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_3), triiodothyronine (T_3), free thyroxine (fT_4), and thyroxine (T_4) 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_3 was significantly ($p<0.05$) higher in group 1 subjects than in group 2 subjects and the group 3. The level of T_4 was significantly higher in group 2 subjects than group 1 and the group 3 subjects. The level of T_3 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^3 in subjects who previously had CD4+ counts lower than 100 – 200 cells/mm^3 (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 eg amiodarone.
- Not pregnant
- Those who gave informed consent

3.3 Method for sample collection

About 8ml of venous blood was collected by venepuncture 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_3, T_4, TSH, fT_3, and fT_4,

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 Hopton *et 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 β-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 the quantitated by the use of a second TSH antibody (of either monoclononal or polyclonal origin) that is directed against a different antigenic site on the TSH molecule (for example, the α-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. 50µl 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 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 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_3 concentrations by Chopra *et al.,* 1971.

Principle

Procedures are similar to those described for T_4, except that a ^{125}I- T_3 tracer and T_3 – specific antibody are used. Solid-phase systems are preferred to liquid-phase separation systems. As with the T_4 methods, most T_3 methods use ANS to release T_3 from serum binding proteins without disturbing the binding of T_3 to antibody. A typical calibration curve ranges from 25 to 800ng/dl.

Nonisotopic assays similar to those described for serum T_4 have been applied to the measurement of T_3. Most commercial methods use peroxidase or alkaline phosphatase to label T_3 antigens or T_3 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_4

Competition principle.

Principle

- Sample, T_4 derivant coated microwells and enzyme labeled Anti-T_4 are combined
- During the incubation, T_4 derivant coated on microwells and fT_4 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_4 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:

EnzAg + Ag + AbC.W. = AgAbC.W. + EnzAgAbC.W.

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 µl of wash buffer was added, and decant. This step was repeated for a total of three times.
10. 100 µl 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 µl 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 SERO STATUS 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 showed the level of various thyroid hormones between the HIV positive and HIV negative subjects. The mean levels of TSH, T_4, T_3 and fT_3 were higher in HIV positive patients than the HIV negative subjects, while the level of fT_4 (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_3 where the difference between the mean was statistically not significant ($p>0.05$). See table 2.

Table 1: Demographic Characteristics and the sero status category of the study population

Variables	Group	Frequency	Percentage	Mean±SD

Gender	Female	84	67.2	
	Male	41	32.8	
		125	100	
Age group	30 below	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

	Serostatus			
Variables	**Positive (n=95)**	**Negative (n=30)**	**t-value**	**p-value**
TSH	3.3±2.7	2±1.2	3.63	<0.001
T_4	10.2±2.1	8.6±1.5	3.68	<0.001
T_3	2.3±0.8	1.4±0.5	5.72	<0.001
fT_4	25.1±7.5	42.5±8.4	10.83	<0.001
fT_3	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^a	2.5 ± 2^b	2 ± 1.2^b	8.61	<0.001
T_4	8.9 ± 2.4^a	11.4 ± 0.5^b	8.6 ± 1.5^a	35.31	<0.001
T_3	2.2 ± 1.1^a	2.4 ± 0.5^a	1.4 ± 0.5^b	17.49	<0.001
fT_4	27 ± 8.5^a	23.1 ± 5.8^b	42.5 ± 8.4^c	64.27	<0.001
fT_3	3.2 ± 1.2^a	2.8 ± 0.7^b	2.7 ± 0.9^b	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 showed 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)[a]	5 (10.6)[a,b]	1 (3.3)[b]	
T_4	Normal	46 (95.8)	47 (100)	30 (100)	0.34
	Abnormal	2 (4.2)	0 (0)	0 (0)	
fT_4	Normal	16 (33.3)	28 (59.6)	0 (0)	<0.001
	Abnormal	32 (66.7)[a]	19 (40.4)[b]	30 (100)[c]	
fT_3	Normal	41 (85.4)	47 (100.)	27 (90.0)	0.01
	Abnormal	7 (14.6)[a]	0 (0)[b]	3 (10.0)[a,b]	
	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 5 showed 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

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_4	Normal	6 (42.9)	7 (30.4)	2 (20.0)	0.46
	Abnormal	8 (57.1)	16 (69.6)	8 (80.0)	
fT_3	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)	

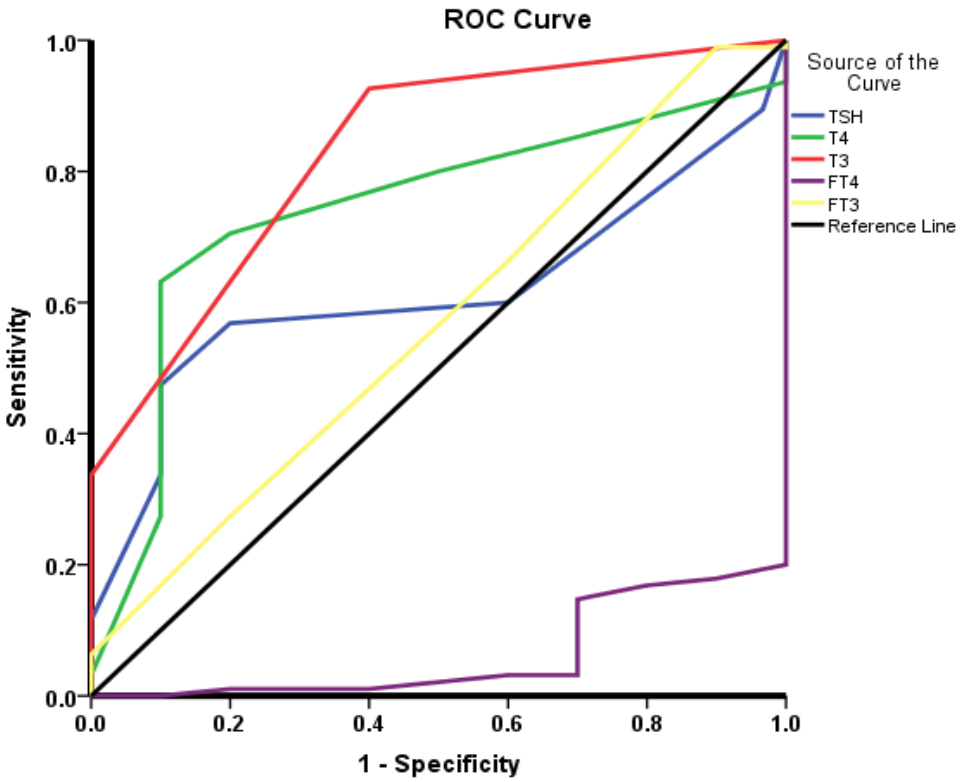

FIGURE 1: MEASURE OF DIAGNOSTIC ABILITY OF THE VARIABLES FOR SEROSTATUS USING RECEIVER OPERATING CHARACTERISTIC (ROC) CURVE

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_4	0.74	0.05	<0.001	0.64 - 0.84
T_3	0.83	0.04	<0.001	0.75 - 0.91
fT_4	0.06	0.02	<0.001	0.02 - 0.10
fT_3	0.57	0.06	0.26	0.45 - 0.69

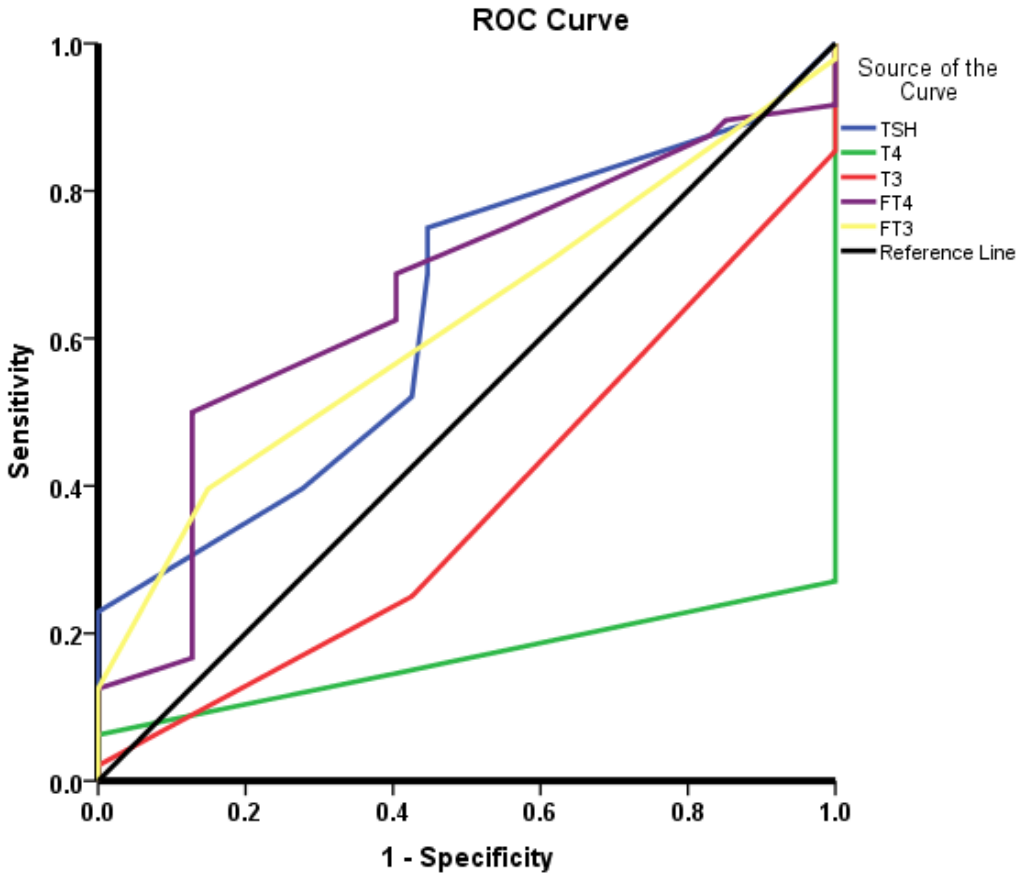

Figure 2: MEASURE OF DIAGNOSTIC ABILITY OF THE VARIABLES FOR HAART ADMINISTRATION USING RECEIVER OPERATING CHARACTERISTIC (ROC) CURVE

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_4	0.166	0.047	<0.001	0.07 - 0.26
T_3	0.375	0.057	0.04	0.26 - 0.49
fT_4	0.66	0.057	0.007	0.55 - 0.77
fT_3	0.62	0.058	0.04	0.51 - 0.73

4.3 DISCUSSION

This study is aimed at evaluation of 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 Collazos *et al.,* (2003) but in agreement with Palanisamy *et al* (2010) in India were fT3 was lower with higher fT4 and TSH among subjects with HIV compared with controls. In Ibadan Southwestern Nigeria, Abbiyesuku *et 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 (Qureshi *et 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 Rajendra *et 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 fT4. 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 fT4 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.

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