Original Article

A Histological Autopsy Study of the Thyroid gland in HIV infected Adults at the University Teaching Hospital in Lusaka, Zambia

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ABSTRACT

Background: Despite the high prevalence of Human Immunodeficiency Virus (HIV) in Zambia, not much is known about the spectrum of thyroid lesions in patients with Acquired Immune Deficiency Virus, (AIDS). Patients with AIDS have increased prevalence of thyroid function abnormalities. It is therefore postulated the thyroid gland would be involved in AIDS patients. This study focused on the histologic appearances of the thyroid gland in HIV and AIDS patients on post mortem. The findings will provide evidence based recommendations on the management of thyroid diseases in the HIV infected in Zambia.

Objective*:* The aim of the study was to determine the histological appearances of adult thyroid glands in patients who died of HIV related diseases at UTH in the period 2010 to 2012.

Materials and Methods: This research was a descriptive retrospective study of adult thyroid glands collected at autopsy during the period 2010 to 2012. The study took place at the University Teaching Hospital (UTH) in the Department of Pathology and Microbiology in Lusaka, Zambia. A total of 200 thyroid gland paraffin blocked samples were obtained from the parent study tissue bank by convenient sampling. The inclusion criteria included samples from adult HIV patients who were admitted to UTH and died in hospital. The Paraffin blocked thyroid tissue samples were processed according to standard histopathology laboratory protocols at UTH which included sectioning and staining with hematoxylin and eosin. The slides were examined at light microscopic level. Clinical data was obtained from the available case notes.

Results: The sample size was 200 thyroid glands of which 123(62%) were males. Thyroid histopathological lesions were seen in 111 (56%) of the cases, of which 60 (54%) were male. The lesions included: Interstitial fibrosis 104 (52%), nonspecific chronic thyroiditis 42 (21%). Infectious diseases included Mycobacterium tuberculosis 2 (1%) and C*ryptococcus* neoformans 1(0.5%).

Conclusion: These findings outline the importance of thyroid function testing in HIV infected patients who may have subclinical thyroid dysfunction and infection.

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

Key Words: *Thyroid histology, HIV infection, Autopsy, Adult*

Acquired Immune Deficiency Syndrome (AIDS).[1,2,3] The most common thyroid abnormalities in these patients include euthyroid sick syndrome and hypothyroidism.[4] However, there is very scant 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.

Histological appearances of thyroid glands in patients who died of HIV related diseases have been described in some studies. These studies were carried out in the South American, North American and Asian continents. Studies done in Brazil, India and USA described the presence of *Mycobacterium tuberculosis, Cryptococcus neoformans,* and *cytomegalovirus* infections in thyroid glands of the HIV infected at autopsy. The Brazilian study described the presence of thyroid papillary carcinoma whereas the Indian and USA studies revealed the presence of Kaposi sarcoma.[5,6,7]

Histological appearances of thyroid glands in the HIV infected have not been described in the African continent.

This study is the first retrospective report to describe the appearance of the thyroid gland in HIV infected patients in Zambia and Africa as a whole. It will determine the types of infections and structural changes in the thyroid gland at autopsy in HIV infected patients. Some of the determinants of thyroid gland pathology in HIV infected adults at autopsy will be evaluated. The findings of this study will provide for evidence based recommendations on the management of thyroid diseases in the HIV infected in Zambia.

METHODOLOGY

Study Design

This research was a descriptive retrospective study of adult thyroid glands collected at autopsy during the period 2010 to 2012.

Study Setting

The study took place at the University Teaching Hospital (UTH) in the Department of Pathology and Microbiology in Lusaka, Zambia. Thyroid gland paraffin blocked samples were obtained from the Neuro-AIDS-Study tissue bank by convenient sampling. A total of 200 paraffin blocks were selected. The inclusion criteria included samples from adult HIV patients who were admitted to UTH and died in hospital. Paraffin blocks which did not have corresponding clinical records and those which contained autolyzed tissues were excluded from this study. The Paraffin blocked thyroid tissue samples were processed according to standard histopathology laboratory protocols at UTH. This included sectioning and staining with hematoxylin and eosin. The slides were examined at light microscopic level. Special staining was done as per microscopy results using ZN and PAS. Clinical data was obtained from the available case notes. The independent variables included age, sex, drug history and CD4 count results. The dependent variables included thyroid lesions seen under microscopy examination.

Statistical Analysis

Data was examined using SPSS version 20. A Chi square test was used to study association between categorical variables. A P-Value of less than 0.05 was considered significant.

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 50cells/μL were 13(42%). The number of cases with CD4 count of less than 200 cells/ μL was 25, representing 81% of patients.

Eighty-six (43%) patients were not on ART while 114 (57%) patients were on combined Anti-Retroviral Therapy (cART). Out of the patients who were on cART, 50% were on Atripla-Tenofovir/Emtricitabine /Efavirenz (TDF/FTC/EFV) and the rest were on TDF/FTC/NVP, D4T/3TC/NVP and ABC/3TC/EVF.

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

Table 1: Demographic and Baseline Characteristics of the Participants

Variable	Category	Frequency n (n=200)	Frequency %
Age	16-30	43	21
	31-45	142	72
	46-60	11	5
	61-75	4	2
	>75	0	0
	Total	**200**	**100**
Sex	Male	123	62
	Female	77	38
	Total	**200**	**100**
Drug History	PreHAART	114	57
	HAART	86	43
	Total	**200**	**100**
Drug Combinations	TDF/FTC/NVP	10	12
	TDF/FTC/EVF	43	50
	D4T/3TC/NVP	8	9
	ABC/3TC/EVF	25	29
	Total	**86**	**100**
CD4 count	Done	31	15
	Not done	169	85
	Total	**200**	**100**
CD4 Follow-up	**<50**	13	42
	51-100	8	26
	101-200	4	13
	201-300	2	6
	301-400	1	3
	401-500	3	10
	>500	0	0
	Total	**31**	**100**

Pathologic Findings

Out of 200 cases, 112 cases (56%) had thyroid lesions. Interstitial fibrosis was the most common histological finding accounting for 104 cases (52%), followed by nonspecific chronic thyroiditis with 42 (21%). Infectious diseases affecting the thyroid gland consisted of Mycobacterium tuberculosis 2 (1%) and C*ryptococcus* neoformans 1(0.5%). [See table 2 and 3]

Table 2: Summary of Pathologic Findings

	Frequency n (n=200)	Frequency %
Present	112	56
Absent	88	44
Total	**200**	**100**

Table 3: Frequency of Thyroid lesions

Thyroid Lesion	Frequency n (n=100)	Frequency %
Interstitial Fibrosis	104	52
Chronic thyroiditis	42	21
Mycobacterium tuberculosis	2	1
Cryptococcus neoformans	1	0.5

The numbers in table 3 will not sum to 100% since the categories are not mutually exclusive.

Description of Pathologic Findings:

Interstitial Fibrosis

Interstitial fibrosis was described as thick fibrous bands compressing thyroid follicles without any inflammatory infiltrates. The fibrous bands stained blue with Masson trichrome. [See pictographs 1 to 4].

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. [See pictographs 5 and 6].

Mycobacterium tuberculosis

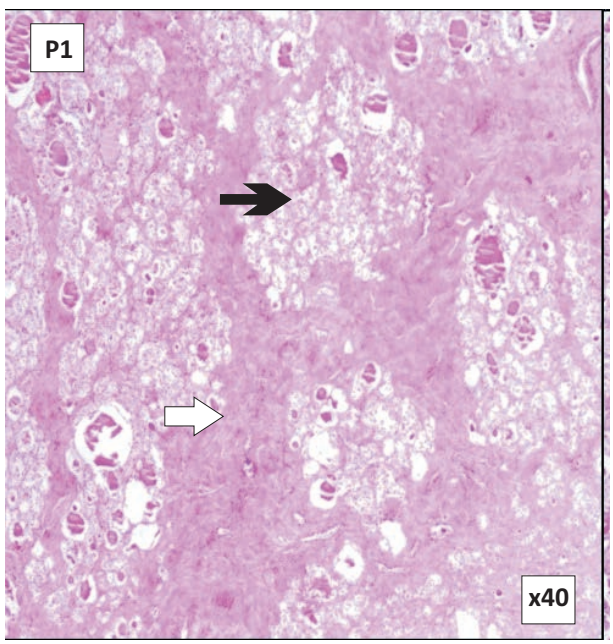
The presence of caseating granulomatous inflammation was used to describe features consistent with tuberculosis. [See pictographs 7 and 8].

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. [See pictographs 9 and 12].

1.PICTOGRAPH SHOWING INTERSTITIAL FIBROSIS (H&E): AT LOW POWER

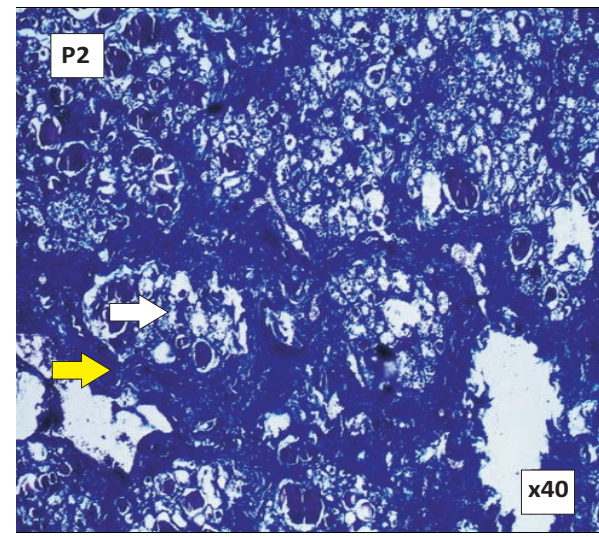
Dense fibrous band with compressed thyroid follicles



Pictograph 1: Pictograph showing interstitial fibrosis at X40
H&E: Low power. x40
Dense fibrous band (Black arrow).
Compressed thyroid follicles (White arrow)

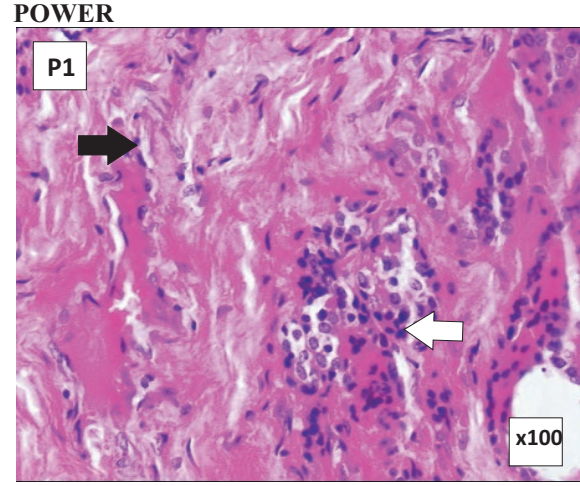
2. PICTOGRAPH SHOWING INTERSTITIAL FIBROSIS

(MASSON TRICHROME): AT LOW POWER
Dense fibrous bands in blue compressing thyroid follicles



Pictograph 2: Pictograph showing interstitial fibrosis
Masson Trichrome: Low power. x40
Dense fibrous band (Yellow arrow).
Compressed thyroid follicles (White arrow)

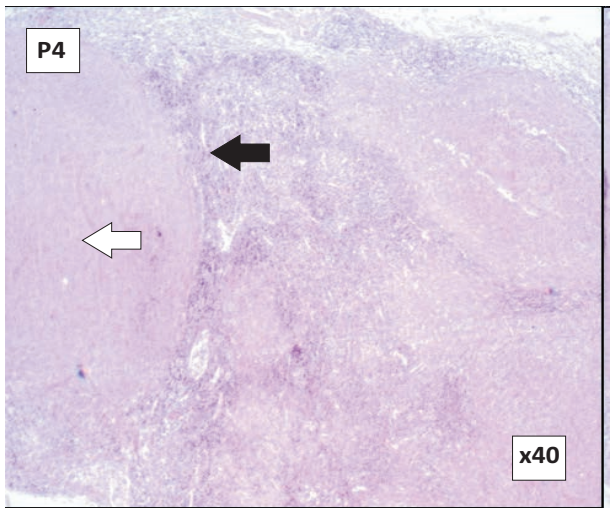
3. PICTOGRAPH SHOWING NON-SPECIFIC CHRONIC THYROIDITIS (H&E): AT HIGH POWER



Pictograph 3: Pictograph showing non-specific chronic thyroiditis with interstitial fibrosis
H&E: Medium power. x100
Dense fibrous band (Black arrow).
Lymphocytes (White arrow)

4. PICTOGRAPH SHOWING GRANULOMATOUS THYROIDITIS (H&E): LOW POWER

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



Pictograph 4: Pictograph showing granulomatous thyroiditis

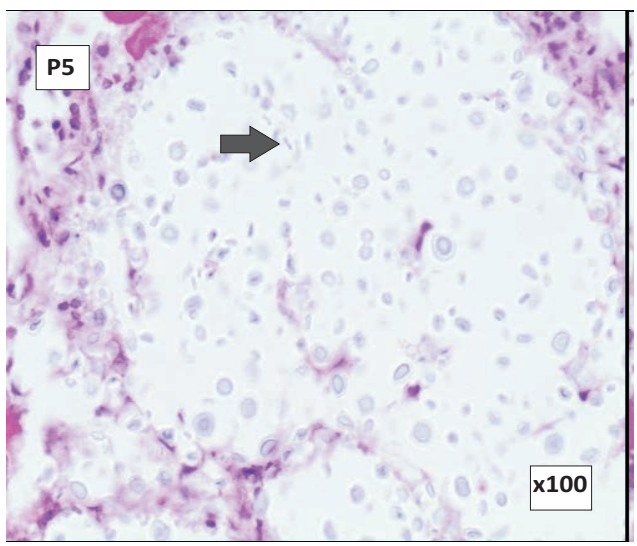
H&E: Low power. x40

Thin rim of lymphocytes.(Black arrow).

Caseating necrosis (White arrow)

5. PICTOGRAPH SHOWING CRYPTOCOCCUS NEOFORMANS (H&E): HIGH POWER

The pictograph below shows *Cryptococcus neoformans* within thyroid follicles



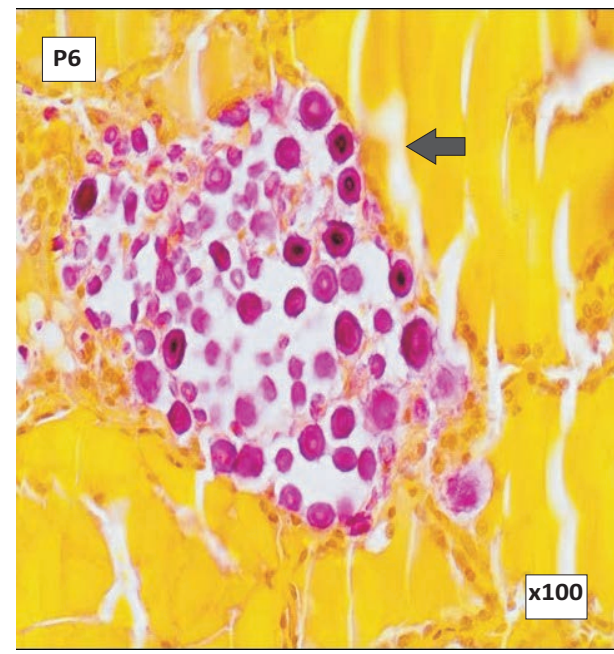
Pictograph 5: Pictograph showing *Cryptococcus neoformans*

H&E: Medium power. x100

Cryptococcus neoformans

6. PICTOGRAPH SHOWING CRYPTOCOCCUS NEOFORMANS (MUCICARMINE): HIGH POWER

The pictograph below shows *Cryptococcus neoformans* within thyroid follicles



Pictograph 6: Pictograph showing *Cryptococcus neoformans*

Mucicarmine: Medium power. x100

Cryptococcus neoformans

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]

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]

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]

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]

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: Determinants of Pathologic findings

Total thyroid gland lesions and gender distribution				
Thyroid Gland Lesion	**Present N (%)**	**Absent N (%)**	**Total N (%)**	**P-value**
Male	62 (50)	61(50)	123 (100)	
Female	50 (65)	27 (35)	77 (100)	**0.04**
Interstitial Fibrosis and Gender Distribution				
Interstitial Fibrosis	**Present N (%)**	**Absent N (%)**	**Total N (%)**	**P-value**
Male	57 (46)	66 (54)	123 (100)	
Female	47 (61)	30 (39)	77 (100)	**0.04**
Nonspecific Chronic Thyroiditis and Gender				
	Present N (%)	**Absent N (%)**	**Total N (%)**	**P-value**
Male	31 (25)	92 (75)	123 (100)	
Female	15 (19)	62 (81)	77 (100)	**0.35**
Nonspecific Chronic Thyroiditis And Specific Drug Combinations				
	Present N (%)	**Absent N (%)**	**Total N (%)**	**P-value**
TDF/FTC/EVF	10 (23)	33 (77)	43 (100)	
TDF/FTC/NVP	2 (20)	8 (80)	10 (100)	
ABC/3TC/EVF	2 (8)	23 (92)	25 (100)	
D4T/3TC/NVP	4 (50)	4 (50)	8 (100)	**0.007**

DISCUSSION

The Brazilian and Indian studies showed that infectious diseases such as *Mycobacterium tuberculosis*, *Cytomegalovirus* and *Cryptococcus neoformans* were more commonly described than in the Zambian and USA studies. As expected, the Zambian and USA studies described fewer infections as they were done more recently; after the introduction of HAART. It has been shown that HAART prevents the contraction of opportunistic infections.[5,6,7]

Unlike the Brazilian study which recorded *Pneumocystis jirovecii* 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.[5,6,7]

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. These differences can partly be explained by differences in ethnicity.[5,6,7] It has been recorded that there is a difference in multiple organ involvement in HIV infected between Black and Whites or Hispanic individuals.[8] However the exact explanation for these discrepancies is not clear.

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.[9] 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 TGF-beta.

Female cases were significantly more affected by interstitial fibrosis than male cases (P = 0.04). More studies need to be done to explain this trend.

Nonspecific chronic thyroiditis was significantly high in drug combination D4T/3TC/NVP (P = 0.007). Stavudine based regimens had more cases compared to the tenofovir based regimens. These results can be partly explained by the higher efficacy of Tenofovir based regimens.[10] However, studies need to be done to establish the relationship of these different ART regimens to inflammation

Two cases were found to have caseating granulomatous thyroiditis. Although ZN stain did not reveal any acid alcohol fast bacilli, the histological features were consistent with tuberculosis. A single case of *Cryptococcus neoformans* was described. These were described as round to oval yeast spores, measuring 4-10 microns and covered by a thick, mucinous capsule which stained bright red with mucicarmine,

CONCLUSION

Subclinical thyroid gland lesions in HIV-infected Zambian population were described and shown that subclinical thyroid gland lesions are significantly high in the HIV-infected Zambian population. These 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* (1%) and *Cryptococcus neoformans* (0.5%) 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.

STUDY LIMITATIONS

There were no documented thyroid function tests from the clinical files. And CD4 count documentation was poor. Further, the study lacked controls.

RECOMMENDATIONS

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.

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