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Prevalence and risk factors of thyroid dysfunction among HAART taking patients at Bethel Teaching General Hospital Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Sibhat W/Kirkos, entitled: **Prevalence and risk factors of thyroid dysfunction among HAART taking patients at Bethel Teaching General Hospital Addis Ababa, Ethiopia** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

AIDS	Acquired Immunodeficiency Syndrome
AITD	Autoimmune thyroid disease
ART	Anti- Retroviral Therapy
CD	Cluster of Differentiation
CDC	Centers for Disease Control and Prevention
ELISA	Enzyme Linked Immunosorbent Assay
fT3	Free Tri-iodothyronine
fT4	Free Thyroxine
GD	Graves' disease,
HAART	Highly active antiretroviral therapy
HIV	Human Immunodeficiency Virus
IRIS	Immune reconstitution inflammatory syndrome
MoH	Ministry of Health
NCASC	National Centre for AIDS and STD Control
NPHL	National Public Health Laboratory
ScH	Subclinical hypothyroidism
STI	Sexually Transmitted Infection
TSH	Thyroid Stimulating Hormone
T3	Triiodothyronine
T4	Tetraiodothyronine
VL	Viral load
WHO	World Health Organization

Abstract

Background: Abnormal thyroid function test results are one of the common complications among HIV infected patients. Although the prevalence of overt thyroid disease does not appear to be significantly increased in HIV-infected patients, compared with the general population, specific patterns of abnormal thyroid function test findings are more frequently identified among HIV-infected patients.

Objective: To assess thyroid dysfunction rate and associated factors among HAART taking patients

Methods: A hospital based cross sectional study was conducted from December 2017 to May 2018 at Bethel Teaching General Hospital, Addis Ababa, Ethiopia. A total of 300 HIV patients were enrolled in the study. Participants' socio-demographic and clinical information was collected from hospital cards. Serum and EDTA whole blood was examined for Thyroid function tests and CD4+ T cell count levels, respectively. Descriptive statistics was used to express the socio demographic characteristics. Binary and multiple logistic regressions were computed to assess association between variables using SPSS version 20. Odds ratio with confidence interval and $P<0.05$ have been be used to determine strength and statistically significant differences.

Results: Of the 300 individuals, 70 (23.3%) were identified with abnormal thyroid function, of whom, 47 (15.7%) were female and 23 (7.7%) male. 14(4.7%) were diagnosed with hyperthyroidism and 56(18.7%) with hypothyroidism. Of these, 35(62.5%) had subclinical hypothyroidism, 21 (37.5 %) had overt hypothyroidism. Out of these subclinical hypothyroid 23 (65.7%) were female and 12 (34.3 %) were males. The thyroid hormone levels however, did not correlate with duration of HAART and HIV duration. The levels of TSH, T3, T4 230(76.7%), 235(78.3%), 272(90.7%) for the majority of the participants were in the normal label respectively. There was a slight negative correlation of CD4 counts with serum TSH levels ($r = -0.1224$ with $p < 0.034$)

Conclusion: High rate of thyroid function test abnormalities was observed in HAART receiving HIV/AIDS individuals. Subclinical hypothyroidism was the most common disorder and females were more exposed, warranting thyroid function tests monitoring.

Keywords: HIV, HAART, TFT, Thyroid Dysfunction, CD4+ T cell

1. Introduction

1.1 Background

Human immunodeficiency virus (HIV) infection can expose to multiple organ involvement including the endocrine system. Endocrine function may be changed in HIV infection because of the possible association between the immune and endocrine systems, direct involvement of the glands by the HIV itself, opportunistic infections or malignancies [1, 2]. In recent years, increasing number of patients with HIV infection are able to survive for long periods because of the extensive application of highly active antiretroviral therapy (HAART) for the suppression of viral replication and also because of the emergence of new medicines and therapeutic regimens. Many non-acquired immune deficiency syndrome- (AIDS-) related diseases now primarily account for the disease burden in patients with HIV infection because of prolonged therapy [3].

Thyroid disorder is one of the common endocrine dysfunctions observed due to alteration in the production of thyroid hormones. Altered production of these hormones often involves dysfunction of thyroid gland, pituitary gland, and hypothalamus [4]. Hyperthyroidism (overproduction of T3 and T4) and hypothyroidism (underproduction of T3 and T4) are regarded as the most common clinical forms of thyroid disorders.T3 and T4, an important hormone regulating metabolism, can also be affected by HIV infection. Besides, HAART therapy can complicate thyroid function further through drug interactions and the immune reconstitution inflammatory syndrome (IRIS) [5].

Abnormal thyroid function test results are also common among HIV infected patients. Although the prevalence of overt thyroid disease does not appear to be significantly increased in HIV-infected patients, compared with the general population, specific patterns of abnormal thyroid function test findings are more frequently identified among HIV-infected patients. Among patients with AIDS, nonthyroidal illness is common [6, 7]. During antiretroviral therapy, the prevalence of two generally asymptomatic conditions (subclinical hypothyroidism, which is characterized by isolated elevated thyroid-stimulating hormone levels, and isolated low free thyroxin levels) is increased [8].

Prevalence of overt primary hypothyroidism in the general population and HIV infected individuals from different studies across the globe has been reported to be 0.3% and 0–2.6%, respectively. Several studies have reported that the incidence of thyroid dysfunction is much higher (about 36%-37%) in patients infected with HIV than in the general population [9, 10]. In Ethiopia, the overall prevalence of thyroid dysfunction among HIV infected patient is still less investigated.

1.2 Statement of the problem

The interaction between HIV disease, treatment and the endocrine system covers a spectrum from subtle abnormalities in hormonal secretion, transport, or metabolism to rare instances of overt glandular failure, with potentially devastating consequences [9].Thyroid dysfunction reduces the quality of life of patients infected with HIV [11].

Overt hypothyroidism leads to the insidious onset of fatigue, weakness, dry skin, cold intolerance, slowed mentation, constipation, hoarse voice, paresthesia, bradycardia, and delayed relaxation of tendon reflexes [12]. similarly Overt hyperthyroidism is characterized by irritability, heat intolerance, and sweating, warm moist skin, palpitations, tachycardia, and fatigue, weight loss with increased appetite, diarrhoea, tremor, muscle weakness, hyperreflexia, and lid retraction[13]. The consequences of subclinical hyperthyroidism include reduced bone mineral density and an increased risk of atrial fibrillation, the risk of which is proportional to the degree of thyroid hyper function [14-16]. Furthermore; subclinical hyperthyroidism may precede overt hyperthyroidism. It is unclear why HIV-infected patients are susceptible to thyroid dysfunction, but HIV infection and it treatment is regarded as a crucial factor [10, 17].

Prolonged antiretroviral therapy has been associated with adverse events such as Grave's disease, sub-clinical hypothyroidism, and sick euthyroidism [18]. A recent study in China indicate that thyroid dysfunction was significantly more frequent in the HAART group (41/104, 39.4%) than in the HAART-naïve group (18/74, 24.3 %;). The mean CD4 cell count was significantly lower in patients with hypothyroidism than in the other patients. The FT4 level was significantly lower in the HAART group than in the HAART-naïve group. FT3/FT4 levels were negatively related to HIV duration and FT3 levels were positively related to CD4+ T cell count [19].

In Ethiopia published studies regarding thyroid dysfunction among HAART taking HIV patients is lacking to the best of my knowledge. As this problem influence the life of HIV infected patients, this study aimed to investigate magnitude of thyroid dysfunction among HAART taking patient and its correlation with immune response.

1.3 Significance of the study

There is little information about the effects of HIV treatment on thyroid function. Although the majority of patients with HIV develop no thyroid function problems, there is some evidence to suggest that an increasing number of patients taking anti-HIV drugs are presenting with thyroid disorders. So that this will generate data on the effect of HAART on thyroid hormones in people living with HIV and its effect on CD4 count. Knowing the effect of HAART on thyroid function level of HIV patients is essential for management and care of patients living with HIV/AIDS. So this study will give additional information to clinicians so that they can choose the correct HAART regimen for their clients.

2. Literature review

HIV infection and its therapy HAART correlated with different complications. For example a study conducted by Lopresti *et al* in Los Angeles USA, explored alterations in serum thyroid hormone indices in patients with HIV infection using data collected from twenty-six AIDS inpatients. The study included outpatients of whom 10 seropositive for HIV, 10 with AIDS-related complex, and 10 with AIDS. Serum triiodothyronine (T3) values generally remained normal until hospitalization, with non survivors having lower values than survivors (0.56 ± 0.1 nmol/L compared with 1.3 ± 0.1 nmol/L, $P < 0.002$, respectively). Reverse triiodothyronine (rT3) levels were low in persons with AIDS-related complex (0.21 ± 0.02 nmol/L, $P < 0.001$) and in AIDS outpatients (0.17 ±0.02 nmol/L, $P < 0.001$). Normalization of rT3 occurred after patients were hospitalized (0.28 ± 0.01 nmol/L). All values were compared with normal values (T3, 2.3 ± 0.04 nmol/L; rT3, 0.28 ± 0.01 nmol/L. Infection with HIV produces unique alterations in thyroid function. The persistence of a normal T3 despite progression of HIV infection may contribute to weight loss. A low serum T3 on admission correlates with mortality [20].

In a study conducted by Carvalho *et al* at the Universidade Federal do Rio de Janeiro, enrolled 153 patients selected from the infectious disease outpatient clinic at a University Hospital in Rio de Janeiro. Patients were characterized based on their circulating CD4+ lymphocytes levels, serum TSH levels, and the presence of FT4. A total of 129 participants were on HAART and 24 were not. The frequency of thyroid disorders was 7.8% (12/153 patients) and all were on HAART at the time of diagnosis, yielding a prevalence of 9.3% in patients receiving HAART compared with 0% in patients not on HAART. Hyper and hypothyroidism were detected in 3.1%, and 4.1% of HAART patients respectively [21].

A cross-sectional study in Rio de Janeiro, Brazil was performed to analyze the records of 117 HIV-infected patients who had their CD4+ cell count, viral load, TSH and free T4 levels collected on the same day. The prevalence of thyroid disease was 34.18%. There was an association of risk between stavudine use and subclinical hypothyroidism (OR = 4.19, 95% CI: 1.29 to 13.59, X^2 = 6.37, p = 0.01). Immune reconstitution achieved protection associated with thyroid disease that was near statistical significance [OR = 0.45, 95% CI: 0.19 to 1.04, X^2 = 3.55, p = 0.059]. The

historical use of stavudine has an association of risk for the presence of subclinical hypothyroidism [22]

Beltran S and his colleagues in north France included 350 HIV-infected patients in their cross sectional study. Sixteen percent of patients had hypothyroidism: 2.6% had overt hypothyroidism, 6.6% had subclinical hypothyroidism, and 6.8% had a low free T4 level. The prevalence of subclinical hypothyroidism was higher among HIV-infected men than among HIV-infected women. Similar case-control study was conducted by these researchers that compared hypothyroid (*n*=56) and euthyroid (*n*=287) patients. In the multivariate analysis, receipt of stavudine and low CD4 cell count were associated with hypothyroidism [10].

Another cross-sectional study was done in France by Grappin M *et al* that include a total of 212 patients, of whom 26 patients (12.3%) presented with at least one abnormal test of thyroid function. On these, no clinical dysthyroidism was noted. Four patients had a previous history of thyroid disorder, requiring the adjustment of levothyroxine doses after starting HAART. Therefore, after excluding the four patients with previous thyroid disease, biological thyroid dysfunction was assessed in 22 (10.4%) patients: 18 (8.5%) with subclinical hypothyroidism. No hyperthyroidism was found**.** . In univariate analysis, excluding patients with a history of thyroid dysfunction (four patients in the group with biological thyroid abnormalities and one patient in the group without abnormalities), the cumulative daily dose of stavudine and lamivudine were found to be significantly ($P = 0.01$ and 0.04, respectively) greater in patients with hypothyroidism than in those without. In multivariate analysis, the cumulative daily dose of stavudine remained associated with subclinical hypothyroidism (annual odds ratio 1.96; confidence interval 1.15–3.33; $P = 0.01$) [23].

A study by Raffi F *et al* in France explained from 98 prospectively evaluated HIV patients. The main abnormalities were sick euthyroid syndrome with low tri-idothyronine and/or thyroxine in 16% of patients with AIDS. These abnormalities related to a functional deficiency of the hypothalamic-pituitary axis and were highly correlated with the degree of illness, i.e. weight loss and low CD4+ cell count [24].

An article by Quirino *et al* in Italy showed that from 687 patients observed, in 51 patients (7.42%), they detected subclinical hypothyroidism; 5 (9.8%) of these patients were treatment naive, and 46 (90.2%) were receiving antiretroviral therapy. The frequency of this disease among male subjects was 7.11%, which was slightly lower than the frequency among female subjects (8.71%) [25].

A total of 190 consecutive HIV-infected individuals were followed and categorized into three groups by Bongiovanni *et al* in Milan, Italy. Group 1(G1), subjects on stable HAART (for at least 1 year) at baseline and at month 24 (n = 97); Group 2(G2), naive at both baseline and month 24 (n = 47); Group 3(G3), individuals starting HAART at baseline (n = 46).The three groups were comparable with respect to age, gender, body weight and prevalence of HCV infection. At baseline, subclinical hypothyroidism was detected in 14 subjects in G1 (14.4%), 5 in G2 (10.6%) and 4 in G3 (8.7%) (P = 0.18) and these were excluded from the analysis. At month 24, 15 participants had developed subclinical hypothyroidism: 4 in G1 (4.8%), 3 in G2 (7.1%) and 8 in G3 (19.0%) [26].

In a study by Madeddu *et al* in Italy which enrolled 202 HIV patients, abnormalities in thyroid function tests were found in 23/182 (12.6%) HAART patients, but not in naïve patients. Most abnormalities were subclinical hypothyroidism, with mean FT4 and TSH levels lower and higher, respectively, in HAART patients compared to naïve patients and controls, FT4 levels being significantly lower than controls. TSH negatively correlated with CD4 count nadir and positively with HAART duration. During follow-up, FT4 and FT3 significantly decreased and TSH increased in patients continuing HAART, whereas CD4 counts were unmodified; subclinical hypothyroid conditions persisted and further cases occurred, whereas the only hypothyroid patient who interrupted HAART shows a normalization of thyroid tests. Patients on stavudine, included in most hypothyroid patient protocols, had significantly lower FT4 levels with prolonged treatment [27]

Another study in Italy Roma, by Olivieri *et al* showed that thyroid function was evaluated in119 human immunodeficiency virus (HIV) infected patients at different stages of infection, compared with euthyroid normal individuals and hepatitis C virus infected blood donors as control groups. The low T3 state, well documented in severe nonthyroidal illnesses, was not found in these HIV infected patients. They showed lower FT4 levels and higher TSH values than euthyroid normal controls [28].

A hospital based HIV cohort study in London which enrolled a total of 2437 individuals had routine thyroid function tests performed. The incidence of hyperthyroidism was 3.4 (95% confidence interval: 1.5 to 6.8) per 10,000 patient-years and of hypothyroidism it measured 10.7 (95% confidence interval: 6.9 to 15.8) per 10,000 patient-years. Of these 2437 individuals, 54 (2.2%) were identified with abnormal thyroid function, of whom, 26 were diagnosed with hyperthyroidism and 28 with hypothyroidism. Twenty-one (80.8%) of 26 hyperthyroid patients were male and 5 (19.2%) were female. Twenty-two (78.6%) of 28 hypothyroid patients were male and 6 (21.4%) were female. The clinical prevalence of hyperthyroidism in their HIV cohort was calculated to be 1.01% and that of hypothyroidism was 1.2%. Of the patients tested, thyroid antibodies were present in 40% of those with hypothyroidism and 66.7% of patients with hyperthyroidism, a finding that may suggest an association with immune restoration after HAART [29].

A retrospective analysis in London, UK by Madge *et al* indicates that a total of 2151 ,1565 patients (73% of the clinic population) had at least one TFT taken since 2001. Overall, 3584 samples were analyzed. Of the patients included in the study, 1233 (79%) were male, 1043 (66%) were white and 365 (23%) were black African, and in 969 (62%) the main risk for HIV was homosexual sex. Median age at baseline was 37 years. 900 patients (58%) were on HAART at the start of the study. Thirty-nine (2.5%) were found to have overt hypothyroidism, and eight (<1%) had overt hyperthyroidism. Sixty-one (4%) had subclinical hypothyroidism, five (<1%) had subclinical hyperthyroidism and 263 (17%) had a nonthyroidal illness. A normal TFT was obtained for 1118 patients (75.5%) [30].

A study by Hommes *et al* in Netherlands (Amsterdam) indicates thyroid hormones and TSH responsiveness to thyrotropin-releasing hormone (TRH) were measured. Triiodothyronine (T_3) and thyroxine (T_4) did not differ between HIV-infected patients and controls, free thyroxin (FT_4) index (94 ± 3 *v* 110 ± 4, $P < .01$), FT_4 (11.8 ± 0.4 *v* 14.3 ± 0.4 pmol/L, $P < .01$), and reverse triiodothyronine (rT_3) values (0.18 ± 0.01 *v* 0.26 ± 0.02 nmol/L, $P < .001$)values. Mean 24-hour TSH levels were increased in HIV patients (2.39 ± 0.33 *v* 1.44 ± 0.16 mU/L, $P < .05$), associated with increased mean TSH pulse amplitude and TSH responsiveness to TRH. No differences were observed between asymptomatic HIV-seropositive and AIDS patients [31].

Feldt- ransmussen *et a*l in Copenhagen, Denmark conducted a study among 18 male patients with AIDS and 12 asymptomatic HIV1-positive persons compared with an age-matched control group.

The study showed that serum total thyroxine was not significantly different between the groups, but both serum total triiodothyronine, triiodothyronine uptake test, and free thyroid hormone indices showed significantly decreasing values from HIV1-positive healthy persons to AIDS patients compared with controls (P value from < 0.05 to < 0.001).The investigators concluded that thyroid tests showed an atypical outcome in HIV1-positive patients with or without AIDS compared with the pattern normally seen in non-thyroid illness, and should, therefore, caution the interpretation of the biochemical changes when diagnosing abnormal thyroid function in these patients [32].

Noureldeen *et al* in Jeddah, Kingdom of Saudi Arabia enrolled a total of 100 newly diagnosed HIV-infected patients having a CD4 cell count of 180-350 cells/mm. Same number of healthy volunteers were also included for comparison. In total, 70% of HIV-infected patients had normal thyroid function tests when compared with control individuals, while 30% of HIV-infected patients had abnormal thyroid function. Of the 30 cases, 11 cases had abnormal TSH values, with increased TSH predominant (7% of HIV cases) than decreased TSH (4% of patients) values. Incidence of thyroid abnormalities ranging from hypothyroidism (subclinical and overt: 6% and 1%, respectively) to hyperthyroidism (2%) and nonthyroidal illness (9%) were estimated in HIV-infected patients [33].

Another cross sectional prevalence study was conducted by Kaneria *et al* in India, Mumbai on HIV positive adult patients admitted to the Department of Medicine at T.N. Medical College and B.Y.L Nair Charitable Hospital over a period of 18 months. According to the study overt hypothyroidism was found in 2 (3%), subclinical hypothyroidism in 10 (13%), isolated low FT4 in 2(3%) and sick euthyroidism in 19 (25%) patients. As the stage of HIV advanced, the FT3 and FT4 levels went on decreasing. The TSH levels however, did not correlate with the stage of infection. A direct correlation was found between FT3 and CD4 counts and an inverse correlation between TSH levels and CD4 counts. The mean TSH levels in patients on HAART were significantly higher than in patients not on HAART [34].

Sherma *et al* from India initially screened 527 patients, 359 patients with (61.44±39.42 months' disease duration), having good immune function (CD4 count >200 cell/mm^3: 88.58% HAART taking), were analyzed. Subclinical hypothyroidism (ScH) was the commonest thyroid dysfunction

(14.76%) followed by sick euthyroid syndrome (SES) (5.29%) and isolated low TSH (3.1%). Burden of thyroid dysfunction in chronic HIV infection with stable immune function is lower compared to pre-HAART era. Thyroid dysfunction is primarily of non-autoimmune origin, predominantly ScH[11].

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The study by Jaint *et al* in India also showed that out of 50 cases, thyroid function abnormalities were observed in substantial number of patients. Nine (18%) patients had FT-3 levels below the normal range, ten (20%) patients had decreased FT-4 levels and twelve (24%) patients had TSH levels above the normal range. When the results were statistically analyzed for the 50 patients enrolled in the study using Pearson's correlation coefficient, there was a direct correlation between CD4 count and FT3 and FT4 values ($r=0.357$ with $p<0.05$; $r = 0.650$ with $p<0.05$ respectively). There was an inverse correlation of CD4 counts with serum TSH levels ($r = -0.470$ with $p < 0.050$). Thyroid dysfunction is frequent in HIV infection and with progression of disease there is a primary hypothyroid like stage that occurs in patients with HIV infection. FT3 /FT4 / serum TSH can be used as a surrogate marker of the progression of the disease [35]

Tripathy, *et al* in India showed that the prevalence of gonadal dysfunction (88.3%) was the most common endocrine dysfunction followed by thyroid (60.4%) and adrenal dysfunction (27.9%). Secondary hypogonadism (68.4%) was more common than primary (31.6%). Low T3 syndrome, that is, isolated low free T3, was the most common (25.6%) thyroid dysfunction followed by secondary hypothyroidism (16.2%) and subclinical hypothyroidism (11.6%). Adrenal excess (16.3%) was more common than adrenal insufficiency (11.6%). The difference in hormonal dysfunction between male and female was statistically insignificant ($P > 0.05$).27.9% of patients had multiple hormone deficiency. There was negligible or no correlation between CD4 count and serum hormone level [36].

A cross-sectional study was done by Dev N and his colleagues in India which enrolled 225 HIV-positive and an equal number of healthy volunteers. The mean (SD) CD4 count in the study group was 147.1 (84) and 70.7% had advanced immune deficiency with CD4 count <200 cells/μL. The overall prevalence of thyroid dysfunction was 75.5% in the study group and 16% in the control group. Subclinical hypothyroidism was the commonest abnormality noted in almost 53%. Signif-

icant correlation was observed between CD4 count and thyroid stimulating hormone, free triiodothyronine, and free thyroxine levels ($r = -0.86$, $r = 0.77$, and $r = 0.84$, respectively, $p < 0.0001$ for all) (37).

A study in Nepal by Joshi *et al* investigated a total of 120 HIV/AIDS infected individuals (including 80 under HAART) for thyroid function disorders. In the HAART receiving group, 92.3% individuals had hypothyroidism while in HAART naïve group only one (7.7%) individual had such disorder. Association between hypothyroidism and HAART was statistically significant (p = 0.038). In addition, the rate of hypothyroidism was significantly higher in HAART receiving females (p = 0.01). Hypothyroidism status in HAART receiving or not receiving individuals and their gender was further fitted in a regression model. After adjusting for gender and HAART status in this model, gender remained as an independent predictor of hypothyroidism (Odds Ratio- OR: 5.097, p = 0.031; CI: 1.15-22.47) [2].

A study by Thaimuta *et al* in Nairobi, Kenya which enrolled a total of 110 patients (only 84 of study participant were on HAART) explained that the TSH levels were within reference range in HAART taking (85.6%) and HAART naïve (92.3%) patient. However, there were no incidences of elevated FT4 1evel among HAART naïve, but 12.0% HAART taking had high TSH levels. There was no statistically significant difference in the levels of TSH (p=0.233) between HAART taking and naïve patients. The frequency of lower FT4 levels in HAART taking (54.8%) compared to HAART naive (30.0%) was not statistically significant (p=0.45). The level of non-thyroidal illness among HAART taking (44%) were comparable with that of HAART naïve (46.2%). There was a higher incidence of euthyroid among HAART taking (42.9%) than HAART naïve (34.6%) patients. Abnormality with thyroidal state was more frequent in HAART naïve than in HAART taking except for sub clinical hypothyroidism which was absent in the former [38]

Taken together, the studies reviewed above revealed derangement in thyroid function levels in association with HAART. There is a gap of information in Ethiopian HAART taking patients in this regard, which this study is trying to address.

Conceptual frame work

HAART, HIV, IRIS, opportunistic infections, malignancies and drug interactions are some of important risk factors associated with Thyroid dysfunction

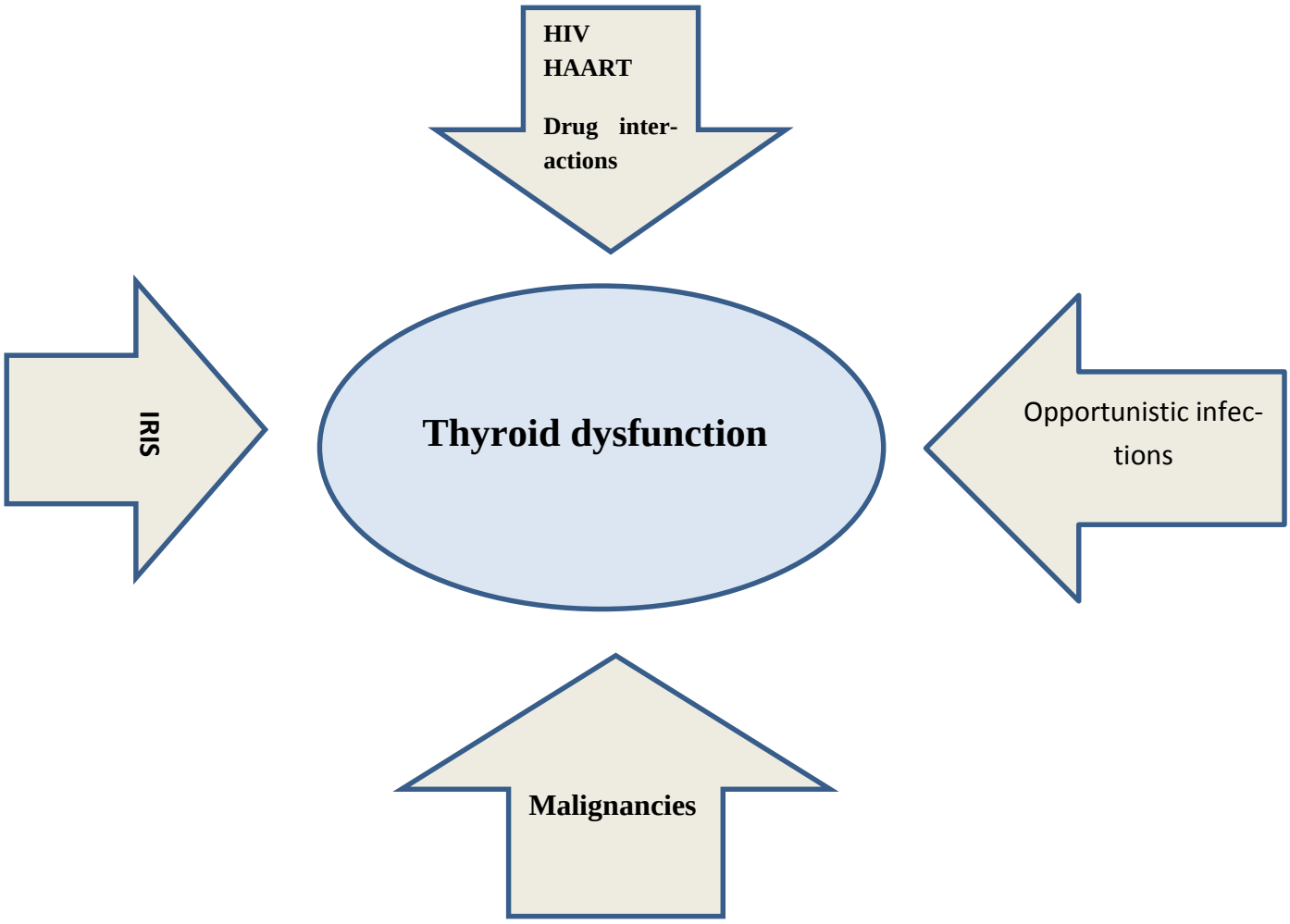

Figure: 1**.** Conceptual frame work for thyroid dysfunction

3. Objective

3.1 General objective

- To assess the magnitude and associated factors of thyroid dysfunction among HAART taking patients at Bethel Teaching General Hospital, Addis Ababa, Ethiopia

3.2 Specific objectives

- To investigate the prevalence of thyroid dysfunction among HAART taking patients
- To determine risk factors associated with the progress of thyroid dysfunction.
- To determine correlation between thyroid function level and CD4 level.

4. Hypothesis

- There is no incidence of thyroid dysfunctions among HAART taking HIV patients as reported by other studies.

5. Materials and Methods

5.1 Study area

The research project was carried out at Bethel Teaching General Hospital in Addis Ababa, Ethiopia. The hospital is founded in 2000, owned and operated by an Ethiopian obstetrician and gynecologist. It is nationally as well as internationally renowned for its high standard and quality services and for availability of specialists from all over the world.

The hospitals endeavor to introduce state-of-the-art equipment and pioneering medical procedures. The hospitals which are located at Bethel area, a place in Kolfe keranyo subcity which gets the name after the hospital's establishment and the second one around Tor Hailoch area are linked to bethel Medical College, which is one of the few private institutions in the country to offer Medical Doctor Degree. Currently the center treats more than 2000 HIV patients each month as 100 clients are being seen daily [39].

5.2 Study design and period

A hospital based comparative cross sectional study was conducted from December 2017 to May 2018 at Bethel Teaching General Hospitals, Addis Ababa, Ethiopia.

5.3 Population

5.3.1 Source population

All ART patients attending Bethel Teaching General Hospital during the study period

5.3.2 Study population

Adult patients that are attending ART clinic of Bethel Teaching General Hospital that fulfill the eligibility criteria

5.4 Eligibility

5.4.1 Inclusion criteria

- Adult patients (≥18 years) who are on HAART at least for one year
- HIV patient on ART follow up and voluntary to permit additional testing of thyroid hormones shall be included from these studies.

5.4.2 Exclusion criteria

- Patient who have previous history of thyroid function problem.

5.5 Study variables

5.5.1 Dependent variables

- T3 level
- T4 level
- TSH level

5.5.2 Independent variables

- Age
- Sex
- Occupation
- Marital status
- OI status
- Regimen type
- Weight/Height (BMI)
- Duration on HAART
- CD4 level
- CD4 category

5.6 Sample size calculation and sampling technique

5.6.1 Sample size calculation,

$$n = \frac{z^2 p(1-p)}{d^2}$$

n: Sample size

z: z -score

p: prevalence

d: allowable error

Using P value 0.13 from study by Thaimuta *et al*, 2010 (taking maximum thyroid dys-function rate of 13%) [38]; with confidence interval of 95% and margin of error (d) =5 % (0.05) allowing 10% for non-response, the, minimum sample size becomes =190

But 300 ART patients attending Bethel Teaching General Hospital ART clinic were enrolled in the study.

5.6.2 Sampling technique

Convenient sampling technique was employed to collect data from those ART patients who fulfill the inclusion and exclusion criteria during the study period

5.7 Data collection and processing

Socio-demographic information and other relevant possible risk factors of the study participants were routinely collected using structured and pre tested questionnaire by trained nurses in the ART clinic. Clinical data were also collected from patients' records. Following the standard procedures of the ART clinic, 8 ml of venous blood was aseptically collected using plain and EDTA vacationer tubes (about 4 ml in each tube) for the determination of thyroid function and CD4.The blood specimen in the plain tube was centrifuged at 3000 RPM for 5 minutes to separate the serum and use for determination of thyroid function within one hour of separation. Thyroid function was assessed by measuring T3, T4 and TSH levels in patient's serum by quantitative Enzyme-Linked Immuno

sorbent Assay (ELISA) (Human, Wiesbaden, Germany).The second tube that contains whole blood was used for the CD4 determination.

5.7.1. Thyroid function tests

Serum thyroid hormones levels (TSH) 0.3-4.0 miu/l, triiodothyronine(T3)0.69-2.02 (µg/dl) and thyroxin (T_4)4.4-10.8(ng/ml) were measured their levels in patient's serum by quantitative Enzyme- Linked Immunosorbent Assay (ELISA) (Human, Wiesbaden, Germany). In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-tT4 (tT3) conjugate and biotinylated tT4 or tT3 antibody are added, and the reactants are mixed. In the case of TSH, the biotinylated and enzyme conjugate are added in one step. A reaction results between the enzyme conjugate, biotinylated conjugate and the native thyroid hormone (tT3, tT4 or TSH) for the antibody combining sites. Immobilization takes place through the reaction of the incorporated biotin and streptavidin coated on the well served as second antibody. After the completion of the required incubation period, the bound enzyme conjugate is separated from the unbound enzyme conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color. The employment of several serum references of known thyroid hormone concentration (s) permits construction of a graph of activity and concentration. From comparison to the dose response curve (s), an unknown

specimen's activity can be correlated with hormone concentration. The test is used for Quantitative Determination of Total Thyroxine, Total Triiodothyronine, Thyroid Stimulating Hormone Concentration for a comprehensive thyroid status of a Human Serum or Plasma sample by a Microplate Enzyme Immunoassay [40].

5.7.2 CD4 determination

The CD4 count was determined by BD FACS Count flowcytometer (Beckton Dickinson, San Jose, California). A single test requires one convenient, ready-to-use reagent tube pair. When whole blood is added to the reagents and incubated at room temperature, fluorochrome-labelled antibodies in the reagents bind specifically to lymphocyte surface antigens. After a fixative solution is added to the reagent tubes, the sample is run on the instrument. Here, the cells come in contact with the laser light, which causes the fluorochrome labelled cells to fluoresce. This fluorescent light provides the information necessary for the instrument to count cells. In addition to containing the antibody reagent, the tubes also contain a known number of fluorochrome-integrated reference beads. These beads function as a fluorescence standard for locating the lymphocytes and also as a quantification standard for enumerating the cells expecting the result to be in the range500-1500cells/μL of blood [41].

5.8 Statistical Analysis

Data was entered, cleaned and analyzed using SPSS version 20 software. Descriptive analysis was done to determine mean and standard deviation for continuous variables and the difference in means was compared with independent-sample t-test. The differences in proportions was evaluated by Pearson's Chi-square test (x2 test), linear regression was employed for effect analysis and P-value of less than 0.05 was considered as statistically significant.

5.9 Data Quality Assurance

Standard operating procedure (SOP) was followed during pre-analytical, analytical, and post analytical phases of the study. Training was given to data collectors to avoid technical errors and to minimize observer bias. All reagents were checked for their expiry date and we confirmed the reagents are prepared according to manufacturer's instructions. All TSH, T_3 and T_4 kits were checked by using known control samples. Similarly the quality of CD4, reagents were regularly monitored by running control materials in each morning before the actual work is done.

5.10 Ethical considerations

Ethical clearance was obtained from departmental research and ethics review committee of department of Medical Laboratory Science of Addis Ababa University. The proposal was also reviewed by ethical review board of Bethel Teaching General Hospital. Written informed consent (signed or thumb print) was obtained from each participant. Confidential identifiers were used to code participant's identities. Results and any information regarding patients were kept confidential during and after the completion of the research project by password protecting electronic and locking hard copy files.

5.11 Dissemination of Results

The results of the study will be disseminated to Addis Ababa University and Bethel Teaching General Hospital so that they work in collaboration to improve the health condition of the patients. It will also be communicated and disseminated to stakeholders, public and concerned bodies through presentation in different professional association meetings and conferences. The final paper will be submitted to a national or international peer reviewed scientific journal for publication.

5.12 Operational definitions

- **Hyperthyroidism:** is a condition in which an overactive thyroid gland is producing an excessive amount of thyroid hormones that circulate in the blood
- **Hypothyroidism:** is a condition characterized by abnormally low thyroid hormone production
- **Subclinical hypothyroidism**: is the elevation of thyroid stimulating hormone (TSH) which is common in individuals with HIV/AIDS
- **Thyroid Dysfunction:** states abnormal production of thyroid hormones
- **Thyroid function test:** Measuring serum hormone concentrations, to assess thyroid function such as TSH, T_4 and T_3.
- **HAART:** HAART also known as combination therapy is a therapy, usually a combination of two reverse transcriptase inhibitors and a protease inhibitor and is the current standard of care for HIV/AIDS in the world
- **Overt hypothyroidism** is defined as a clinical syndrome of hypothyroidism associated with elevated TSH and decreased serum levels of T_4 or T_3

6. Results

6.1. Socio-demographic and clinical characteristics

A total of 300 participants aged 23 to 93 years participated in this cross sectional study. Of them 59.3 % (178) were female, most in the age group 40-49 years (127, 42.3%). The mean and standard deviation age was 44.5 (10.5) years. Among those receiving HAART 292(97.3%) were at WHO stage1.When they started the treatment they were in different WHO staging but after 1 year their immune status became stable and automatically categorized under stage 1.The majority of the participants had CD4 level above 500 cells per microliter 141(47.0%). The majority were taking first line regimen 270(90.0%). Detailed clinical and socio demographic characteristics are shown in Table 1.

Table 1. Socio-demographic and clinical characteristics of patients taking combination antiretro-viral therapy for at least one year, Addis Ababa, June 2018 (n=300)

Variables	Frequency (n)	%
Sex		
Male	122	40.7
Female	178	59.3
Age		
18-29	13	4.3
30-39	75	25.0
40-49	127	42.3
50-59	59	19.7
60 and above	26	8.7
BMI		
<18.5	12	4.0
18.5-24.9	130	43.3
25.0-29.9	122	40.7
30 and above	36	12.0
Staging		
I	292	97.3
II	4	1.3
III	3	1.0
IV	1	0.3
CD4 (Cells/ µL)		
<350	90	30.0
350-500	69	23.0
>500	141	47.0
Regimen type		
First line	270	90.0
Second line	30	10.0
HAART duration (Years)		
1-5	105	35.0
6-10	163	54.3
>10	32	10.7
Opportunistic infection		
Yes	132	44.0
No	168	56.0

6.2. TSH, T3, T4 profile of patients taking HAART for at least one year

Of the 300 individuals, 70 (23.3%) were identified with abnormal thyroid function, of whom, 47 (15.7%) were female and 23 (7.7%) male. Fourteen (4.7%) were diagnosed with hyperthyroidism as defined by decreased TSH below normal and 56(18.7%) with hypothyroidism as defined by TSH above normal. Of them, 35(62.5%) were having subclinical hypothyroidism, while 21 (37.5 %) had overt hypothyroidism, as defined by elevated TSH and decreased serum levels of T_4 or T_3. The levels of TSH, T3, T4, 230 (76.7), 235 (78.3), 272 (90.7) for the majority of the participants were in the normal range, respectively Figure 2.

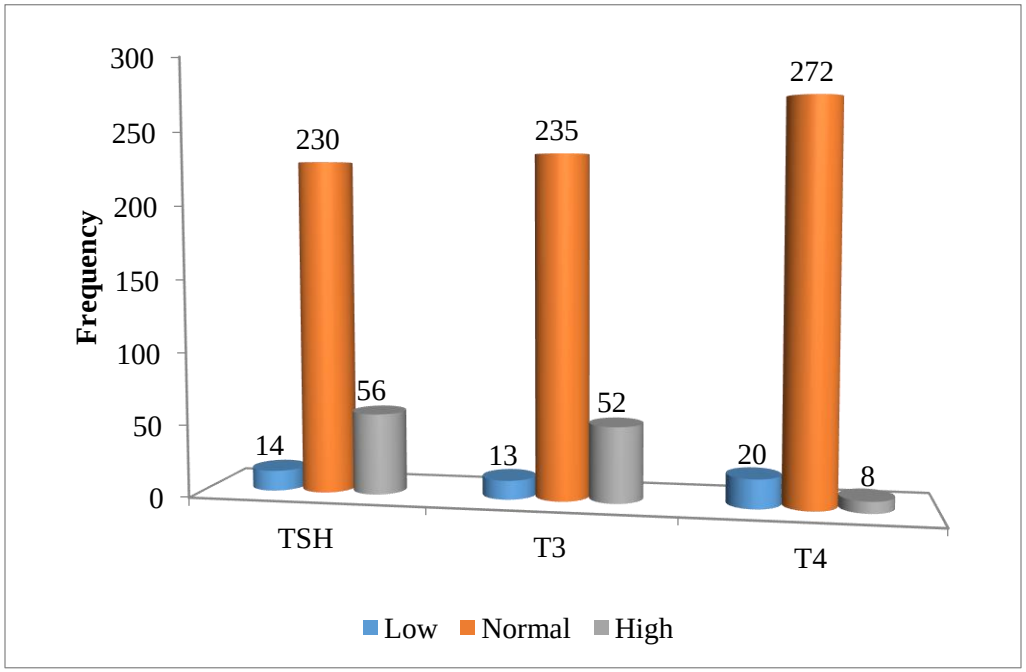

Figure 2. TSH, T3, T4 profile of patients taking HAART for at least 1 year, Addis Ababa, June 2018 (n=300)

6.3. Analysis of Thyroid hormone profile abnormality by CD4 levels

Among ART-receiving group, 30.0% (90/300) had CD4+ T-lymphocyte count < 350 cells/ µL and 23% (69/300) had CD4 count in the range of 350-500 cells/ µL and the remaining 47% (141/300) had CD4>500 cells/ µL.

When analyzed by CD4 categories, 30.4% (21/69) of those in the CD4 category 350-500 cells/ µL had abnormal TSH levels while for those in the CD4 category < 350 cells/ µL, 20(22.2%) had T3 abnormality and 11(12.2%) had T4 abnormality, . Among those in the highest CD4 category (>500 cells/µL) 22.0%, 22.0%, and 8.5% had abnormal TSH, T3 and T4 levels, respectively. Chi square test revealed no significant association (Table 2).

T**able 2.** Distribution of Thyroid profile abnormality by CD4 categories among patients taking HAART for at least 1 year, Addis Ababa, June 2018 (n=300) categories

Variable	**CD4<350 cells/ µL**	**350-500 cells/ µL**	**>500 cells/ µL**	**Total**	**P value***
TSH					
Abnormal	18 (20.0%)	21(30.4%)	31(22.0%)	**70 (23.3%)**	0.266
Normal	72(80.0%)	48(69.6%)	110 (78%)	230 (76.7%)	
Total	90	69	141	300	
T3					
Abnormal	20(22.2%)	14(20.3%)	31(22.0%)	**65 (21.7%)**	0.950
Normal	70(77.8)	55(79.7)	110(78.0)	235(78.3)	
Total	90	69	141	300	
T4					
Abnormal	11(12.2%)	5(7.3%)	12(8.5%)	28(9.3%)	0.508
Normal	79(87.8%)	64(92.7%)	129(91.5%)	272(90.7)	
Total	90	69	141	300	

*chi square test for association between CD4 categories and abnormal TSH, T3, T4 levels

As shown in the whisker plot below (Figure 3), the median TSH level does not significantly vary between the three CD4 categories (P>0.05). However, when data analysis was restricted to those with abnormal TSH values only (n=70), those in the CD4 category of >500 cells/ µL had significantly lower median TSH levels compared to those with CD4 count 350-500 cell/ µL (P=0.04) and those with CD4 count<350 cells/ µL (P=0.03) (Figure 4).

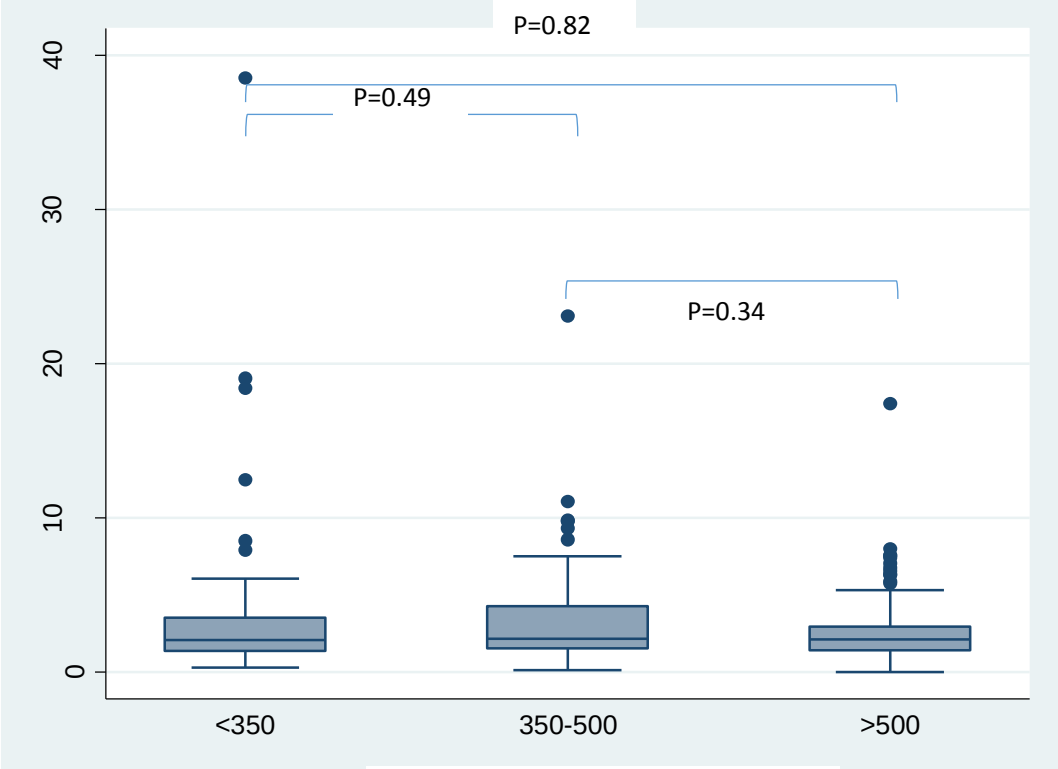

Figure 3. Thyroid stimulating hormone levels by CD4 categories of patients taking HAART for at least 1 year, Addis Ababa, June 2018 (n=300)

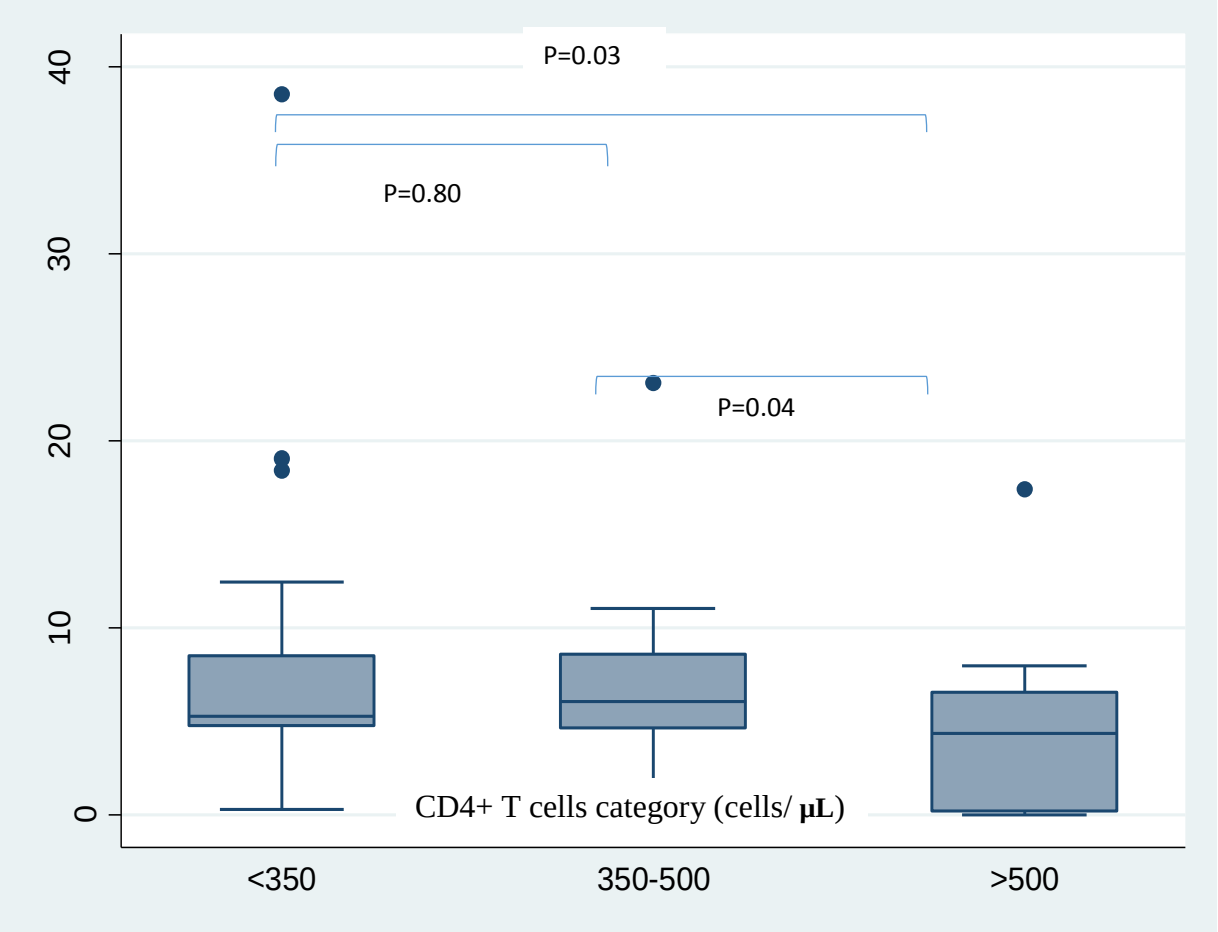

Figure 4. Abnormal TSH hormone Levels profile by CD4 categories of patients taking HAART for at least 1 year, Addis Ababa, June 2018 (n=70)

Correlation analysis between TSH levels and CD4 was carried out. There is a slight but statistically significant (r=-0.1224, p=0.034) negative correlation between the two parameters as shown in Figure 5.

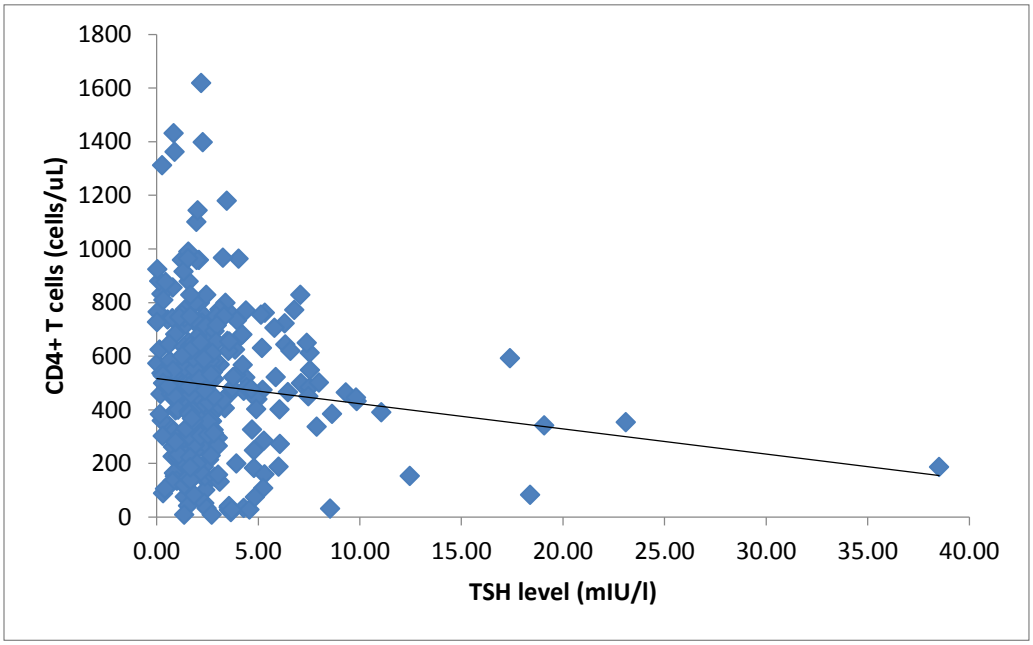

Figure 5. Correlation of TSH hormone level with CD4 T cells of patients taking HAART for at least 1 year, Addis Ababa, June 2018 (n=300)

6.4. Analysis of Thyroid hormone profile abnormality by duration of HAART

Figures 6-8 show thyroid hormone levels by duration of treatment: The TSH level was around the mean during the first five years but as the treatment progressed beyond 6 year it declines below the mean levels (Figure 6). However the mean T3 and T4 levels tend to rise without decline as the year of duration of HAART increases (Figure 7 & 8), though statistically not significant.

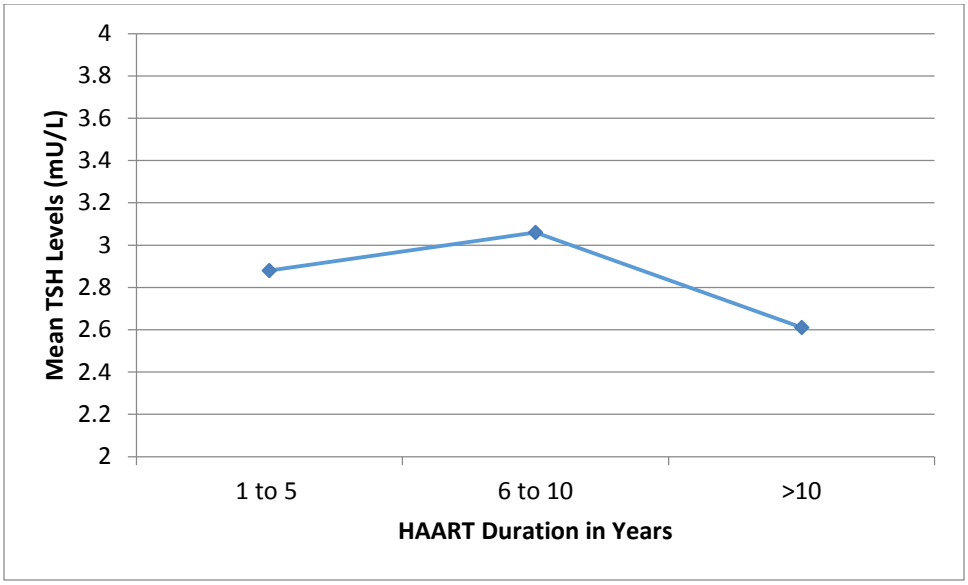

Figure 6. TSH Levels by Duration of HAART among patients taking combination antiretroviral therapy for at least one year, Addis Ababa, June 2018 (n=300)

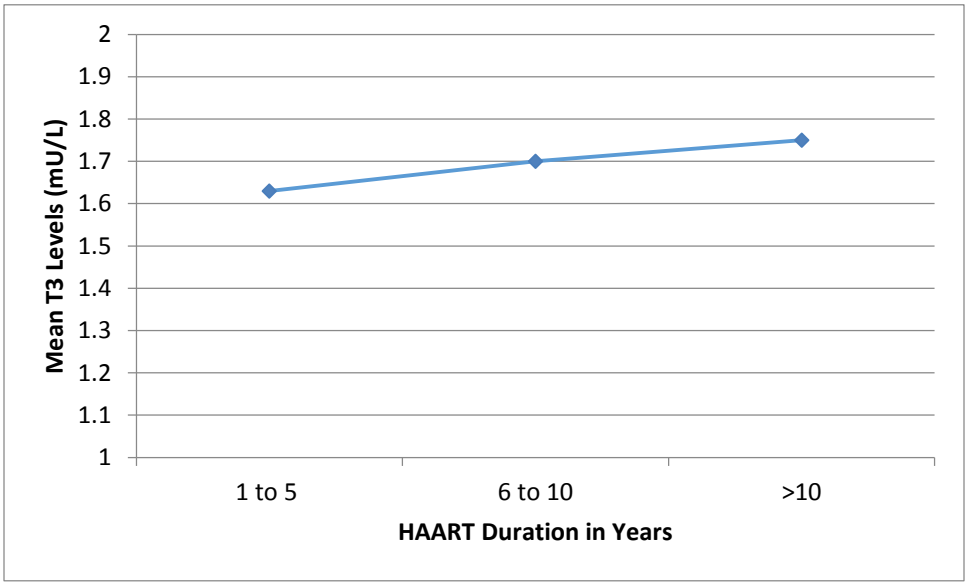

Figure 7. T3 Levels by Duration of HAART among patients taking combination antiretroviral therapy for at least one year, Addis Ababa, June 2018 (n=300)

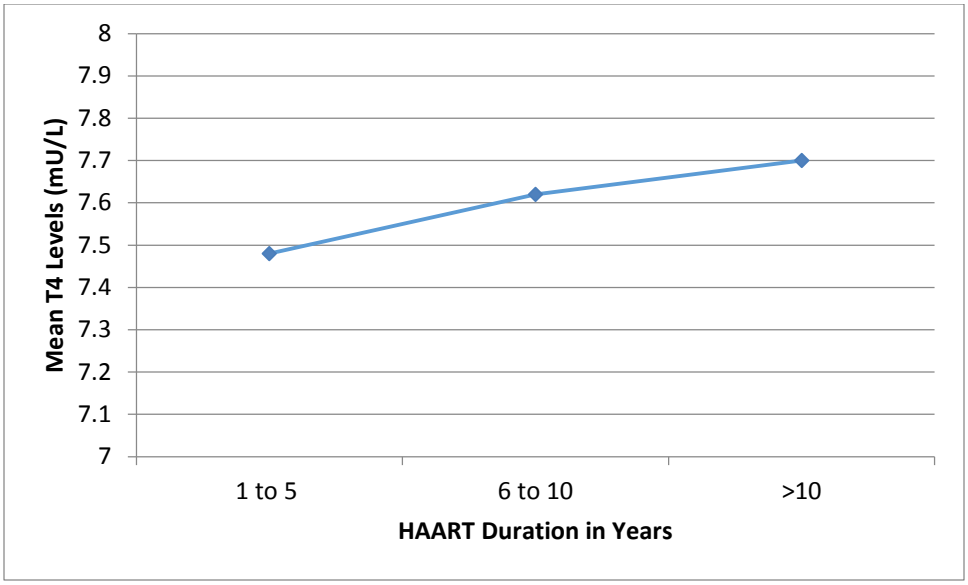

Figure 8. T4 Levels by Duration of HAART among patients taking combination antiretroviral therapy for at least one year, Addis Ababa, June 2018 (n=300)

6.5 Bivariate and Multivariate Logistic Regression Fitted for Thyroid Dysfunction.

Bivariate logistic regression analysis was carried out to determine factors associated with hypothyroidism. To control for possible confounding variables, multivariate logistic regression analysis was conducted. Potential risk factors including age, sex, opportunistic infection, regimen type, BMI, CD4 cell count, were tested but none of them showed significant association with hypothyroidism (Table 3).

Table 3. Bivariate and Multivariate analysis of factors associated with hypothyroidism among patients taking combination antiretroviral therapy for at least one year, Addis Ababa, June 2018 (n=286)

Variables	n	Hypothyroidism (n=56)	Normal TSH (n=230)	COR	P value	AOR (95%CI)	P value
Sex							
Male	122	22	99	1		1	
Female	178	34	131	1.17[0.64-2.12]	0.61	1.05[0.54-2.07]	0.88
Age							
18-29	13	2	10	1		1	
30-39	75	17	53	1.60[0.32-8.05]	0.57	1.78[0.30-10.50]	0.526
40-49	127	23	98	1.17[0.24-5.72]	0.84	1.31[0.22-7.78]	0.767
50-59	59	9	48	0.94 [0.18-5.01]	0.94	0.99[0.15-6.36]	0.99
60 and above	26	5	21	1.19 [0.20-7.23]	0.85	01.46[0.19-10.84]	0.71
BMI							
<18.5	130	2	9	1		1	
18.5-24.9	130	25	98	1.15 [0.23-5.65]	0.87	1.66[0.28-9.88]	0.58
25.0-29.9	122	23	96	1.08 [0.22-5.33]	0.93	1.53[0.25-9.25]	0.65
30 and above	36	6	27	1.00[0.17-5.87]	1.00	1.52[0.21-10.96]	0.68
CD4 (Cells/ µL)							
<350	90	17	72	1		1	
350-500	69	18	48	1.59 [0.75-3.39]	0.23	1.57[0.70-3.49]	0.27
>500	141	21	110	0.81 0.40-1.63	0.56	0.75[0.47-1.61]	0.47
Regimen type							
First line	270	53	206	1		1	
Second line	30	3	24	0.49 [0.14-1.67]	0.25	0.59[0.17-2.17]	0.44
HAART duration (Years)							
1-5	105	17	79	1		1	
6-10	163	35	123	1.32 [0.69-2.52]	0.40	1.48[0.75-2.94]	0.26
>10	32	4	28	0.66 [0.21-2.14]	0.49	0.57[0.16-2.04]	0.39
Opportunistic infection							
Yes	132	34	126	1		1	
No	168	22	103	0.79[0.44-1.44]	0.44	0.69[0.37-1.29]	0.25

7. Discussion

Thyroid disorder is one of the common endocrine dysfunctions observed due to alteration in the production of thyroid hormones. Thyroxine (T4) and Tri-iodothyronine (T3), altered production of these hormones often involves dysfunction of thyroid gland, pituitary gland, and hypothalamus. Hyperthyroidism (overproduction of T3 and T4) and hypothyroidism (underproduction of T3 and T4) are regarded as the most common clinical forms of thyroid disorders [5]. Highly active antiretroviral therapy (HAART) has changed the clinical evolution of HIV infection. However, its adverse effects are increasingly being recognized, particularly those concerning protease inhibitors, and some of the secondary effects are probably not known yet. Therefore, this cross-sectional study conducted to evaluate the prevalence of thyroid dysfunction in patients treated with HAART.

The overall burden of thyroid dysfunction among HIV infected patients who were on HAART for at least 1 year in this study was 23.3% which is higher than a study in France (16%), Italy (12.6%), Brazil (9.3%) and Kenya (12.0%) [10,27,22,38]. The finding is close to what has been reported from Jeddah, Kingdom of Saudi Arabia (30%) by Noureldeen *et al* [33]. The observed thyroid dysfunction rate was lower compared with the study in Nepal which reported 92.3% [2]. The reason for such variations among HIV infected patients could possibly be due to difference in the treatment guideline and regimen of HAART in the various geographic locations, recruitment setting, sample size and ethnic variation. In this study the prevalence of thyroid dysfunction was slightly higher in females than males (15.7% vs. 7.7%) which also resembles with the study by Joshi et al [2].

Subclinical hypothyroidism of thyroid dysfunction in this study was 18.7 % which is lower than studies in India (53%) [37].On the other hand in contrary to this study non statistically significant lower prevalence of Subclinical hypothyroidism was also observed in another study India (13%), UK (4%), Spain (3.5%) [34,30,6] which supports a study in France by Beltran *et al* (6.6%) and Merenich *et al*, (8%) [16, 4]. The reason for Subclinical hypothyroidism variation in HAART taking individuals in the different studies might share the factors responsible for thyroid dysfunction prevalence variation discussed above.

This study indicates that hyperthyroidism (high T3) level were also common with 52 (17.3 %) person developing the disorder; among these 30 (57.69%) were female and 22 (41.25%) were male

which was higher than the findings reported by Jaint *et al* (38.7%) [35].The finding is close to what has been reported in London [29] and India [37] where the prevalence of hyperthyroidism is 22.5%. However, the finding of this study was not lower when compared to other studies because they have done the studies in small sample size.

Our result is in agreement with the observation made by Beltran *et al*. (2003) and Quirino *et al*. (2004) [16,25]. A number of other reports show higher rate of hypothyroidism even in general female population [30,31] and data on males having higher rate of hypothyroidism in HIV-infected population is low [13].

It can be concluded from our observation that the hypothyroidic individuals that took HAART might have not more severe HIV/AIDS disease progression because this study found higher value of absolute CD4+ T-lymphocyte counts among hypothyroid HIV patients under ART. Out of 56(18.67%) hypothyroid patients 21(37.50%) have CD4+ T-lymphocyte counts > 500. In contrary study from India shows 70.7% had advanced immune deficiency with CD4 count <200 cells/μL [37]. Also Jaint *et al* in India inverse correlation of CD4 counts with serum TSH levels ($r = -0.470$ with $p < 0.050$) was reported [35]. Though weak, our study also demonstrated an inverse relation between TSH levels and CD4 count. Correlation of low levels of T4 and subclinical hypothyroidism with low CD4+ T-lymphocyte counts were also reported in Spanish [14] and French population [18]. It has also been reported that CD4+ T-lymphocyte count correlates well with T3 and T4 values, while an inverse correlation exists between CD4+ T-lymphocyte count and serum TSH levels [32, 33]. Besides ART, several other factors including opportunistic infection, local neoplasm [34], severe systemic illness, or caloric deprivation [35] can also contribute to thyroid disorders among HIV/AIDS patients.

Studies suggested that the cumulative dose of TDF/3TC/EFV was associated with an increased risk of developing subclinical hypothyroidism even though it is not significant. This cross sectional study found neither HAART regimen nor specifically TDF/3TC/EFV use was significantly associated with either overt hypothyroidism or subclinical hypothyroidism. Beltran et al. [16] and Collazos et al. [6] found a similar prevalence of hypothyroidism to that found in this work (2.6% and 3.5%, respectively). The numbers screened in this work are greater than those in previous studies. Previous work on 84 patients also found an increased prevalence of subclinical hypothyroidism, and this was in patients treated with HAART. Quirino *et al*. [25] investigated 687 patients, of

whom 7.4%.had subclinical hypothyroidism, but they found no significant relationship between the condition and drugs or CD4 cell counts.

According to this study duration of HAART was not statistically significant with thyroid hormone level (TSH, T3, T4) of HIV patients which supports the study conducted by Jaint *et al*, (2016) and Joshi et al who reported that there were no correlations between thyroid hormone levels and duration of HAART, but reported that HAART was an important factor affecting the incidence of thyroid dysfunction [2, 35].

In line with Sherma *et al [*11] in India this study did not find the effects of HAART on thyroid function. Therefore no significant differences in thyroid hormone levels were found among patients on different HAART regimens, indicating that there were no significant differences in the effects of First line or Second lined regimen on thyroid hormone levels. However a follow up experiment by Madeddu *et al*. illustrated that prolonged stavudine treatment significantly decreased T4 levels [27]. Besides Beltran *et al* [16] found on multivariate analysis that stavudine treatment and low CD4 cell counts were associated with hypothyroidism.

According to this study (97.3%) of the participants were in stage I and no incidence of thyroid dysfunction in HAART taking patients at different WHO staging. In line with this study Quirino *et al* had found no correlation between stage of infection and thyroid function abnormalities [25]. Neither TSH nor T4 levels were different among patients based on WHO staging. However Kaneria *et al* shows subclinical hypothyroidism went on increasing from stage I to stage IV [34]. We also analyzed the incidence of thyroid dysfunction among patients with OIs in comparison with duration of HAART. No significance incidence of thyroid dysfunction and different levels of thyroid hormones were found in patients with OIs in relation to their duration ($p>0.05$).

7. Strength and Limitation

Even if all laboratory analysis was done in an accredited laboratory with competent personnel, the lack of availability of thyroid auto-antibodies for all patients is the possible limitation of our study. In addition, it is still unclear whether the cause of thyroid dysfunction in HIV patients is the HIV infection itself, its complications, therapy, or progression.

8. Conclusion

Although ART treatment improves immunological status of HIV patients, this may also increase the possibility of side effects due to longer ART duration. This study shows a higher rate of hypothyroidism in individuals under ART at this specific site and also shows hyperthyroid individuals which may suggest the failures of negative feedback mechanism loop. Among the categories of hypothyroidism, subclinical hypothyroidism was the most common thyroid disorder and females were found to be more susceptible towards thyroid disorders.

9. Recommendation

From these findings patients on HAART may need regular monitoring of thyroid function tests however, larger studies are needed to examine the epidemiology and health consequences of thyroid dysfunction in HIV-infected patients and to better inform screening and treatment guidelines and also to assess the implications of these findings for practice and in particular how they relate to response to therapy.

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Annex 1: Information Sheet (English version)

Title of the Research Project: Prevalence and risk factors of thyroid dysfunction among HAART taking patients at Bethel Teaching General Hospital Addis Ababa, Ethiopia

Name of Investigator: Sibhat W/Kirkos (BSc, Msc candidate)

Name of the Organization: Addis Ababa University, College of Health Science, Department of Clinical Laboratory Science.

Introduction

You are invited to participate in a study to be conducted by MSC student at Addis Ababa University, College of health sciences, School of Allied Health Science, Department of Medical Laboratory Sciences. It is aimed at to investigate the prevalence and risk factors of thyroid dysfunction among HAART taking adult patients at Bethel Teaching General Hospital. After the result of the study is disseminated, strategies will be designed to prevent and control the predisposing factors. Moreover, it will also be a useful reference for drug choice. Please read the following statements and ask any unclear points before you agree to participate.

Participation in the study is exclusively voluntary. If you are not willing to participate in the study or if you want to withdraw even after deciding to participate, there will be no consequences and you will get all the services provided in the hospital with no problem. If you decide to participate, you have to sign the consent form and you can get a copy of this information sheet.

What is expected from you as a participant of the study?

As a participant of this study you are expected to give about 8 ml blood (one table spoon). In addition you are expected to give answers for some questions about your health and socio demographic conditions. You need to know that the results might be discussed with appropriate individuals out of this hospital. But your name, address and phone number will not be disclosed to anyone and to be more precise, identification code will be used in such conditions.

How long participation will take you?

You will spend 20-35 minutes until the specimen is collected, the questionnaire is filled and the consent is signed.

What are the risks of participating in this study?

There are no anticipated risks to your participation except minor discomfort during venipuncture because well experienced professionals will collect blood samples.

How the information is to be kept confidential?

All information that you give and the results from your specimen will be used for this study only. Only limited number of professionals will have access to the information. All the information will be encoded in a computer and will be password protected.

What are the benefits from participation?

Since this study is MSc student research, there will not be payment for participants. But your participation is important for studying the prevalence and associated factors of thyroid dysfunction that will be useful in the improvement of management of HIV positive patients.

What are your rights as a participant of this study?

You can ask any questions for further explanation. The principal investigator and the data collectors are responsible to clear any doubt that you may have during participation. You have the right to get the results of the analysis.

What can I do if I have a problem or a question?

Please forward any question or problems you may encounter during this study to

Sibhat W/kirkos

Department of medical laboratory science

School of Allied health sciences

College of health sciences

Addis Ababa University

Mob: +251-911 41 38 52

Email:sibhatme@gmail.com

Agree to participate?

□ Yes

□ No

Annex 2: Information sheet (Amharic version)

አዲስ አበባ ዩኒቨርሲቲ,የጤና ሣይንስ ኮሌጅ,የአላይድ ጤና ሣይንስ ት/ቤት,የሕክምና ላቦራቶሪ ሣይንስ ክፍል እድሜያቸው ከአስራ ስምንት አመት በላይ ከሆኑ አዋቂዎች ላይ የደም ናሙና ተወስዶ ለሚሰራው ከ ኤች አይ ቪ መድሃኒቶች ጋር ተያይዞ ለሚመጣው የእንቅርት በሽታና የ ሲዲ ፎር ምርመራ በኤችአይቪ ህሙማን ላይ ያሉትን ሁኔታዎች ለማጥናት ታስቦ ለተሳታፊዎች የተዘጋጀ መረጃ ሲሆን እርሶም በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል የማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል፡፡ እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነዉን/ኑትን ማንኛዉም ሃሳብ ይጠይቁ፡፡

መግቢያ

የጥናቱ ርዕስ፡- ‹‹ የኤች አይ ቪ መድሃኒቶች በእንቅርት ላይ በተጓዳኝ የሚያመጡትን ህመም እንዲሁም በሲዲ ፎር ላይ ያለውን ሁኔታ ለማጥናትና ለማየት››

እርስዎ በዚህ ጥናት ላይ የሚኖሮት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኋላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋ በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት አይቋረጥም፡፡በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል፡፡ከፈለጉ ይህንን መረጃ አንድ ቅጅ ለራስዎ ሊያስቀሩ ይችላሉ፡፡

የጥናቱ ተሳታፊ በመሆኖ የሚጠበቅቦት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ 8 ሚ.ሊ በግምት አንድ የሾርባ ማንኪያ የደም ናሙና ለመስጠት መስማማት ይጠበቅብዎታል፡፡ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤ አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎቸን አይጨምርም፡፡ ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉልና ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡በተጨማሪም ስለርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይጠበቅብዎታል፡፡

በዚህ ጥናት መሳተፍ ምን ያህል ጊዜ ይፈጃል?

የተዘጋጀዉን መጠይቅ ለመሙላት፤የስምምነት ቅጹ ላይ ለመፈረምና ናሙና ለመስጠት ከ20-25 ደቂቃ ያስፈልጋል፡፡

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ቸግሮቸ ምንድን ናቸዉ?

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም ምክንያቱም ናሙናው የሚወሰደው Gምድ ባላቸው የጤና ባለሙያዎች በመሆኑ ነው፡፡

የእኔ የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

የሰጡት ማንኛውም መረጃና ከተወሰደው ናሙና ላይ የተገኘው የላቦራቶሪ ውጤት የሚውለው ለጥናቱ አላማ ብቻ ነው፡፡ ይህንን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰራተኞች ብቻ ናቸው፡፡ ከዚህም በላይ ስለእርሶ ያለውን ማንኛውም መረጃ የተለየ የይለፍ ቃል ባላው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረገል፡፡

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው ?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ ፅሁፍ እንደመሆኑ መጠን ለተሳታፊዎች ገንዘብ አይሰጥም፡፡ ሆኖም ከጥናቱ የሚገኘው መረጃ የኤች አይ ቪ ህሙማንን ህክምና ለማሻሻል አስተዋፅዎ ያደርጋል፡፡ በተጨማሪም ትኩረት ያልተሰጣቸውን ኢንፌክሽየስ በሽታዎችን ለማህበረሰቡ ማስገንዘብ፡፡

የዚህ ጥናት ተሳታፊ መብቱ ምንድን ነው ?

ከዚህም በተጨማሪ ጥናቱ በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለዎት፡፡የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ፡፡

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ፡፡

ስብሐት ወ/ቂርቆስ

የህክምና ላባራቶሪ ሳይንስ ት/ክፍል

የአላይድ ጤና ሳይንስ ት/ቤት

የጤና ሳይንስ ኮሌጅ

አዲስ አበባ ዩኒቨርሲቲ

ሞባይል +251- 911 41 38 52

ኢሜይል sibhatme@gmail.com

ለመሳተፍ ይስማማሉ?

እስማማለሁ □ አልስማማም □

Annex 3: Consent Form (English version)

Code number----------

Name of the participant----------

I have been informed about the study which is aimed on the Prevalence and risk factors of thyroid dysfunction among HAART taking patients at Bethel Teaching General Hospital Addis Ababa, Ethiopia. For this study blood sample is required from a participant. The aims of the study and possible risks were explained to me as well.

I am also informed that all the information contained within the questionnaire is to be kept confidential. Moreover I have been well informed of my right to keep hold of information, decline to cooperate and make withdrawal from the study.

It is therefore with full understanding of the situation that I gave the informed consent voluntarily to the researcher to use my blood sample for the investigation. In addition, I have had the opportunity to ask questions about it and received clarification to my satisfaction. I have also been informed that the benefit of participation is to get the results of analysis from my sample measured for free via the counselor nurse.

Participant's signature /finger print ----------

Name of Data collectors ---------- signature---------- Date----------

Please direct any questions or problems you may encounter during this study to:

Sibhat W/Kirkos

Department of medical laboratory science

School of Allied health science

College of health sciences

Addis Ababa University

Mob: +251-911- 41 38 52

Email: sibhatme@gmail.com

For additional information, please contact Addis Ababa University, College of Health Science institutional review board (IRB) office at:

Tell. +251-11-8-96-13-96

Fax +251-11-5-51-1-51-30-99

P.O. Box 9086, Addis Ababa, Ethiopia

Email: aau.mf.irb@yahoo.com

Annex 4: Consent Form (Amharic version)

የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ በዓዋቂነት የዕድሜ ክልል በሚገኙ የኤድስ ቫይረስ ተጠቂ ግለሰቦች ላይ ለሚሰራው የደም ናሙና ተወስዶ ከ ኤች አይ ቪ መድሃኒቶች ጋር ተያይዞ ለሚመጣው የእንቅርት በሽታና የ ሲዲ ፎር ምርመራ ያሉትን ሁኔታዎች ለማጥናት ታስቦ በቂ ገለጻ ተደርጎልኛል፡፡ ለጥናቱም ከእኔ የተወሰደ የደም ናሙና እንደሚያስፈልግ ተገልጾልኛል፡፡ የጥናቱንም አላማዎች በሚገባ ተረድቻለሁ፡፡

በመጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል፡፡በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል፡ ፡

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡፡ የምሰጠውም ናሙና ለምርምር ብቻ እንደሚውልም ተረድቻለው፡፡ በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ፡፡ የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ፡፡

የተሳታፊዉ ፊርማ /የጣት አሻራ ----------

የምስክር ሙሉ ስም ፊርማ

1. ---------- ----------
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(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የመረጃ ሰብሳቢው ስም ---------- ፊርማ ---------- ቀን----------

ጥናቱን የሚያካሂደዉ ሰዉ ማረጋገጫ

ይህን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካሎዎት በሚከተለዉ አድራሻ ይጠቁሙን፡፡

ስብሐት ወ/ቂርቆስ

የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል

የአላይድ ጤና ሳይንስ ት/ቤት

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SOPs

SOP for Thyroid Stimulating Hormone (TSH)
PURPOSE

For the determination of TSH concentration which is a glycoprotein hormone synthesized and secreted by thyrotrope cells in the anterior pituitary gland, which regulates the endocrine function of the thyroid gland.

PRINCIPLE: An anti-TSH coating antibody is adsorbed onto a microtiter plate. TSH protein present in the sample or standard binds to the antibodies adsorbed on the plate; a biotinylated anti-TSH antibody is added and binds to the antigen captured by the first antibody. Following incubation and wash steps, a streptavidin-enzyme conjugate is added and binds to the biotinylated anti-TSH antibody. Unbound streptavidin-enzyme conjugate is removed during a wash step, and substrate solution is added to the wells. A colored product is formed in proportion to the amount of TSH present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from purified TSH and sample concentration is then determined.

Analytical detection limit

Defined as the smallest concentration of TSH which is significantly different from the zero concentration with a probability of 95% 0.05 60µlU/ml

SAMPLE: The specimens shall be blood, serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells. Samples may be refrigerated at 2-8°C for a maximum period of five days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20 °C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0. 05ml of the specimen is required for TSH.

Materials

1. TSH Sample: serum, plasma, cell or tissue lysate
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips

4. Multichannel micropipette reservoir

5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

6. Centrifuge

Reagents

1. Anti-TSH Antibody Coated Plate One strip well 96-well plate.

2. Biotinylated Anti-TSH Antibody (1000X): One 20 µL vial.

3. Streptavidin-Enzyme Conjugate: One 20 µL vial.

4. Assay Diluent: One 50 mL bottle.

5. 10X Wash Buffer: One 100 mL bottle.

6. Substrate Solution: One 12 mL amber bottle.

7. Stop Solution : One 12 mL bottle.

CALIBRATION: TSH calibrator

PROCEDURE:

1. Prepare and mix all reagents thoroughly before use.

2. Add 100 µL of TSH sample or standard to the Anti-TSH Antibody Coated Plate. Each TSH sample, standard, blank, and control should be assayed in duplicate.

3. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.

4. Remove plate cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.

5. Add 100 µL of the diluted Biotinylated Anti-TSH Antibody to each well.

6. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.

7. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 4 above.

8. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well.

9. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.

10. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 4 above. Proceed immediately to the next step.

11. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 minutes.

Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

12. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).

13. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

INTERFERENCES. Gross haemolysis, lipemic and icterus specimen.

BIOLOGICAL REFERENCE INTERVALS

Analyte	**Reference Range**		**Units**
TSH	**0.3 – 4.0**		**µUI/ml**

Clinical Utility: TSH stimulates the thyroid gland to secrete the hormone thyroxine (T4), which has only a slight effect on metabolism. T4 is converted to triiodothyronine (T3), which is the active hormone that stimulates metabolism. About 80% of this conversion is in the liver and other organs, and 20% in the thyroid itself.

The hypothalamus, in the base of the brain, produces thyrotropin-releasing hormone (TRH). TRH stimulates the pituitary gland to produce TSH.

Somatostatin is also prooduced by the hypothalamus, and has an opposite effect on the pituitary production of TSH, decreasing or inhibiting its release.

The concentration of thyroid hormones (T3 and T4) in the blood regulates the pituitary release of TSH; when T3 and T4 concentrations are low, the production of TSH is increased, and, conversely, when T3 and T4 concentrations are high, TSH production

SAFETY PRECAUTION

•Use Universal safety precaution (wearing gloves lab coat and washing hands) when handling infectious materials

•Refer to the National Health and Safety Guidelines for standard safety procedure.

SOPS of Triiodothyronine (T3) and thyroxine (T4)

Purpose

The thyroid gland controls how quickly the body uses energy, makes proteins, and controls how sensitive the body is to other hormones. It participates in these processes by producing thyroid hormones, the principal ones being triiodothyronine (T3) and thyroxine which can sometimes be referred to as tetraiodothyronine (T4). These hormones regulate the rate of metabolism and affect the growth and rate of function of many other systems in the body. T3 and T4 are synthesized from both iodine and tyrosine. The thyroid also produces calcitonin, which plays a role in calcium homeostasis.

Abbreviations

T3 = triiodothyronine

STAT = Short Turnaround Time

SST = Serum separator tube

T4 = thyroxine or tetraiodothyronine

Materials

1. Pipette capable of delivering 25 and 50µl volumes with a precision of better than 1. 5%.

2. Dispenser(s) for repetitive deliveries of 0. 100ml and 0. 300ml volumes with a precision of better than 1. 5%.

3. Adjustable volume (20-200µl) and (200-1000µl) dispenser(s) for conjugate and substrate dilutions.

4. Microplate washer or a squeeze bottle (optional).

5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.

6. Test tubes for dilution of enzyme conjugate and substrate A and B.

7. Absorbent Paper for blotting the microplate wells.

8. Plastic wrap or microplate cover for incubation steps.

9. Vacuum aspirator (optional) for wash steps.

10. Timer.

11. Quality Control Materials.

Sample

The specimens shall be blood, serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells. Samples may be refrigerated at 2-8°C for a maximum period of five days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20 °C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0. 05ml of the specimen is required for tT4 and 0. 10ml for tT3.

Limitations: Gross hemolysis, lipemic and icterus specimen.

Safety Precaution

Use Universal safety precaution (wearing gloves lab coat and washing hands) when handling infectious materials

Refer to the National Health and Safety Guidelines for standard safety procedures

Kit contents

1. Anti-T4 and T3 Antibody Coated Plate One strip well 96-well plate for each test.

2. Biotinylated Anti- T4 and T3 Antibody (1000X)

3. Streptavidin-Enzyme Conjugate One 20 μL vial for each test.

4. Assay Diluent One 50 mL bottle for each test.

5. 10X Wash Buffer One 100 mL bottle for each test.

6. Substrate Solution One 12 mL amber bottle for each test.

7. Stop Solution (Part. No. 310808): One 12 mL bottle.

PROCEDURE:

1. Format the microplates' wells for each serum calibrator, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.

2. Pipette 100µl of the appropriate serum reference, control or specimen into the assigned well for tT4. Pipette 100µl for tT3.

3. Add 100µl of Working Reagent A, tT4 or tT3 -enzyme conjugate solution to the appropriate wells.

4. Swirl the microplate gently for 20-30 seconds to mix and cover.

5. Add 100µl of biotinylated tT4 or (tT3) -antibody conjugate solution to the appropriate wells.

6. Swirl the microplate gently for 20-30 seconds to mix and cover.

7. Incubate 60 minutes at room temperature.

8. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

9. Add 300µl of wash buffer, decant (tap and blot) or aspirate. Repeat two additional times for a total of three washes. An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and Repeat two additional times.

10. Add 0. 100 ml (100µl) of working substrate solution to all wells. Always add reagents in the same order to minimize reaction time differences between wells. DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION.

11. Incubate at room temperature for 15 minutes.

12. Add 100µl of stop solution to each well and gently mix for 15-20 seconds. Always add reagents in the same order to minimize reaction time differences between wells.

13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within 30 minutes of adding the stop solution. Note: For reassaying specimens with concentrations greater than highest calibrator, dilute 12. 5µl (tT4) or 25µl tT3 of the specimen and 12. 5µl (tT4) or 25µl tT3 of the 0 serum reference into the sample well (this maintains a uniform protein concentration). Multiply the read out value by 2 to obtain the thyroxine concentration.

Result Interpretation

Analyte	**Reference Range**		**Units**
T3	**0.69 - 2.02**		**µg/dl**
T4	4.4 – 10.8 (M) 4.8 – 11.6 (F)		

Limitations : Gross haemolysis, lipemic and icterus specimen

Principle

The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate, native antigen and a substrate that emits light. Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. 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, measured by reaction with luminol, is directly proportional to the native 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.

Clinical Utility: Thyroid disorders include hyperthyroidism (abnormally increased activity), hypothyroidism (abnormally decreased activity) and thyroid nodules, which are generally benign thyroid neoplasms, but may be thyroid cancers. All these disorders may give rise to goiter, that is, an enlarged thyroid.

Hyperthyroidism

Hyperthyroidism, or overactive thyroid, is the overproduction of the thyroid hormones T3 and T4, and is most commonly caused by the development of Graves' disease, an autoimmune disease in which antibodies are produced which stimulate the thyroid to secrete excessive quantities of thyroid hormones. The disease can result in the formation of a toxic goiter as a result of thyroid growth in response to a lack of negative feedback mechanisms. It presents with symptoms such as a thyroid goiter, protruding eyes (exopthalmos), palpitations, excess sweating, diarrhea, weight loss, muscle weakness and unusual sensitivity to heat. The appetite is often increased.

Hypothyroidism

Hypothyroidism is the underproduction of the thyroid hormones T3 and T4. Hypothyroid disorders may occur as a result of congenital thyroid abnormalities (see congenital hypothyroidism), autoimmune disorders such as Hashimoto's thyroiditis, iodine deficiency (more likely in poorer countries) or the removal of the thyroid following surgery to treat severe hyperthyroidism and/or thyroid cancer. Typical symptoms are abnormal weight gain, tiredness, baldness, cold intolerance, and bradycardia.

Negative feedback mechanisms result in growth of the thyroid gland when thyroid hormones are being produced in sufficiently low quantities as a means of increasing the thyroid output; however, where the hypothyroidism is caused by iodine insufficiency, the thyroid is unable to produce T3 and T4 and as a result, the thyroid may continue to grow to form a non-toxic goiter. It is termed non-toxic as it does not produce toxic quantities of thyroid hormones, despite its size.[40]

Enumeration of CD4+ lymphocytes (FACS Count flowcytometr)

Purpose

The test is mainly used for baseline assessment and monitoring response to treatment.

Abbreviations

CD4: cluster of differentiation

Materials Reagents

- BD FACScount reagent kit
- BD FACScount control kit
- BD multicheck control
- BD FACScount sheath fluid
- BD FACScount rinse
- BD FACScount clean

Reagents preparation: N/A

Reagents stability and storage:

1. Stock reagent, stored at 2-8°C, until expiry date.

Supplies

Reagent bottle

Yellow tips and Blue tips

Micropipettes

Equipment

- - BD FACS count instrument
- Automatic electronic pipette and tips
- Vortex mixer
- Coring station

• Cleaning tubes

• FACS count workstation

• Disposable clothing

• Biohazard waste container or bag

• Safety Cabinet class II (optional)

Sample

Sample type	whole blood
Amount required	4mL
Transport	Transport whole blood at RT
Storage & Stability	no later than 72 hours after the blood specimen Keep at room temperature

Limitations: Gross hemolysis, lipemic and icterus specimen.

Sample retention: Specimens are discarded in accordance with Specimen retention policy. This refers to both Specimens in the Primary and Secondary Containers.

Safety Precautions: Use Universal safety precaution (wearing gloves, lab coat and washing hands) when handling infectious materials

Refer to the National Health and Safety Guidelines for standard safety procedure

Quality Control

Control	Level	Stability	Frequency	Preparation (y/n)
High	1	: Until Expiry Date	Once per a testing date	no
Medium Low	1			

Note:

• Commercially available BD multicheck controls or fresh peripheral blood from blood donors must be run every morning to verify both the reagents and methodology.

• Control specimens must be tested in the same manner as patient samples.

• Control samples that fall out of range need to be investigated, and patient results from the same test run are suspect until the reason for the control sample failure is resolved.

• All control data must be documented and the results verified for acceptability before reporting results.

Procedure

Refer to how to order tests of FACS count

1. Label the tab of one reagent tube pair with patient laboratory number. Vortex the reagent tube pair upside down for 5 seconds, then upright for 5 seconds.

2. Open the reagent tube pairs with the coring station.

3. Transfer the reagent tube pair from the coring station to the workstation, keeping the tubes upright.

4. Close the workstation cover to protect the reagents from light.

5. Mix the whole blood by inverting the BD Vacutianer tubes five times.

6. Pipette 50 µL of blood into each of the four reagent tubes. Change the tips between each tube.

7. Cap the reagent tube pairs and vortex upright for 5 seconds.

8. Replace the reagent tube pairs in the FACScount workstation, close the cover to protect reagent from light, and incubate for 60–120 minutes at room temperature (20–25ºC).

9. After the incubation step is complete, uncap the tubes and pipette 50 µL of fixative solution into each reagent tube. Change tips between tubes.

10. Seal the reagent tube pair with new caps and vortex upright for 5 seconds. (Fixed samples can be held up to 12 hours before adding the control beads.)

11. Run the tubes on the FACSc ount instrument within 2 hours of adding control beads to the reagent tubes.

12. Store samples at room temperature in the workstation until they are run on the instrument. Vortex upright for 5 seconds immediately before running and run on the BD FACScount instrument following the instructions in the user's manual.

Expected Values	

Analyte	**Reference Range**		**Units**
CD4	500	1,500	cells/ **µL** of blood

Principle:A single test requires one convenient, ready-to-use reagent tube pair. When whole blood is added to the reagents, fluorochrome-labelled antibodies in the reagents bind specifically to lymphocyte surface antigens. After a fixative solution is added to the reagent tubes, the sample is run on the instrument. Here, the cells come in contact with the laser light, which causes the fluorochrome labelled cells to fluoresce. This fluorescent light provides the information necessary for the instrument to count cells. In addition to containing the antibody reagent, the tubes also contain a known number of fluorochrome-integrated reference beads. These beads function as a fluorescence standard for locating the lymphocytes and also as a quantification standard for enumerating the cells.

Clinical Utility: The lower the CD4 count, the more the disease has progressed. Treatment with ARVs will be initiated when counts are below 500 cells/ **µL**.

- AIDS is diagnosed when CD4 cell counts are below 200 cells/ **µL**. [41]

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Sibhat W/Kirkos (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with my approval as advisors.

Advisor: Aster Tsegaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Mistre Woldie (MSc,PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia