

## **Short Research Article**

### **EVALUATION OF THYROID OF HIV PATIENTS IN UMUNZE, ANAMBRA STATE, NIGERIA.**

#### **Abstract**

**BACKGROUND:** Human immunodeficiency virus (HIV) and thyroid function has been described. Prevalence pattern, and atherogenic status significantly differ from HIV negative control in several studies. Unfortunately, few studies have determined the prevalence of thyroid function among Nigerians living with HIV.

**Objective:** This study is to evaluate thyroid hormones in HIV positive subjects compared with HIV negative control.

**Materials and Methods:** the serum concentration of thyroid stimulating hormone (TSH), free triiodothyronine (fT<sub>3</sub>), triiodothyronine (T<sub>3</sub>), free thyroxine (fT<sub>4</sub>), and thyroxine (T<sub>4</sub>) was determined in 95 HIV positive subjects which include 48 patients who were on HAART- group 1 and 47 not on HAART- group 2; and compared to 30 HIV negative controls – group 3.

**Results:** The level of TSH and fT<sub>3</sub> was significantly ( $p < 0.05$ ) higher in group 1 subjects than in group 2 subjects and the group 3. The level of T<sub>4</sub> was significantly higher in group 2 subjects than group 1 and the group 3 subjects. The level of T<sub>3</sub> was significantly lower in Control subjects in comparison to both HAART and non-HAART patients.

**CONCLUSION:** The results obtained from this study indicate that serum levels of thyroid hormones maybe used as baseline periodic markers during antiretroviral therapy and many people living with HIV may benefit from supplementation if appropriate.

**Keywords:** HIV infection, HAART, Thyroid hormones.

#### **Introduction**

HIV is an emerging disease and is one of the largest health problems today because of its pandemic status and severity characteristics (Guilherme et al., 2015). This disease is mainly characterized by a progressive loss of CD4+ T lymphocytes (CD4+), which cause immunosuppression and involvement by opportunistic diseases. The natural history of AIDS has been altered considerably by high activity antiretroviral therapy (HAART), which prevents the evolution of the loss of CD4+ to its final stage. Along with prevention campaigns, HAART

contributes to the decline of the transmission and stabilization of the epidemic in many countries (Miller et al., 2014).

Nevertheless, several complications have been reported with the use of HAART, among them are hypertriglyceridemia, lipodystrophy, type 2 Diabetes mellitus, gonadal dysfunction and osteoporosis (Brockmeyer et al., 2000). The mechanism by which HAART causes these changes has not been fully elucidated (Pacici R., 1996). Another complication is immune reconstitution inflammatory syndrome (IRIS). This condition occurs in some patients receiving HAART who develop clinical deterioration by the reestablishment of immunity despite high CD4+ counts and a low plasma viral load. Immune reconstitution (IR) can be defined as an increased CD4+ count above 200cells/mm<sup>3</sup> in subjects who previously had CD4+ counts lower than 100 – 200 cells/mm<sup>3</sup> (da Silva GAR., et al 2012).

## 2.0 MATERIALS AND METHODS

### 2.1 Study location/site

The study was carried out at the Immaculate Heart Mission Hospital, Umunze, in Orumba South Local Government Area of Anambra State Nigeria.

### 3.2 Study population

Participants in this study are

- Patients diagnosed with HIV on treatment and not on treatment
- Patients aged between 27 and 57years,
- Apparently healthy individuals HIV negative adults aged between 27 and 57years
- Not on any drug that can cause thyroid dysfunction e.g.amiodarone.
- Not pregnant
- Those who gave informed consent

### 3.3 Method for sample collection

About 8ml of venous blood was collected by ~~venepuncture~~venipuncture from the cubital fossa into plain specimen tubes. It was allowed to clot, centrifuged and the resultant serum stored at -20°C until analyses was carried out for T<sub>3</sub>, T<sub>4</sub>, TSH, fT<sub>3</sub>, and fT<sub>4</sub>,

Formatted: Superscript

### 3.6 Informed consent and ethical clearance

Informed consent was obtained from each subject and ethical clearance was obtained from the management committee of Immaculate Heart Mission Hospital, Umunze before commencing the research.

### 3.7 Sample size

For calculating the sample size, the formular proposed by Naing et al (2006) was adopted. It states:

$$N = Z^2PQ / D^2$$

Where N = minimum sample size

Z = standard normal deviate at 95% confidence interval which is 1.96

P = least estimate of population prevalence from literature review

D = test difference between two sub samples regarding a proportion, assuming an equal number of cases (D = 0.10).

$$Q = 1 - P$$

$$N = \frac{1.96^2 \times 0.5 (1 - 0.5)}{0.10^2}$$

$$N = 96.04$$

### 3.8 Research design

This is a cross sectional study to study evidence of overt and subclinical thyroid disorders especially hypothyroidism as well as Lipid profile and glucose level among HIV positive subjects attending Immaculate Heart Mission Hospital, Umunze, Nigeria.

### 3.9 Methodology

#### 3.9.1 Estimation of TSH

Measurement of serum TSH was determined using the standard Immunoassay procedure described by Hoptonet *al.*, (1986).

#### Principle

The TSH test applies immunometric sandwich configuration in which a serum TSH molecule forms a bridge between two or more distinct anti-TSH antibodies. The first antibody (of monoclonal origin) often is directed at the specific  $\beta$ -subunit and is anchored to a solid-phase separation system. This antibody is present in excess and selectively immune extracts the majority of TSH molecules from the serum specimen.

Bound hormone is quantitated by the use of a second TSH antibody (of either monoclonal or polyclonal origin) that is directed against a different antigenic site on the TSH molecule (for example, the  $\alpha$ -subunit). Most procedures label the detection antibody with peroxidase or alkaline phosphatase; sensitive photometric, fluorescent, or chemiluminescent substrates commonly are used to measure enzyme activity.

#### **Assay procedure**

1. The desired number of coated wells was secured in the holder
2. 50  $\mu$ l of standards, specimen and controls was dispensed into appropriate wells
3. 100  $\mu$ l of enzyme conjugate reagent was dispensed into each well.
4. It was thoroughly mixed for 30 seconds
5. Incubated for 60 minutes at room temperature (18-22°C)
6. The incubation mixture was removed by flicking plate contents into a waste container
7. It was rinsed, the microtiter wells flicked for 5 times with washing buffer(1X)
8. The wells were stroke sharply onto absorbent paper to remove all residual water droplets.
9. 100  $\mu$ l of TMB solution was dispensed into each well, gently mixed for 5seconds.
10. It was incubated for 20 minutes at room temperature.
11. The reaction was stopped by adding 100  $\mu$ l of stop solution to each well.
12. It was gently mixed for 30 seconds while all the blue colour changes to yellow colour completely.
13. The optical density was read at 450nm with microtiter well reader.

#### **3.9.2 Estimation of thyroxine**

Serum thyroxine was determined using isotopic and non-isotopic (competitive) Immunoassay method (Chopra *et al.*, 1971) which measures both free and protein-bound  $T_4$ .

#### **Principle**

Heterogenous assays require physical separation of free and bound  $T_4$  and a variety of solid-phase supports are used. An assortment of photometric, fluorescent and luminescent substrates is available for monitoring of the enzyme activity of the antibody-bound fraction. In contrast, homogenous enzyme immunoassays do not require physical separation of free and bound  $T_4$ . These procedures are rapid and simple to use and also have been applied to several major automated instruments.

### Assay procedure

1. The desired number of coated wells was secured in the holder
2. 25µl of standards, specimen and controls was dispensed into appropriate wells
3. 100 µl of enzyme conjugate reagent was dispensed into each well.
4. It was thoroughly mixed for 10 seconds
5. Incubated for 60 minutes at room temperature (18-22°C)
6. The incubation mixture was removed by flicking plate contents into a waste container
7. It was rinsed, the microtiter wells flicked for 5 times with washing buffer(1X)
8. The wells were stroke sharply onto absorbent paper to remove all residual water droplets.
9. 100 µl of TMB solution was dispensed into each well, gently mixed for 5seconds.
10. It was incubated for 20 minutes at room temperature without shaking.
11. The reaction was stopped by adding 100 µl of stop solution to each well.
12. It was gently mixed for 5 seconds
13. The optical density was read at 450nm with microtiter well reader.

### 3.9.3 Estimation of triiodothyronine

Isotopic and nonisotopic immunoassays are the methods of choice used to measure total T<sub>3</sub> concentrations by Chopra *et al.*, 1971.

#### Principle

Procedures are similar to those described for T<sub>4</sub>, except that a <sup>125</sup>I- T<sub>3</sub> tracer and T<sub>3</sub> – specific antibody are used. Solid-phase systems are preferred to liquid-phase separation systems. As with the T<sub>4</sub> methods, most T<sub>3</sub> methods use ANS to release T<sub>3</sub> from serum binding proteins without disturbing the binding of T<sub>3</sub> to antibody. A typical calibration curve ranges from 25 to 800ng/dl.

Nonisotopic assays similar to those described for serum T<sub>4</sub> have been applied to the measurement of T<sub>3</sub>. Most commercial methods use peroxidase or alkaline phosphatase to label T<sub>3</sub> antigens or T<sub>3</sub> antibodies; enzyme activity is determined commonly by use of a variety of sensitive photometric, fluorescent, or chemiluminescent substrates.

### Assay procedure

1. The desired number of coated wells was secured in the holder, data sheet made with sample identification

2. 50µl of standards, specimen and controls was dispensed into appropriate wells
3. It was thoroughly mixed for 30 seconds and 100 µl of enzyme conjugate reagent was dispensed into each well.
4. It was thoroughly mixed for 30 seconds
5. Incubated for 60 minutes at room temperature (18-22°C)
6. The incubation mixture was removed by flicking plate contents into a waste container
7. It was rinsed, the microtiter wells flicked for 5 times with washing buffer(1X)
8. The wells were stroke sharply onto absorbent paper to remove all residual water droplets.
9. 100 µl of TMB solution was dispensed into each well, gently mixed for 5seconds.
10. It was incubated for 20 minutes at room temperature.
11. The reaction was stopped by adding 100 µl of stop solution to each well.
12. It was gently mixed for 15 seconds while all the blue colour changes to yellow colour completely.
13. The optical density was read at 450nm with microtiter well reader within 15 minutes.

### 3.9.4 Estimation of free thyroxine(fT<sub>4</sub>)

#### Competition principle-

##### Principle

- Sample, T<sub>4</sub>derivant coated microwells and enzyme labeled Anti-T<sub>4</sub> are combined
- During the incubation, T<sub>4</sub>derivant coated on microwells and fT<sub>4</sub> present in the sample compete for binding to the enzyme labeled antibodies
- After washing, a complex is generated between the solid phase and enzyme-linked antibodies by immunological reactions.
- Substrate solution is then added and catalyzed by this complex, resulting in a chromogenic reaction. The resulting chromogenic reaction is measured as absorbance.
- The colour intensity proportional to the amount of fT<sub>4</sub> in the sample.

##### Assay procedure

1. The desired number of coated wells was secured in the holder, data sheet made with sample identification
2. 50µl of samples was added to each well
3. 50µl of enzyme conjugate was added to each well
4. The microplate was shake gently for 30 seconds to mix
5. It was covered with plate lid and incubated for 60 minutes at 37°C
6. The contents of the micro plate was discarded into a waste container
7. It was rinsed, the microtiter wells flicked for 5 times with washing buffer(1X)

8. The wells were stroke sharply onto absorbent paper to remove all residual water droplets.
9. 100 µl of TMB solution was dispensed into each well, gently mixed for 5seconds.
10. It was incubated for 20 minutes at room temperature.
11. The reaction was stopped by adding 100 µl of stop solution to each well.
12. It was gently mixed for 15 seconds while all the blue colour changes to yellow colour completely.
13. The optical density was read at 450nm with microtiter well reader within 15 minutes.

### 3.9.5 Estimation of free triiodothyronine( $ft_3$ )

#### Principle

Competition enzyme immunoassay. Upon immobilized antibody, enzyme-T3 conjugate and a serum containing the native free T3 antigen, a competition reaction results between the native free T3 and the enzyme-T3 conjugate for a limited number of insolubulized binding sites. The interaction is illustrated by the following reaction:



AbC.W. = Monospecific Immobilized Antibody (Constant Quantity)

Ag = Native Antigen (Variable Quantity)

EnzAg = Enzyme-Antigen Conjugate (Constant Quantity)

AgAbC.W. = Enzyme-angtigen Conjugate-Antibody Complex

Ka = Rate Constant of Association

k-a = Rate Constant of Disassociation

k = ka /k-a = Equilibrium Constant

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native free antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

#### Assay procedure

1. The desired number of coated wells was secured in the holder, data sheet made with sample identification
2. 50µl of samples was added to each well
3. 100µl of enzyme conjugate was added to each well
4. The microplate was shake gently for 30 seconds to mix
5. It was covered with plate lid and incubated for 60 minutes at 37°C

6. The contents of the micro plate was discarded into a waste container
7. It was rinsed, the microtiter wells flicked for 5 times with washing buffer(1X)
8. The wells were stroke sharply onto absorbent paper to remove all residual water droplets.
9. 300  $\mu$ l of wash buffer was added, and decant. This step was repeated for a total of three times.
10. 100  $\mu$ l of working substrate solution was added to all wells
11. It was incubated for 15minutes at room temperature
12. The reaction was stopped by adding 50  $\mu$ l of stop solution to each well and gently mixed for 20seconds
13. It was gently mixed for 25 seconds while all the blue colour changes to yellow colour completely.
14. The optical density was read at 450nm with microtiter well reader within 30 minutes.

### 3.9.9.2 Statistical analysis: (using IBM SPSS version 20)

#### Data Analysis/ Table interpretation

Data were analyzed. Distributions of variables were reported in frequency and percentages. Comparison of proportion of distribution of the dependent variable across the independent variables were analyzed using chi-square and Fisher's exact. Post hoc analysis involved pair wise comparisons using the z-test of two proportions was done after statistical significant chi-square of Fisher exact analysis. The choice of fisher's exact was based on the assumption minimum expected frequency is violated in choosing Pearson chi-square. Analysis of mean difference between groups was done using t-test for two groups and One-way analysis of variance (anova) for groups more than two. Statistical significant differences were considered at p-value less than 0.05. Post-hoc analysis for statistical significant comparison was done in anova, the result of post-hocs were express in superscript within the tables. Values with same superscript are not significantly different at p-value of 0.05. Thyroid hormone was classified to both normal and abnormal (dysfunction) based on the value within the reference range. The categorical outcome was used as cross-tabulation against serostatus category to study the prevalence of hormonal dysfunction across the group of the serostatus.

## 4.0 RESULTS AND DISCUSSIONS

The results obtained in this study are presented in tables 1 to 5 and figures 1 to 2

#### 4.1 DEMOGRAPHIC CHARACTERISTICS AND THE SEROSTATUS CATEGORY OF THE STUDY POPULATION

The characteristics of the study population such as gender, age group, HIV Status, HAART Status and HAART Duration are shown in table 1 below. The number of subjects for the total study is 125, with 95 (76%) being seropositive which include 48 (38.4%) patients who were on HAART and 47 (37.6%) not on HAART; and 30 (24%) were seronegative individuals that served as control. 84(67.2%) were female and 41 (32.8%) were male. [See table 1.](#)

#### 4.2 COMPARISON OF THE AVERAGE LEVEL OF THE THYROID HORMONE BETWEEN SEROPOSITIVE AND SERONEGATIVE PATIENT

The table [2 showed shows](#) the level of various thyroid hormones between the HIV positive and HIV negative subjects. The mean levels of TSH, T<sub>4</sub>, T<sub>3</sub> and fT<sub>3</sub> were higher in HIV positive patients than the HIV negative subjects, while the level of fT<sub>4</sub> (25.1±7.5) in HIV positive patients was lower than in HIV negative subjects (42.5±8.4). The comparison of mean using independent sample t-test showed that there was a significant (p<0.05) difference between the serostatus in the various thyroid hormone level except for fT<sub>3</sub> where the difference between the mean was statistically not significant (p>0.05). [See table 2.](#)

**Table 1: Demographic Characteristics and the serostatus category of the study population**

Variables	Group	Frequency	Percentage	Mean±SD
-----------	-------	-----------	------------	---------

Gender	Female	84	67.2	
	Male	41	32.8	
	<u>total</u>	125	100	
Age group (years)	<del>30</del> -below <u>30</u>	32	25.6	27.0±3.7
	31-40	64	51.2	36.1±2.7
	41-50	18	14.4	45.9±3.2
	51-60	11	8.8	57.0±1.9
HIV Status	Positive	95	76	
	Control Negative	30	24	
HAART Status	On HAART	48	38.4	
	Not On HAART	47	37.6	
	Control	30	24	
	Total	125	100	
Duration	1-2yrs	14	29.8	
	3-4yrs	23	48.9	
	5-6yrs	10	21.3	
	Total	47	100	

**Table 2: level of the Thyroid hormone in Seropositive and seronegative persons**

Variables	Serostatus		t-value	p-value
	Positive (n=95)	Negative (n=30)		
TSH	3.3±2.7	2±1.2	3.63	<0.001
T <sub>4</sub>	10.2±2.1	8.6±1.5	3.68	<0.001
T <sub>3</sub>	2.3±0.8	1.4±0.5	5.72	<0.001
fT <sub>4</sub>	25.1±7.5	42.5±8.4	10.83	<0.001
fT <sub>3</sub>	3±1	2.7±0.9	1.46	0.14

#### 4.3 THYROID HORMONE LEVEL ACROSS THE GROUPS

Table 3 shows the average thyroid hormone level across three groups based on HAART administration (those on HAART, Those not on HAART and the control subjects). The analysis of mean difference was done with one-way ANOVA. The result showed that the level of TSH and  $fT_3$  was significantly ( $p < 0.05$ ) higher in patient on HAART than in the control and the HIV positive not on HAART. The level of  $T_4$  was significantly higher in HIV patients not on HAART than the controls and the HIV patients on HAART. The level of  $T_3$  was significantly lower in the Controls subjects than both on HAART and non-HAART patients.

**Table 3: Thyroid Hormone level across the groups**

Variables	On HAART	Not On HAART	Control	F-value	p-value
TSH	4±3.1 <sup>a</sup>	2.5±2 <sup>b</sup>	2±1.2 <sup>b</sup>	8.61	<0.001
$T_4$	8.9±2.4 <sup>a</sup>	11.4±0.5 <sup>b</sup>	8.6±1.5 <sup>a</sup>	35.31	<0.001
$T_3$	2.2±1.1 <sup>a</sup>	2.4±0.5 <sup>a</sup>	1.4±0.5 <sup>b</sup>	17.49	<0.001
$fT_4$	27±8.5 <sup>a</sup>	23.1±5.8 <sup>b</sup>	42.5±8.4 <sup>c</sup>	64.27	<0.001
$fT_3$	3.2±1.2 <sup>a</sup>	2.8±0.7 <sup>b</sup>	2.7±0.9 <sup>b</sup>	3.851	0.024

a,b,c value with different superscript are significantly different from one another at  $p < 0.05$

#### 4.4 DISTRIBUTION OF HORMONAL DYSFUNCTION ACROSS THE GROUPS

The table [4 showed](#) shows the distribution of thyroid hormone dysfunction (abnormal) across the groups. The comparison of the prevalence showed that there was a significant difference ( $p < 0.05$ ) in the distribution of TSH,  $fT_4$  and  $fT_3$  dysfunction across the group. The TSH dysfunction was significantly lower in controls when compared to the patients on HAART but not significant with patients who are not on HAART. The prevalence of  $fT_4$  dysfunction across the groups was significantly different from each other. The prevalence of  $fT_3$  dysfunction in patients on HAART was significantly higher than those not on HAART, but there was no

significant difference in the prevalence of  $fT_3$  dysfunction between control and patients on HAART.

**Table 4: Distribution of Hormonal dysfunction across the groups**

Variables	No	On HAART (%)	Not On HAART (%)	Control (%)	p-value
TSH	Normal	36 (75.0)	42 (89.4)	29 (96.7)	0.02
	Abnormal	12 (25.0) <sup>a</sup>	5 (10.6) <sup>a,b</sup>	1 (3.3) <sup>b</sup>	
T <sub>4</sub>	Normal	46 (95.8)	47 (100)	30 (100)	0.34
	Abnormal	2 (4.2)	0 (0)	0 (0)	
fT <sub>4</sub>	Normal	16 (33.3)	28 (59.6)	0 (0)	<0.001
	Abnormal	32 (66.7) <sup>a</sup>	19 (40.4) <sup>b</sup>	30 (100) <sup>c</sup>	
fT <sub>3</sub>	Normal	41 (85.4)	47 (100.)	27 (90.0)	0.01
	Abnormal	7 (14.6) <sup>a</sup>	0 (0) <sup>b</sup>	3 (10.0) <sup>a,b</sup>	
	Total	48 (100)	47 (100)		

a,b,c value with different superscript across the row are significantly different from one another at  $p < 0.05$

#### 4.5 DISTRIBUTION OF THYROID HORMONE DYSFUNCTION ACROSS THE DURATION OF HAART FOR THE PATIENTS ON HAART

The table ~~showed~~ shows the prevalence of the thyroid dysfunction across the duration on HAART for the patients on HAART. The distribution table showed that the distribution of each hormonal dysfunction was statistically not significant  $p > 0.05$ .

The area under the curve (AUC) of the ROC quantifies the ability of the test to correctly classify the seropositive and the controls. The curve showed the AUC of all the biochemical markers

**Comment [M1]:** Figure??  
Table??

against the Serostatus (patient and control). The curve showed that  $T_3$  have the highest AUC which can be said to have the best validity test for serostatus among others with statistical significant AUC values of 0.83 followed by  $T_4$  (0.74). Other variables have poor diagnostic validity value.

The AUC of the  $T_3$  is 0.83 with 95% confidence interval of (0.75 - 0.91). This indicates that we would expect minimum 83% of the seropositive patient to be correctly identified by the by  $T_3$ . And the best cutoff point of  $T_3$  that maximizes (sensitivity + specificity) is 1.5. The cutoff point has 92.6% true positive rate and 60% false positive rate.

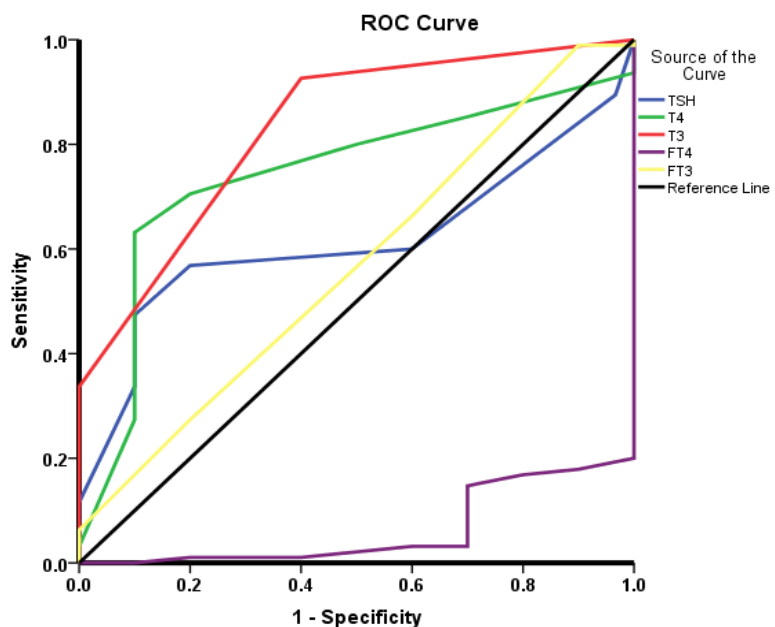
The AUC of  $T_4$  is 0.74 with 95% confidence interval of 0.64 to 0.84. This indicates that we would expect 74% of the seropositive to be correctly identified by the by  $T_4$ . And the best cutoff point of  $T_4$  that maximizes (sensitivity + specificity) is 7.50. The cutoff point has 85.2% true positive rate and 30% false positive rate (1-specificity).

The area under the curve (AUC) of the ROC quantifies the ability of the test to correctly classify the HAART and Non-HAART patients. The curve showed the AUC of all the biochemical markers against the HIV HAART status (on HAART and Non-HAART). The marker with highest AUC value was  $fT_4$  (0.66) followed by TSH (0.63) The AUC values of the parameters are not good validity test to distinguish HIV on HAART and those that are not.

**Table 5: Distribution of Thyroid Hormone Dysfunction across the duration of HAART for the patients on HAART**

Variables	Group	1-2yrs	3-4yrs	5-6yrs	p-value
TSH	Normal	8 (57.1)	19 (82.6)	8(80.0)	0.21
	Abnormal	6 (42.9)	4 (17.4)	2(20.0)	
$T_4$	Normal	13 (92.9)	22 (95.7)	10(100)	1.00
	Abnormal	1 (7.1)	1 (4.3)	0 (0)	

fT <sub>4</sub>	Normal	6 (42.9)	7 (30.4)	2 (20.0)	0.46
	Abnormal	8 (57.1)	16 (69.6)	8 (80.0)	
fT <sub>3</sub>	Normal	11 (78.6)	19 (82.6)	10(100)	0.43
	Abnormal	3 (21.4)	4 (17.4)	0 (0)	
Total		14 (100)	23 (100)	10(100)	



**FIGURE 1: MEASURE OF DIAGNOSTIC ABILITY OF THE VARIABLES FOR SEROSTATUS USING RECEIVER OPERATING CHARACTERISTIC (ROC) CURVE**

Table 6. Standard error of the variables for serostatus with p-value.

Variables	AUC	Std. Error	p-value	95% CI (Lower-Upper)
-----------	-----	------------	---------	----------------------

TSH	0.61	0.05	0.06	0.52 - 0.71
T <sub>4</sub>	0.74	0.05	<0.001	0.64 - 0.84
T <sub>3</sub>	0.83	0.04	<0.001	0.75 - 0.91
fT <sub>4</sub>	0.06	0.02	<0.001	0.02 - 0.10
fT <sub>3</sub>	0.57	0.06	0.26	0.45 - 0.69

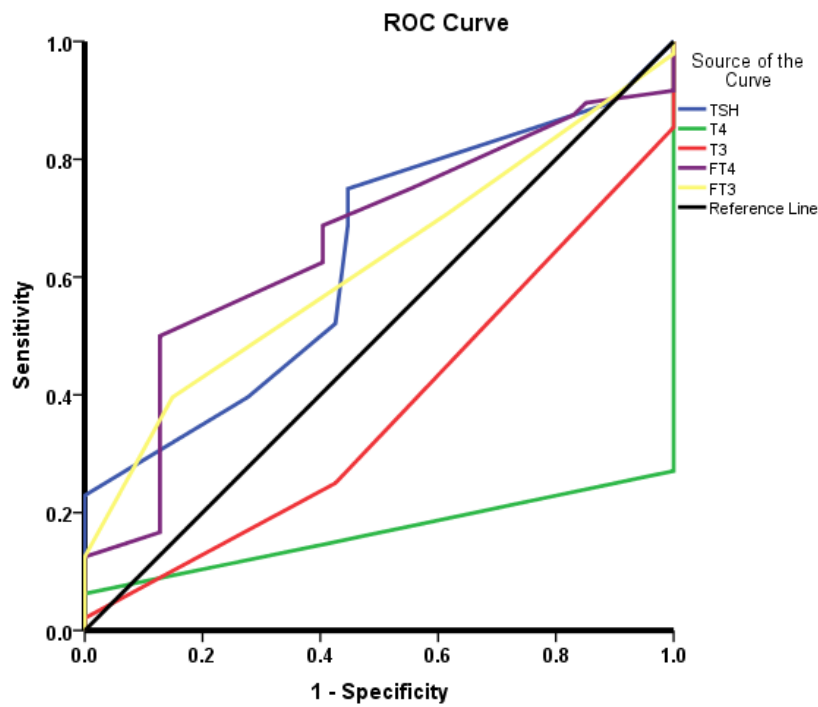


Figure 2: MEASURE OF DIAGNOSTIC ABILITY OF THE VARIABLES FOR HAART ADMINISTRATION USING RECEIVER OPERATING CHARACTERISTIC (ROC) CURVE

Table 7.. Standard error of the variables for haart administration with p-value

Variables	AUC	Std. Error	p-value	95% CI (Lower-Upper)
TSH	0.636	0.057	0.02	0.52 - 0.75
T <sub>4</sub>	0.166	0.047	<0.001	0.07 - 0.26
T <sub>3</sub>	0.375	0.057	0.04	0.26 - 0.49
fT <sub>4</sub>	0.66	0.057	0.007	0.55 - 0.77
fT <sub>3</sub>	0.62	0.058	0.04	0.51 - 0.73

#### 4.35. DISCUSSION

This study is aimed ~~at toevaluation evaluateof the~~ thyroid dysfunction and to determine the relationship between thyroid hormone levels in HIV and HAART duration. The HIV positive subjects are grouped into 1 and 2 for those on HAART and naïve subjects respectively while group 3 are the negative control subjects.

On the basis of age group of the subjects, the subjects were grouped into four; 30years and below, between 31 and 40years, between 41 and 50years and between 51 and 60years. Their mean ages are 27.0±3.7, 36.1±2.7, 45.9±3 and 57.0±1.9 respectively. The study also grouped the HIV positive subjects according to their HIV duration; between 1-2yrs (29.8%), 3-4yrs (48.9%), and 5-6yrs (21.3%).

The sociodemographic data of the study population shows that more than 50% of the study population were young subjects aged 31 to 40years. This is similar to reports from two studies conducted in Osun and Enugu states were subjects aged 30-39years were found to have the highest percentage [58% and 40.9% respectively]. Mean age of the two study populations also supported the fact that many of the subjects were young individuals. This finding is also consistent with higher prevalence of HIV infection seen in the reproductive age group [15-49years] compared to other age groups [WHO 2006 and Ayodele EO et al., 2012]. In terms of gender, females were more than twice the number of males. This suggests that females were twice more likely to have HIV infection than males.

Higher prevalence in females compared to males were also found in some studies carried out in the central and southern parts of Nigeria [Reng R et al., 2016 and Ayodele EO et al., 2012]. This is however contrary to what were found in some studies carried out in foreign countries where men dominated more than half of the study population [Palacios R et al., 2006]. The reason for the disparity may partly be due to increased homosexuality practice outside Nigeria [CDC 2015]. Another reason for the gender disparity seen in this study may be due to the cultural practice in our society, in which a man is allowed to marry more than one wife. Therefore, an HIV infected man can infect all his wives. Another reason may be natural events which give females more opportunity to be screened than their male counter parts e.g. during antenatal care, child birth, child care, immunization and so on.

The mean levels of TSH,  $T_4$ ,  $T_3$  and  $fT_3$  were found to be higher in HIV positive than the HIV negative control. This is in contrast to what was reported in Collazoset *al.*, (2003) but in agreement with Palanisamyet *al* (2010) in India where  $fT_3$  was lower with higher  $fT_4$  and TSH among subjects with HIV compared with controls. In Ibadan Southwestern Nigeria, Abbiyesuket *al.* (2014) also found higher TSH levels among HIV patients compared with controls. It has been shown that abnormal thyroid function is not uncommon in HIV and there may be a number of contributory factors (Qureshiet *al.*, 2005; Hoffmann and Brown, 2007; Noureldeen et al, 2012). However, the level of  $fT_4$  in HIV positive was lower than HIV negative compared to  $fT_3$  that is still within the normal reference range. Statistically using independent sample t-test there is a significant difference between the serostatus in the various thyroid hormone levels except for  $fT_3$  that is not statistically significant.

Furthermore, the level of TSH and  $fT_3$  is significantly higher in group 1 subjects than the group 2 and 3 subjects. Similar observation was reported by Rajendraet *al.*, (2017) and Shujing et al., (2016) in which thyroid dysfunction was significantly more frequent in the HAART group 1 than in group 2. Also  $T_4$  is significantly higher on group 2 subjects than the group 1 and 3 subjects.  $T_3$  is significantly lower in group 3 subjects than group 1 and 2 subjects.

In the distribution of TSH,  $fT_4$  and  $fT_3$  dysfunction across the group; TSH dysfunction is significantly lower in group 3 subjects compared to subjects in group 1 but not with group 2

subjects. The prevalence of  $fT_4$  dysfunction across the groups is significantly different from each other. while the prevalence of  $fT_3$  dysfunction in patients on HAART. There is no significant difference in the prevalence of  $fT_3$  dysfunction between group 2 and 3 subjects. Across the duration for subjects on HAART which are grouped into 1-2yrs, 3-4yrs and 5-6yrs, statistically there is no significant difference on the thyroid hormone dysfunction.

The most common pattern of thyroid dysfunction among subjects in this study was primary hypothyroidism, followed by isolated low  $fT_4$ . Among the controls the most common thyroid dysfunction was subclinical hypothyroidism. Similar findings were reported by Ketsamathi et al. (2006) in Bangkok. Several studies have also found primary hypothyroidism as the most frequent thyroid abnormality among their study population Uloko et al., (2020). However Gagnon et al.,(2006) in Toronto, Canada and Guilherme et al.,(2015) in Rio de Janeiro, Brazil, reported subclinical hypothyroidism as the most common pattern of thyroid dysfunction among their subjects. The longer duration of HIV infection among subjects in those studies and the fact that many of the patients were not on HAART may explain the difference. Some studies have reported association between HAART use and overt hypothyroidism Uloko et al 2020. The isolated  $fT_4$  found in this study, were also reported by Rasoolinejad et al (2004) in Tehran, Iran and Abbiyesuku et al (2014) in Ibadan, Nigeria as the most common thyroid dysfunction among their subjects. This abnormality could be due to sick euthyroid syndrome in the setting of advanced HIV infection. They could also be due to clinical and subclinical opportunistic infection.

## REFERENCES

- Abbiyesuku F.M., Osuji K.C., Kuti M.O., and Atiba AS. (2014). *Thyroid function tests in Nigerian HIV-Seropositive patients on highly active antiretroviral therapy (HAART)*. International Journal of Medical and Clinical Research. 5(1), 277-81.
- Abubakar UI, Uloko AE and Gezama ID. (2020). *Thyroid Disorders and Autoimmunity among Patients with HIV/AIDS in Northern Nigeria*. Ann of Clin Diabetes Endocrinol. 3(1): 1014

Achila OO, Abrhaley F, Kesete Y, Tesfaldet F, Alazar F, Fisshaye L, et al. (2022). *Dyslipidemia and associated risk factors among HIV/AIDS patients on HAART in Asmara, Eritrea*. PLoS ONE 17(7): e0270838. <https://doi.org/10.1371/journal.pone.0270838>

Comment [M2]: ?????

Adewole OO, Eze S., Betiku Ye, Anteyi E, Wada I, Ajuwon Z et al. (2010). *Lipid profile in HIV/AIDS patients in Nigeria*. Afr Health Sci. 10(2); 144-149.

Comment [M3]: ?????

Allain, C.C., Poon, L.S., Chan, C.S., Richmond, W., & Fu, PC. (1974). *Enzymatic determination of total serum cholesterol*. Clin Chem., 20(4):470-475.

Amadi, K, Sobo A.M, Ogunkeye, O.O and Oluwole, F.S. (2008). *Thyroid Hormone: A prime suspects in human immunodeficiency virus (HIV/AIDS) patients?* Nigeria Journal of physiological sciences **23** (1-2): 61-66.

Anthony S. Fauci H. Clifford L. (2008). *Human Immunodeficiency Virus Disease. AIDS and Related Disorders*. In; *Harrison's Textbook of Internal Medicine*. 17<sup>th</sup> ed. USA: McGraw-Hill Inc; 1137-1138.

Comment [M4]: How to write book???

Aukrust P, Liabakk NB, Muller F, Lien E, Espevik T, Froland SS. (1994). *Serum levels of tumor necrosis factor (TNF)  $\alpha$  and soluble TNF receptors in human immunodeficiency virus type 1 infection-correlations to clinical, immunologic and virologic parameters*. J Infect Dis; 169 (2): 420-4.

Ayodele EO, Akinboro AO, Adepeju AA, Akinremi SO, Alao C, Popoola A. (2012). *Prevalence and clinical correlates of metabolic syndrome in Nigerians living with HIV/AIDS*. MetabSyndrRelat Disorder. 10(5):373-379.

Baggaley R Boily C, White R, Alary M. (2006). *Risk of HIV-1 transmission for parenteral exposure and blood transfusion: a systematic review and meta analysis*. AIDS, **20**(6): 805–812.

Bell C, Nelson M, Kaye S.(2002). *A case of immune reconstitution rheumatoid arthritis*. Int'l J STD AIDS.13(8): 560-1.

Beltran S, Lescure FX, El Esper I, Schmit JL, Desaillood R: (2006). *Subclinical hypothyroidism in HIV Infected patients is not an autoimmune disease*. HormRes. 66(1): 21-6.

Beltran S, Lescure F-X, Desaillood R. (2003). *Increased prevalence of hypothyroidism among human immunodeficiency virus-infected patients a need for screening*. Clin Infect Dis. 37:579-83.

Bongiovanni, M, Adorni, F., Casana, M. (2006). *Subclinical hypothyroidism in HIV-infected subjects*. Br SocAntimicrob Chemotherapy 58 (5): 1086-1089.

Brockmeyer N, Kreuter A, Bader A, Seemann U, Reimann G. (2000). *Prevalence of endocrine dysfunction in HIV-infected men*. Horm Res. 54(5-6): 294-5.

Brown TT, Cole SR, Li X, Kingsley LA, Pallela FJ, Riddler SA et al. (2005). *Antiretroviral therapy and the prevalence and incidence of diabetes mellitus in the multicenter AIDS cohort study*. Arch Intern Med. 165: 1179-1184.

Comment [M5]: ????

Calza L, Manfredi R, Chiodo F. (2002). *Subclinical hypothyroidism in HIV-infected patients receiving highly active antiretroviral therapy*. J Acquire Immune Deficiency Syndrome; 31(3): 361-3. 30.

Carl AB, Edward RA, David EB (2006): *Teitz Textbook of Clinical Chemistry and Molecular Diagnostic*. 4<sup>th</sup> Edition Pp: 1065-95.

Comment [M6]: How to write book????

Carr A, Samaras K, Burton S, et al. (1998). *A syndrome of peripheral lipodystrophy, hyperlipidaemia and insulin resistance in patients receiving HIV protease inhibitors*. AIDS;12:F51-F58.

Comment [M7]: ????

Carr A, Cooper DA (2000): *Adverse Effects of Antiretroviral Therapy*. Lancet; 356: 1423-1430

Centres for Disease Control and Prevention (2014): *HIV surveillance Report*. (26):101. <http://www.cdc.gov/hiv/library/reports/surveillance/>.

Comment [M8]: How to write report???

Chopra, JC Nelson, DH Solomon, GN Beal (1971). *Production of antibodies specifically binding triiodothyronine and thyroxine*. J. Clin. Endocrinol.Metab, 32, 299-308.

Christeff N, Melchior JC, de Truchis P, Perronne C, Nunez EA, Gougeon ML. (1999).

*Lipodystrophy defined by a clinical score in HIV-infected men on highly active antiretroviral therapy: correlation between dyslipidaemia and steroid hormone alterations.* AIDS, 13: 2251–2260.

Collazos J, Ibarra S, Mayor J. (2003). *Thyroid hormones in HIV infected patients in the highly active antiretroviral therapy era: Evidence of an interrelationship between the thyroid axis and the immune system.* AIDS. 17(5):763-65.

Corcoran C, Grinspoon S. (1999). *Treatments for wasting in patients with the acquired immunodeficiency syndrome diagnosis and treatment of endocrine disorders in the HIV-infected patient.* New England Journal of Medicine, 340(22): 1740-1750.

Cuellar ML (1998). *HIV infection associated inflammatory musculoskeletal disorders.* Rheum Dis Clinics N Am; 24(2): 403-21.

Crowther's Tenth Martini. (2015). Endocrine System

Comment [M9]: ???not mentioned in the text (no citation)

Cristal Limon and Britnee Bond (2105). Myxedema

Comment [M10]: ???not mentioned in the text (no citation)

Crum NF, Ganesan A, Johns ST, Wallace MR (2006). *Graves' disease: An increasingly recognized immune reconstitution syndrome.* AIDS. 20(3): 466-9.

da Silva Gar, Azevedo MCV, Motta RN, Pinto JFC, Sa Cam, Ferry FRA (2012). *Herpes zoster oftalmicocomo manifestacao de syndrome de reconstituicao immune em um paciente com AIDS.* Relato de Caso Cad Bras Med. 25:15-8.

Das DK et al., (1984). *Thyroid hormone regulation of beta adrenergic receptors and sensitive adenylate cyclase in foetal heart.*

Comment [M11]: ?????

Dave JA, Lambert EV, Badri M, West S, Maartens G, Levitt NS (2011). *Effect of nonnucleoside reverse transcriptase inhibitor-based antiretroviral therapy on dysglycaemia and insulin sensitivity in South African HIV-infected patients.* Acquir Immune Defic Syndr; 57(4):284-289.

Deas JE, Liu LG, Thompson JJ (1998). *Reactivity of sera from systemic lupus erythematosus and Sjogren's syndrome patients with peptides derived from human immunodeficiency virus p24 c apsid antigen.* ClinDiag Lab Immunol. 5(2):181 -5.

Denise P. Carvallo., Corinne Dupuy (2017). *Thyroid hormone biosynthesis and release: Molecular*

and Cellular Endocrinology [Volume 458 Pg 6-15](#).

Comment [M12]: ????

Dobs AS, Dempsy MA, Ladenson PW, Polk BF (1988). *Endocrine disorders in men infected with the human immunodeficiency virus*. Am J Med. (3Pt 2): 611-6.

Dwyre D, Fernando L, Holland P. (2011). *Hepatitis B, Hepatitis C and HIV Transfusion-Transmitted Infections in the 21st Century*. Vox Sang, **100**(1): 92-98.

Ebuehi O.A.T., Awolola A., Akanmu A.S., (2015). *Changes in Serum Cortisol, Thyroid Hormones and Lipid Profiles in Nigerian Men and Women on 1<sup>st</sup> and 2<sup>nd</sup> Line Antiretroviral Therapy for 52 Weeks*. International Journal of Virology and Molecular Biology, Vol.4 No. 1, 2015, pp.12-18. [doi: 10.5923/j.ijvmb.20150401.03](#).

El-Sadr WM, Mullin CM, Carr A, [et al](#) (2005). *Effect of HIV disease on lipid, glucose and insulin levels: results from a large antiretroviral-naïve cohort*. HIV Med. 6:114-121.

Comment [M13]: ???

Feleke Y, Fekade D, Mezegebu Y. (2012). *Prevalence of HAART-associated metabolic abnormalities and lipodystrophy in HIV infected patients*. Ethiop Med J.50(3):221-230.

Flint OP, Noor MA, Hruz PW, Hylemon PB, Yarasheski K, Kotler DP. (2007). *The Role of Protease Inhibitors in the Pathogenesis of HIV-Associated Lipodystrophy. Cellular Mechanisms and Clinical Implications*. Toxicology Pathology; 37: 65-77.

Friedewald, W.T., Levy, R. I., & Fredrickson, D.S. (1972). *Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge*. Clinical Chemistry, 18 (6): 499-502.

Giovanni Bucolo and Harold David (1973). *Quantitative Determination of Serum Triglycerides by the Use of Enzymes*. Clinical Chemistry 19(5): 476-482.

Goddard GZ.,Shoenfeld Y (2002). *HIV and autoimmunity: Autoimmunity Reviews*: 1:329-337.

Golden SH, Robinson KA, Saldanha I, Anton B, Ladenson PW (2009). *Prevalence and incidence of endocrine and metabolic disorders in the United States: A comprehensive review*. J ClinEndocrinolMetab. 94(6): 1853-78.

Grappin, M., Piroth, L., Verge, L., Bruno, Sgro, C., Mack, G. Bulsson, M. Marielle, D, Michel, C.,

Pascal, P, H. (2000). *Increased prevalence of subclinical hypothyroidism in HIV patients in treated with highly active antiretroviral therapy*. AIDS, 14 ([898](#)): 1070.

Grunfeld C, Pang M, Doerrler W, Shigenaga JK, Jensen P, Feingold KR et al (1992). *Lipids, lipoproteins, triglyceride clearance, and cytokines in human immunodeficiency virus infection and the acquired immunodeficiency syndrome*. J Clin Endocrinol Metab;74:1045-1052.

Comment [M14]: ?????

Guilherme AR, Mayra Christina, Daniel de Alvarenga, Rafaef, Jorge FC, Walter de Araujo Eyer Silva, Fernando Raphael de Almeida Ferry, Marcelo CV, Rogerio NM (2015). *Association between antiretroviral and thyroid disease: A cross sectional Study*. Arch Endocrinol Metab. 59 (2):240-44.

Hariri S, Me Kenna M. (2007). *Epidemiology of Human immunodeficiency virus in the United States*. Clin. Microbiol. Rev., 20: 474-488.

He rnandez, HF, Gascuena, RR, Escrbano SP, Yelazquez MT, Lombera RT, Rubic GR, Putido OF, Ramon-Costal, Perez HJ, Saenz-De-La-Cazada C. (2001). *Diastolic Dysfunction in Human immunodeficiency virus infection*: Rev. Esp. Cardiol. 54 (10) 1183 – 89.

Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA (2002). *Serum TSH, T(4) and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III)*. J. Clin Endocrinol Metab. 87(2): 489-99.

Hoffman CJ, Brown TT (2007). *Thyroid function abnormalities in HIV infected patients*. Clin infect Dis. 45(4): 488-94.

Hopton, M.R and Harrop J.S. (1986). *Immunoradiometric assay of thyrotropin as a "first-line" thyroid-function test in routine laboratory*. Clinical Chemistry, 32 (4): 691 – 693.

Howard AA, Floris-Moore M, Amsten JH, Santoro N, Fleischer N, Lo Y, et al. (2005). *Disorders of glucose metabolism among HIV-infected women*. Clin Infect Dis. 40:1492-1499.

Comment [M15]: ????

[https://www.unaids.org/en/resources/presscentre/pressreleaseandstatementarchive/2019/march/20190314\\_nigeria](https://www.unaids.org/en/resources/presscentre/pressreleaseandstatementarchive/2019/march/20190314_nigeria)

Comment [M16]: ????

Iwuala SO, Lesi OA, Olamoyegun MA, Sabir AA, Fasanmade OA (2015). *Lipoatrophy among patients on antiretroviral therapy in Lagos, Nigeria: Prevalence, pattern and association with cardiovascular risk factors*. Niger J Clin Pract. 18: 626-632.

Jain G., Devpura, G, Guputa B.S (2009). *Abnormalities in thyroid function tests as surrogate marker*

of advancing HIV infection in infected adults. Journal of the association of Physicians of India 57: 508-5510.

Jain N, Dandu H, Verma SP, Tripathi AK, Khanna A, Gutch M (2013). *An observational study on the prevalence of dyslipidaemia and dysglycaemia in human immunodeficiency virus patients.*

Annals of Tropical Medicine and Public Health. 6(1):84-88.

Justman JE, Benning L, Danoff A, Minkoff H, Leuine A, Greenblatt RM et al. (2003). *Protease inhibitor use and the incidence of diabetes mellitus in a large cohort of HIV-infected women.* J Acquir Immune Defic Syndr 32: 298-302.

Comment [M17]: ????

Jyothi I, Ravindra'n G, D'Souza J, Givija S, Sultana F (2011). *Diabetes Mellitus, Insulin Resistance and Metabolic Syndrome in HIV positive patients in South India.* Int J. Gen Med 4: 73-78.

Ketsamathi C, Jongjaroenprasert W, Chailurkit LO, Udomsubpayakul U, Kiertiburanakul S (2006). *Prevalence of thyroid dysfunction in Thai HIV-infected patients.* Curr HIV Res 4(4): 465-7.

Kilby JM, Tabereaux PB. (1998). *Severe hyperglycaemia in an HIV clinic: preexisting versus drug-associated diabetes mellitus.* J Acquir Immune Defic Syndr Hum Retroviral; 17:46-50.

Kofler D (2004). *low dose maintenance therapy with recombinant human growth low testosterone levels.* Int'l J. STD. 12(1): 75-83.

Kotegoda R., Madge S., Smith C.J., Lampe F.C., Thomas M. and Harvey R. (2005). *No association between HIV disease and its treatment and thyroid function; 55th Meeting of the British Thyroid Association, London.*

Lanzafame M, Trevenzoli M, Faggian F, et al. (2002). *Interaction between levothyroxine and indinavir in a patient with HIV infection.* Infection 30: 54-5.

Lesserman J, Petitto JM, Golden RN, et al. (2000). *The impact of stressful life events, depression, social support, coping and cortisol on progression to AIDS.* Am J Psychiatry; 157: 1221-1228.

Lesserman J, Jackson ED, Petitto JM, et al., (1999). *Progression to AIDS: the effects of stress,*

Comment [M18]: ????

*depressive symptoms, and social support.* Psychosom Med.61:397-406.

Leserman J. (2008). *Role of depression, stress and trauma in HIV disease progression.* Psychosomatic Medicine, **70**: 539-545.

Lisandro Irizarry *et al.*, (2016). *Thyroid hormone toxicity, treatment and management.*

Comment [M19]: ?????

IoPresti JS, Fried JC, Spencer CA, Nicolof JT (1989). *Unique alterations of thyroid hormone indices in the acquired immunodeficiency syndrome.* Ann Intern Med.; 110(12): 970-5.

Louise Gagnon (2006). *Thyroid function in older men who are HIV-positive or at risk for HIV.* AIDS, **20**(7): 544-49.

Lucia P, Stefano V. (2011). *A brief history of antiretroviral therapy of HIV infection: success and challenges.* Ann First Super Sanita, **47**: 44-48.

Madeddu G, Spanu A, Chessa F, Calia GM, Lovigu C, Solinas P, *et al* (2006). *Thyroid function in human immunodeficiency virus patients treated with highly active anti retroviral therapy (HAART): A longitudinal Study.* ClinEndocrinol. **64**(4): 375-83.

Comment [M20]: ????

Madge S, Smith CJ, Lampe FC, Thomas M, Johnson MA, Youle M, *et al.* (2006). *No association between HIV disease and its treatment and thyroid.* HIV Med. **8**(1): 22-7.

Comment [M21]: ????

Madhu N. Rao, Kathleen Mulligan, Viva Tai, Michael J. Wen, ArtemDyachenko, Melissa Weinberg, Xiaojuan Li, Thomas Lang, Carl Grunfeld, Jean-Marc Schwarz, and Morris Schambelan (2013). *Effects of Insulin-like Growth Factor (IGF) –I/IGF-Binding Protein-3 Treatment on Glucose metabolism and fat distribution in HIV infected patients with Abdominal Obesity and Insulin Resistance.* The journal of clinical endocrinology & metabolism **96**(9): 2009-2023.

Manuthu EM, Joshi MD, Lule GN, Karari E (2008). *Prevalence of dyslipidaemia and dysglycaemia in HIV-infected patients.* East Afr Med J **85**: 10-17.

Marianne Belleza RN (2021). *Congenital hypothyroidism – Nursing care management.*

Comment [M22]: ???not mentioned in the text (no citation)

Massabki PS, Accetturi C, Nishie IA, da Silva NP, Sato EI, Andrade LE (1997). *Clinical implications of autoantibodies in HIV infection.* AIDS. **11** (15): 1845-50.

Matthew Hoffman MD (2021). *Human Anatomy – Picture of the thyroid.*

Comment [M23]: ???not mentioned in the text (no citation)

Mauss S. (2000). *HIV-associated lipodystrophy syndrome*. *AIDS*; 14(Suppl 3): S197-S207.  
May M, Sterne J, Co stagiola D (2006). *HIV treatment response and prognosis in Europe and North America in the first decade of highly active antiretroviral therapy. A collaborative study*. *Lancet*; 368: 451-458.

Merenich J, McDermott T, Asp A, Harrison SM, Kidd GS (1990). *Evidence of endocrine involvement early in the course of human immunodeficiency virus infection*. *J.ClinEndocrinolMetab*. 70(3): 566-71.

Miller V, Hodder S (2014). *Beneficial impact of antiretroviral therapy on non- Aids mortality*. *AIDS*. 28(2): 273-4.

Murtala H, Hruz PW, Mueckler M (2002). *Indinavir inhibits the glucose transporter isoform at physiologic concentrations*. *AIDS*; 16: 859-863.

National Agency for the Control of AIDS –NACA (2012). *Women, Girls and HIV in Nigeria*. <http://naca.gov.ng/index2.php?option=com>

**Comment [M24]:** ???not mentioned in the text (no citation)

Noureldeen A, Qusti SY, Khoja GM (2014). *Thyroid function in newly diagnosed HIV-infected patients*. *ToxicolInd Health*. 30(10): 919-25.

Nwankwo UV, Nduka OS, Iliodigwe EE, Ogbonna B, Uzodimma US, Okonta JM (2014). *Assessment of Highly Active Antiretroviral Therapy (HAART) adherence among HIV patients in a tertiary health institution in Nigeria*. *African Journal of Pharmacy and Pharmacology*; 8 (47): 1192-1199.

Ogundele M, Coulter J. (2003). *HIV transmission through breastfeeding: problems and prevention*. *Ann Trop Paediatr*; 23: 91-106.

Oluboyo, AO., Okogun GRA., Duru, LAD, Oluboyo BO., Emenike EF., Obasikene, CN (2006). *Pattern of blood pressure, CD4, T cells count and some Cardiac Enzymes in HIV Seropositive Subjects*. *Journal of Biomedical Investigation*. 4(2): 37- 41.

Oluboyo AO, Onyenekwe CC, Okeke AC., Oluboyo BO., Odeyemi SO, Chukwuanukwu, TO., (2009). *Pattern of Lipid Profiles in HIV Seropositive Subjects on Antiretroviral Therapy*. *Journal of Biomedical Investigation*. 7 (1):32-35.

Onyancha O, Ocholla D. (2009). *Is HIV/AIDS in Africa Distinct? What Can We Learn From an Analysis of the Literature?* *Scientometrics*; 79(1): 277-296.

Palacios R, Merchante N, Macias J, Gonzalez M, Castillo J, Ruiz J (2006). *Incidence of and risk factors for insulin resistance in treatment-naïve HIV-infected patients 48 weeks after starting highly active antiretroviral therapy*. *AntivirTher* 11(4):529-535.

Palanisamy P, Perisamy M, Uma M, Mathiyalagan D (2010). *Thyroid function, cardiac risk assessment profile and haematological changes during HIV infection and AIDS patients.* J Medicine. 11(2):131-36.

Palios J, Kadoglou P, Lampropoulos S. (2012). *The Pathophysiology of HIV-/HAART-Related Metabolic Syndrome Leading to Cardiovascular Disorders: The Emerging Role of Adipokines.* Exp. Diabetes Res., 103063.

Parsa AA, Bhangoo A. (2013). *HIV and thyroid dysfunction.* Rev EndocrMetabDisord; 14(2):127-31.

Poli V, Balena R, Fattori E, Markatos A, Yamamoto M, Tanaka H, et al (1994). *Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion.* EMBO J. 13(5): 1189-96.

Comment [M25]: ????

Raff F, Brisseau JM, Planchon B, Remi JP, Barrier JH, Grolleau JY (1991). *Endocrine function in 98 HIV infected patients: A prospective study.* AIDS; 5(6): 729-33.

Rasoolinejad M, Afhami S, Izadi M, Hajabdolbaghi M, Kjairandish P (2004). *Clinical and paraclinical manifestation of thyroid dysfunction among patients with HIV/AIDS Tehran, Iran.*

Comment [M26]: ?????

Reng R, Uloko AE, Puepet FH, Onwugbuezie GA, Ramalan MA (2016). *Prevalence and determinants of Glucose Intolerance among HIV/AIDS patients in North-central Nigeria.* Nigerian Journal of Medicine. 25(2): 128-133.

Reveille JD (2000). *The changing spectrum of rheumatic disease in human immunodeficiency virus infection.* Semin Arthritis Rheum. 30(3):147-66.

Riddler SA, Smit E, Cole SR, Li R, Chmiel JS, Dobs A, et al. (2003). *Impact of HIV infection and HAART on serum lipids in men.*JAMA. 289: 2978-2982.

Comment [M27]: ????

Rosenfeld CR, Calabrese LH (1999): *Progression of autoimmune thyroiditis in an HIV-infected woman on HAART.* AIDS Read. 9(6):393-4.

Rugby B., Balshem H, Sehgal R., et al.,(2011). *Screening and treatment of subclinical Hypothyroidism or Hyperthyroidism.* Rockville (MD): Agency for Health Research and Quality (US).

Comment [M28]: ????

Safrin S, Grunfeld C. (1999). *Fat distribution and metabolic changes in patients with HIV infection.* AIDS.13;2493-2505.

Comment [M29]: ?????

Salazar-Gonzalez J, Salazar M, Learn G, Fouda G, Kang H, Mahlokozera T, Wilks A, Lovingood R,

Stacey A, Kalilani L, Meshnick S, Borrow P, Montefiori D, Denny T, Letvin N, Shaw G, Hahn B, Permar S. (2011). *Origin and Evolution of HIV-1 in Breast Milk Determined by Single-Genome Amplification and Sequencing*. *J. Virol.*, **85**(6): 2751-2763.

Saravanan P, Dayan CM (2001). *Assessment of thyroid function disease: thyroid autoantibodies*. *EndocrinMetabClin*. 30(2):315-37.

Serrano S, Marinoso ML, Soriano JC, Rubies-Prat J, Aubia J, Coll J *et al*(1995). *Bone remodeling in human immunodeficiency virus-1 infected patients. A histomorphometric study*. *Bone*. 16(2):185-91.

Comment [M30]: ????

Sellmeyer, DE and Grunfeld, C. (2013). *Endocrine and Metabolic Disturbances in Human Immunodeficiency Virus Infection and the Acquired Immune Deficiency Syndrome*. *Endocrine Reviews*, 17 (5): 116-120.

Sharp PM, Hahn BH.(2011). *Origins of HIV and the AIDS Pandemic*. Cold Spring Harbor Perspective in Medicine. 1.

Comment [M31]: ????

Shen Y, Wang Z, Lilia, RefangZhanga, Hongzhou Lu (2013). *Prevalence hyperglycaemia among adults with newly diagnosed HIV/AIDS in China*. *BMC Infect.Dis*.13:79.

Shoenfeld Y (1996). *Common infections, idiotypicdysregulation, auto-antibody spread and induction of autoimmune diseases*. *J Auto-immun*. 9(2): 235-9.

Shoenfeld CY (1995). *The viral autoimmunity relationship*. *Viral Immunol*. 8(1): 1-9.

ShujingJi, Changzhong Jin *et al.*,(2016). *Prevalence and influencing factors of thyroid dysfunction in HIV-infected patients*. *Biomed Research* Vol.2016., Article ID 3874257, 11pages.

Comment [M32]: ????

Comment [M33]: ????

Spinola-Castro AM, Siviero-Miachon AA, da Silva MTN, Guerra- Junior G. (2008). *O papel do hormonio de crescimento no tratamento dos disturbios endocrine-metabolicos do paciente com a sindrome da imunodeficienciaadquirida*. *AIDS.Arq Bras EndocrinolMetab*. 52(5):818-32.

Stefano V, Bernard S, Salif P. (2012). *The history of antiretroviral therapy and of its implementations for a public health approach*; 26: 1231-1241.

Tien PC, Schneider MF, Cole SR, Levine AN, Cohen M, De Hovit ZJ *et al.* (2007). *Antiretroviral*

Comment [M34]: ????

*therapy exposure and incidence of diabetes mellitus in the Women's Interagency HIV Study.* AIDS. 21: 1739-1745.

Touzot M, Le Beller C, Touzot F, Lou M, Piketty C (2006). *Dramatic interaction between levothyroxine/lopinavir/ritonavir in an HIV-infected patient.* AIDS. 20(8) :1210-2.

UNAIDS [2015]. *HIV and AIDS estimates Nigeria.* Fact sheet-2015.  
<http://www.unaids.org/en/regionscountries/nigeria.assessed 26th july 2016>.

**Comment [M35]:** ???not mentioned in the text (no citation)

UNAIDS (2015).*Report on the Global AIDS Epidemic.*  
<http://www.unaids.org/en/media/unaids/contentassets/documents/epidemiology/2012>.  
Assessed 11<sup>th</sup> May 2015.

**Comment [M36]:** ???not mentioned in the text (no citation)

Villette J, Bourin P, Doinel C, Mansour I, Fiet J, Boudou P, Dreux C, Roue R, Debord M, Levi F.

(1990). *Circadian variations in plasma levels of hypophyseal, adrenocortical and testicular hormones in men infected with human immunodeficiency virus.* *J. Clin.Endocrinol.Metab.*,**70**: 572-577.

WHO (2010).*Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public approach;* 19-20.

**Comment [M37]:** ??? not mentioned in the text (no citation)

WHO (2006).*Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycaemia.* Report of a WHO/IDF Consultation. Geneva: World Health Organization;21.

WHO, "Key Facts HIV 2021," Geneva, 2021.

**Comment [M38]:** ??? not mentioned in the text (no citation)

UNDER REVIEW