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Molten paraffin wax</td>
<td>Impregnation</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Molten paraffin wax</td>
<td>Impregnation</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Station 1
Cut up bench of samples fixed in 10% formalin

Station 2

Station 3
Embedding.
Sectioning (3 μm).
Slide mounting.

Station 4
Staining
H&E stain.

Station 5
Microscopy

Station 6
Additional staining as indicated.
Special stains and/or immunohistochemistry
APPENDIX F: HEMATOXYLIN AND EOSIN (H&E) STAINING PROTOCOL

Hematoxylin and Eosin (H&E) Staining Protocol

Principle

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

Haematin exhibits indicator-like properties, being blue and less soluble in aqueous alkaline conditions, and red and more soluble in alcoholic acidic conditions. In acidic conditions, haematin binds to lysine residues of nuclear histones by linkage via a metallic ion mordant, in this case aluminium. To ensure saturation of chemical binding sites, the stain is applied longer than necessary, resulting in the overstaining of the tissues with much non-specific background colouration. This undesirable colouration is selectively removed by controlled leaching in an alcoholic acidic solution, (acid alcohol), the process being termed "differentiation". Differentiation is arrested by returning to an alkaline environment, whereupon the haematin takes on a blue hue, the process of "blueing-up". The haematin demonstrates cell nuclei.

Full cellular detail is obtained by counterstaining with the eosin mixture. Colour enhancement is achieved by fortifying the stain with phloxine, a chemical member of the same family as eosin (halogenated fluoresceins). The mechanism of their staining is not fully understood, but is believed to be of an electrostatic nature. Visualisations most acceptable to the histologist are obtained by applying the dyes in acidic conditions, whereby more intense specific colourations are
obtained, the more acidic tissue components taking up the dye to a greater intensity, hence the addition of acetic acid.

Technical Points

1. (step 2) - The length of time necessary to over-stain the tissues will depend upon fixation and the type of alum haematoxylin employed. Lillie Mayer's alum haematoxylin-formalin fixed tissues should take 5 mins.

Tissue Type

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Haematoxylin</th>
<th>Acid alcohol 0.3%</th>
<th>Eosin</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine tissues</td>
<td>4 minutes</td>
<td>See technical point 2</td>
<td>2 minutes</td>
<td></td>
</tr>
<tr>
<td>Renal biopsies</td>
<td>10 minutes</td>
<td>1-2 seconds</td>
<td>2-4 minutes</td>
<td>Check staining</td>
</tr>
<tr>
<td>Decals</td>
<td>10 minutes</td>
<td>1-2 seconds</td>
<td>30 seconds</td>
<td>Check staining after blueing.</td>
</tr>
<tr>
<td>Hx step may need to be repeated if prolonged decal.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. (step 4) - Differentiation with acid alcohol requires some practical experience to ascertain the correct end-point, since the acid solution alters the colour of the
tissue to red. The correct end-point is when, after blueing up, the background is almost colourless. For renal biopsy sections, two quick dips in 0.3% acid alcohol are all that is required.

3. (step 6) - If Scott’s tap water substitute is employed, blueing up is achieved in a much shorter time.

4. (step 8) - Eosin is highly soluble in water. Over-staining is removed by washing in running water.

5. Fixation - Not critical. Acidic fixatives will give a more eosinophilic result. Picric acid containing fixatives give an overall enhanced result. Acidic decalcifying fluids give poor nuclear staining.

6. Renals - 10% buffered formalin. Sections cut at 2μ.

Method

1. Bring sections to distilled water
2. Stain nuclei with the alum haematoxylin (see note)
3. Rinse in running tap water
4. Differentiate with 0.3% acid alcohol (see note)
5. Rinse in running tap water
6. Rinse in Scott’s tap water substitute (see note)
7. Rinse in tap water
8. Stain with eosin 2 mins
9. Dehydrate, clear and mount.
Results

collagen.............................................pale pink

muscle.............................................deep pink

acidophilic cytoplasm............................red

basophilic cytoplasm..............................purple

nuclei...............................................blue

erthrocytes.......................................cherry red

Reagent Formulae

1. Lillie Mayer alum haematoxylin

aluminium ammonium sulphate ---- 200 g

haematoxylin (CI 75290) ---------- 20 g

ethanol ------------------------------- 40 ml

sodium iodate ----------------------- 4 g

acetic acid -------------------------- 80 ml

glycerol --------------------------- 1200 ml

distilled water ----------------------- 2800 ml
In a 4L Erlenmeyer flask, to 1000 mls of the distilled water, add the aluminium ammonium sulphate. Place the flask on a heater/stirrer, turn on the heater and allow to mix until the alum dissolves - this takes about 15 mins. Remove the flask from the heater/mixer, allow to cool, and then add the remaining 1800 mls distilled water - this will further cool the solution. Add the haematoxylin powder to the alcohol and dissolve as much of the powder as possible by shaking for a few minutes. Pour the strong alcoholic solution of haematoxylin into the cooled alum solution and stir to ensure all the Hx powder is dissolved, preferably overnight. Add the sodium iodate, acetic acid, and finally the glycerol. Mix well, plug loosely and store.

It is appropriate to make up a batch of the required amount, dependent upon the usage rate.

2. Acid alcohol 0.3% Acid Alcohol

commercial grade ethanol ----- 2800 ml
distilled water ------------------ 1200 ml
conc hydrochloric acid -------- 12 ml

In a sufficiently large container, add the acid to the water, then add the alcohol and mix thoroughly. The generation of fine bubbles is an indication that mixing is thorough.

3. Scott's tap water substitute

sodium hydrogen carbonate --- 10 gm
magnesium sulphate ----------- 100 gm
distilled water ------------------ 5 L

Dissolve the salts in the water. Store stock solutions at room temperature.

4. alc acetified eosin/phloxine     TQEH

1% eosin Y (CI 45380) -------- 400 ml

1% aqphloxine (CI 45405) ------ 40 ml

95% alcohol -------------------- 3100 ml

gl acetic acid ------------------ 16 ml

Mix the above reagents together, and stir well. The solution keeps well.

16.1.1 References

Mayer P,(1896),Mitt. zool. Stn. Neapel.,12,303


IHC Protocol for Paraffin Tissue

1. Label slides with antibody, dilution, and date (Month and Year)
2. Melt Wax - Place slides on hotplate for 30 minutes
3. Fill in antibody dilution sheet
4. Cool slides – Leave on bench for 20 minutes
5. Dissolve wax - De-paraffinize tissues (change solutions once a week)
   • Xylene 3X, 5 minutes
6. Re-hydrate
   • 100% Ethanol 2X, 5 minutes
   • 95% Ethanol 1X, 5 minutes
   • 80% Ethanol 1X, 5 minutes

****If tissue is lymph node, tonsil, spleen, or liver - soak in alkylphenol ethoxylate (APE) for 30 minutes ***. SEE APE PROTOCOL.
Continue with step 7.

Remove the slide(s) labeled H&E AND REFER TO H&E PROTOCOL. CAN START H&E PROCEDURE WHILE CONTINUING WITH IMMUNO PROCEDURE.

7. Remove endogenous peroxidase -- 20 minutes (Prepare fresh each time)
   - 200 mL methanol and 2 mL Hydrogen peroxide
8. Rinse - Distilled water, 3X, 3 minutes
9. Antigen retrieval
   - Fill steamer water reservoir with tap water to “MAX” marking line
   **Do not overfill**
   - Place Bowl/drip tray onto the base
   - Fill coplin or slide holders with unmasking retrieval/distilled water
   - Pre-heat for 20 minutes
   - Place slides in plastic storage container filled with antigen retrieval buffer (ARB) that is already preheated
   Use specified antigen retrieval buffer (see Antibody-dilutions and ARB)
   Allow 20 minutes
10. Cool slides- place containers on bench and allow cool down at room temperature for 30 minutes.
11. PBS 1X– rinse for 5 minutes
12. Pap pen- rim tissues with pap pen (make sure slides do not dry)
   ** be careful not to get ink on tissue**

IHC Protocol for Paraffin Tissue January 01, 2011

13. Block with serum - Add 10% Normal serum (NS) (type of serum depends on secondary antibody) to PBS.
- 30 minutes at room temperature.

14. Primary antibody (1Ab)
   - Flick excess 10% NS/PBS off
   - Add primary antibody
   - Place slides in humidity chamber
   - **add tap water to chamber before loading the slides**
   - Leave slides for 60 minutes at room temperature or leave overnight at 4°Celsius (refer to antibody incubation time sheet).
   If overnight, allow slides to warm up at room temperature for 1 hour before rinsing.

15. Rinse in PBS 1X– 3X, 3 minutes

16. Biotinylated Secondary Antibody (2Ab)
   - Flick off excess PBS
   - Apply secondary antibody (1:200 dilution in 10% NS/PBS)
   - Incubate for 30 minutes

**Prepare ABC solution**

1. Add 2 drops A to 5ml PBS 1X, mix
2. Add 2 drops B to the mixture of A and PBS 1X solution, mix
3. Allow to sit for 30-40 minutes at room temperature before use.

****If need less than 2.5ml of ABC solution, then prepare as follows****

1. Add 1 drop A to 2.5ml PBS 1X, mix
2. Add 1 drop B to the mixture of A and PBS 1X solution, mix
3. Allow to sit for 30-40 minutes at room temperature before use.

17. Rinse in PBS 1X– 3X, 3 minutes

18. ABC solution - Flick off excess PBS add ABC complex solution, allow to sit for
   30 minutes.

19. Rinse in PBS 1X– 3X, 3 minutes
20. 3,3'-diaminobenzidine (DAB)

1. DAB – To 1 mL buffer add one drop DAB-mix well

2. Pipette 200 µL DAB solution to slide

3. Verify one slide under microscope to determine time DAB exposure (reaction time is dependent of antibody)

4. Use same time to develop the rest of the slides

IHC Protocol for Paraffin Tissue January 01, 2011

21. Rinse in distilled water – Rinse slides 2X, 5 minutes

22. Counter stain hematoxylin– dip in hematoxylin for 20 secs (if shiny gold specs, filter solution)

23. Rinse in tap water – allow running water until clear water (no coloring)

24. Dip in ammonia water for 10 seconds

25. Rinse in tap water – allow running water for 2 minutes

26. De-hydrate

   - 80% Ethanol, 5 minutes
   - 95% Ethanol, 5 minutes
   - 100% Ethanol, 5 minutes

27. Clearing

   - Xylene, 3X, 3 minutes

28. Cover slip

- mount slides using cytoseal and allow to dry 20 minutes before examining under microscope.
Antibody Dilutions and Calculation

Blocking Solution, 1 Ab and 2 Ab

DATE: _____/_____/___________ Initials __________

- __________ 1 Ab, raised in _______________

- __________ 2 Ab, raised in _______________

__________ Serum (depends on 2 Ab)

_____ slides X 200 □L = _________ □l, ~ _______ □L

Blocking solution needed: Blocking, 1 Ab, and 2 Ab

__________ □L X 3 = _________ □l

__________ □l Total BS needed (round up)

Blocking solution: 10% NS + buffer (PBS 1X)

__________ □l 10% NS + _________ □L PBS 1X = _________ □l

---------------------------------------------------------------------------------------------

1 Ab: □- __________

__________ □L BS / _____ dilution = _________ 1 Ab needed

_____ □l 1 Ab + _________ □l BS = _________ □l 1 Ab & BS Total

---------------------------------------------------------------------------------------------

2 Ab: □-______________

2 Ab Dilution: 1:200

_____ □l 2 Ab + _________ □l BS = _________ □l 2 Ab & BS Total

Avidin and Biotinylated Horseradish Peroxidase Macromolecule Complex

January 01, 2011

Avidin Biotin Complex Procedure

1. Add 2 drops of Reagent A into 5 mL PBS 1X, MIX

2. Add 2 drops of Reagent B into the 5 mL PBS 1X containing

Reagent A, vortex

3. Leave on counter at room temperature for 30 to 40 minutes

XVIII
4. Pipette 200 μL each slide
5. Incubate for 30 minutes
6. Rinse with PBS

Counterstaining and cell structure sharpening

3,3’-diaminobenzidine (DAB)
1. Multiple 200 μL by number of slides = Total μL
2. One drop per 1 mL solution
3. Take one slide and pipette 200 μL DAB mixture
4. Watch reaction develop under microscope
5. Time the reaction
6. Stop reaction by immersing slide in tap water
7. Develop all the remaining slides using the same time
8. **Be consistent**
APPENDIX H: INFORMATION SHEET

Information sheet for the next of kin in sub-type c Neuro-AIDS and pathogenesis in Zambia project.

This information is being provided to regarding the Zambia Neuro-AIDS study to enable to give informed and voluntary consent to participate in this study.

Kindly read it carefully or let someone read it to you before you sign the consent form.

Introduction

People infected with HIV, the virus that causes AIDS, with time may lose their memory and sometimes become confused or demented. Although sub African has highest level of HIV infection in the world, there is very little known of how HIV affects a person’s brain function in this region. This research seeks to understand how HIV infests the brain, and how it affects it. In order to do this, we will test samples from people who have already died of various causes and ran tests on their blood samples to see if they are HIV positive. If they are, we will use the samples collected normally at autopsy to see the effects, if any that HIV has had on the brains. These samples collected will include will include brain samples, cerebrospinal fluid, the fluid that washes over the brain and blood samples. On these samples, we will do tests to verify if the virus (HIV) is present or not, and at what stage of infection the person was at by determining their CD4 counts (a type of white blood cell) and viral loads(amount of virus in these samples.)
Benefits and risks

There is no risk to the family or the deceased as no more will be done than the normal procedure for autopsy. The immediate benefit to the family is that the autopsy will be expedited and should the family need transport to move the body from the point of death to the hospital, the project will provide this at no cost to the family. There is, however, benefit to the community as doctors get to better understand how HIV affects the brain and so can treat those with HIV to better prevent the deleterious effects of HIV on the brain and mental function.

Should you have any questions, please contact Dr Constantine Malama on 097-9-070477 and/or the research ethics committee (REC).

Confidentiality

All the information gathered in this study will be used in privacy and known only to members of the research team. The identity of the persons will not be disclosed to anyone outside the research team.

Declaration.

I give consent to have the deceased have tissues and blood samples be taken at the time of autopsy for the purposes of taking part in this Zambia HIV neuro AIDS study. I give my consent indicating voluntary and informed consent as next of kin to the deceased. I may withdraw my consent at anytime without penalty or loss of benefits or treatment to which I am entitled. Doctors may choose not proceed to collect specimens
or use such specimens for such study without my consent. Additionally, my withdrawal will not in any way affect how doctors treat me in regard to the deceased / next of kin

Declaration:

I understand what the study is all about and what is expected of if I participate in this study.

Interviewees name.......................................................... ..........................................................

Signature / Thumb print...........................................................................................

Date...................................................................................................................

Witness’ name...................................................................................................

Signature...........................................................................................................

Date..................................................................................................................
Should you have any questions, you can contact Dr Constantine Malama, Kalingalinga Health Centre or Dr Victor Mudenda, at the UTH, Department of Pathology. You can also contact the Research and Ethics committee (REC).

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University of Zambia Biomedical Research Ethics Committee

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Zambia.
APPENDIX I: CONSENT FORM FOR AUTOPSY, FOR THE NEXT OF KIN

CONSENT FORM FOR AUTOPSY, FOR THE NEXT OF KIN.

I give consent to have the deceased have tissues and blood samples be taken at the time of autopsy for the purposes of taking part in this Zambia HIV neuro AIDS study. I give my consent indicating voluntary and informed consent as next of kin to the deceased. I may withdraw my consent at anytime without penalty or loss of benefits or treatment to which i am entitled. Doctors may choose not proceed to collect specimens or use such specimens for such study without my consent. Additionally, my withdrawal will not in any way affect how doctors treat me in regard to the deceased / next of kin

Declaration:

I understand what the study is all about and what is expected of if I participate in this study.

Interviewees
name..........................................................................................................................

Signature / Thumb print.....................................................................................................................

Date..........................................................................................................................
Witness’ name........................................................................................................................................

........

Signature............................................................................................................................................

............... 

Date..................................................................................................................................................

............... 

Should you have any questions, you can contact Dr Constantine Malama, Kalingalinga Health Centre or Dr Victor Mudenda, at the UTH, Department of Pathology. You can also contact the Research and Ethics committee (REC).

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Department of medicine
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APPENDIX J: ZAMBIA HIV NEUROPATHOGENESIS STUDY

ID ___ ___ ___ - ___ ___ ___ Post-mortem Information

Zambia HIV Neuropathogenesis

PATHOLOGY

Ward: ___ ___ ___ ___

AGE: ___ ___ SEX: O Male O Female

Clinical

Diagnosis:_____________________________________________________

DOD: __/__ __/__ __ ___ Time of D: ___ ___:___ ___ hrs

Date of AUTOPSY: __/__ __/__ __ ___ ___ ___ ___ ___

Specimen collected: Date: ___ ___ /___ ___/___ ___ ___ ___

O Blood Time: ______________

Purple top: ______ ml, # of vials: ___

Red top: ______ ml, # of vials: ___

Analysis: O HIV

Results: HIV: O Positive O Negative

O Plasma stored in -80 degree freezer

O CSF Time: ______________
XXXX ml, # of vials: ______

Analysis: O HIV

Results: HIV: O Positive       O Negative

O CSF stored in -80 degree freezer

Tissues collected for MOLECULAR ANALYSIS

BRAIN REGION: Hemisphere

□ Frontal Lobe □ Right □ Left
□ Parietal Lobe □ Right □ Left
□ Temporal Lobe □ Right □ Left
□ Occipital □ Right □ Left
□ Hippocampus □ Right □ Left
□ Cerebellum □ Right □ Left
□ Basal Ganglia □ Right □ Left
(Caudate/Putamen)

□ Lymph node □ Choroid Plexus □ Gut tissue
□ Stored in cryovials, -80 degree freezer

Tissues collected for HISTOLOGICAL ANALYSIS

BRAIN REGION

□ Frontal Lobe □ Right □ Left
□ Hippocampus □ Right □ Left
□ Parietal Lobe □ Right □ Left
□ Temporal Lobe □ Right □ Left
□ Occipital □ Right □ Left
□ Basal Ganglia □ Right □ Left
☐ Spleen       ☐ Choroid Plexus       ☐ Gut tissue       ☐ Liver       ☐ Lungs
☐ Lymph node       ☐ Kidneys/adrenals       ☐ Pancreas       ☐ Thyroid

☐ Stored in normal buffered formalin

Pathologist (Print)

Technician

Date and Time picked up