Assessment of Thyroid Profile (TSH, T3 and T4) of HIV Positive Subjects Visiting Central Hospital, Benin City, Edo State

Abstract

Human Immunodeficiency Virus (HIV) infection is caused by human immunodeficiency virus, a lentivirus within the family. Thyroid hormones regulate the basal metabolic rate of hepatocytes, and thereby modulate hepatic function; low total and free T3 levels may be regarded as an adaptive hypothyroid state that serves to reduce the basal metabolic rate within hepatocytes and preserve liver function and total body protein stores. The aim of this study is to evaluate the thyroid profile (TSH, T3 and T4) of HIV positive subjects visiting Central Hospital in Benin City, Edo State. The subjects in this study comprise of HIV positive volunteers aged between 18 to 50 years attending Central Hospital Benin City, Edo State, Nigeria. A total of one hundred and twenty (120) subjects were recruited for this study. The study comprises of eighty (80) HIV positive subjects (test samples) and forty (40) apparently healthy subjects (controls). This study was designed as a prospective and cross-sectional study to evaluate the thyroid profile (TSH, T3 and T4) of HIV positive subjects visiting Central Hospital in Benin city, Edo State, Nigeria. HIV serostatus was determined according to centre for disease and prevention (CDC – UMD). Plasma total TSH, T_4 & T_3 was quantitatively determined using enzyme immunoassay. The result showed that TSH levels were significantly higher ($p<0.05$) in subjects (2.75±1.59 miu/ml) when compared with the control (1.92±1.11 miu/ml). On the contrary, T_3 levels were not significantly lower ($p>0.05$) in subjects (1.32±0.63 ng/ml) when compared with the control (1.49±0.35 ng/ml). T_4 levels were significantly lower ($p<0.05$) in subjects (6.06±1.83 µg/dl) when compared with the control (7.09±1.78 µg/dl). The results showed a significant non-increased in TSH ($p>0.05$) in subjects who had been on drugs within 11-15years, when compared with subjects who have been on drugs for a period of 0-5 months, 1-5 years, 6-10 years and 16-20 years. T_3 levels were higher ($p>0.05$) in subjects who had been on drugs within 16-20 years, when compared with subjects who have been on drugs for a period of 0-5 months, 1-5 years, 6-10 years and 11-15 years. T_4 levels were higher ($p>0.05$) in subjects who had been on drugs within 16-20 years, when compared with subjects who have been on drugs for a period of 0-5 months, 1-5 years, 6-10 years and 11-15 years. In conclusion, this study highlights that TSH levels were significantly higher ($p<0.05$) in subjects when compared with the control, and T_4 levels were significantly lower ($p<0.05$) in subjects when compared with the control. Larger studies are needed to examine the epidemiology and health consequences of mild thyroid dysfunction in HIV-infected subjects and to better inform screening and treatment guidelines.

Abstract:

This study evaluated the thyroid profile (TSH, T3, and T4) of 120 subjects, including 80 HIV positive individuals and 40 healthy controls, visiting Central Hospital in Benin City, Edo State, Nigeria. Conducted as a prospective, cross-sectional study, HIV serostatus was confirmed per CDC guidelines, and plasma levels of TSH, T4, and T3 were measured using enzyme immunoassay. Results showed significantly higher TSH levels (2.75±1.59 miu/ml) and significantly lower T4 levels (6.06±1.83 µg/dl) in HIV positive subjects compared to controls (1.92±1.11 miu/ml and 7.09±1.78 µg/dl, respectively), with T3 levels showing no

significant difference. Additionally, subjects on antiretroviral drugs for 11-15 years exhibited a non-significant increase in TSH, while T3 and T4 levels were higher in those on drugs for 16-20 years compared to other durations. The findings suggest altered thyroid function in HIV positive individuals, emphasizing the need for larger studies to understand the epidemiology and health impacts of mild thyroid dysfunction in this population, and to inform appropriate screening and treatment guidelines.

Keywords: Thyroid Profile, TSH, T3, T4, HIV

1. INTRODUCTION

Human Immunodeficiency Virus (HIV) infection is caused by human immunodeficiency virus, a lentivirus within the family Retroviridae (Prescott, Harley, & Klein, 2019). It causes progressive impairment of the body's cellular immune system leading to increased susceptibility to infection and tumors and the fatal condition Acquired Immune Deficiency syndrome, (AIDS) [1]. Acquired Immunodeficiency Syndrome (AIDS) was recognized as an emerging disease only in early 1980, but has rapidly established itself throughout the world, and likely to endure and persist well in 21st century. AIDS has evolved from a mysterious illness to a global pandemic which has infected tens of millions in less than 20 years period [2]. Total number of HIV-AIDS in world is continuously rising. According to UNAIDS 2018 data 37.9 million people (out of these, 2.1 million are Children under 15 years) are living with HIV/AIDS worldwide, the vast majority of whom are in low- and middle-income countries, out of these only 24.5 million people were assessing antiretroviral therapy [3]. India has the third largest HIV epidemic in the world, 2.1 million people living with HIV in 2015. In India, HIV epidemic shifts from high-risk group to bridge population and then to general population. In 2017, 79% of people lining with HIV were aware of their status of whom only 56% were on ART [3]. Increasing experience with this syndrome has led to the recognition of a variety of HIV related endocrine disorder that occurs during both the early and late stages of the disease. Among these disorders a high prevalence of abnormalities in thyroid function tests is reported in previous cross-sectional studies. Unique abnormalities of thyroid function tests were reported by Lambert et al, [4]. They described a progressive elevation in serum thyroxin binding globulin (sr.TBG) but not in other binding proteins such as cortisol binding globulin (CBG) that accompanies a decline in CD4+ count with advancing HIV infection. Feldt-Rasmussen et al, [5] reported elevation of sr. TSH and s. TBG concentration in conjunction with low FT-4 that occurs frequently and correlates with CD4+ cell depletion in AIDS patients. In addition, this thyroid dysfunction correlated with the degree of immunosuppression and viral replication and preceded the worsening of the disease. Subtle alterations in thyroid function tests (TFT) are more common in HIV infection and are sometimes already detectable in the early phase of disease. The changes in thyroid function tests are HIV specific and are consistent with an abnormal response to acute illness. Various mechanisms have been proposed to explain such abnormalities in TFT [6]. These include direct infection of thyroid gland by opportunistic organisms such as Pneumocystis carinii, infiltration of the gland by tumors such as Kaposi sarcoma, effect of humoral factors such as IL-1α and TNF-α, side effect of the drugs used in the course of HIV infection for e.g. rifampicin, ketoconazole, steroids etc. and direct infection of gland by HIV.

There are three groups of enzymes that regulate thyroid hormone metabolism, forming part of the iodothyronine seleno-deiodinase enzyme system, Type 1 = D1 (deiodinase 1), Type 2 = D2 (deiodinase 2) and Type 3 = D3 (deiodinase 3). They are responsible for the activation of T4 to T3, inactivation of T4 to rT3 and the conversion of rT3 and T3 to T2.3 The type 1 deiodinase is mainly found in the liver and kidney and accounts for approximately 30–40% of extra thyroidal production of T3 (12 nmol). Type 3 deiodinase system primarily exhibits inner-ring deiodination. It is found in the liver, skin and CNS, where it catalyses the conversion of T4 to rT3 and T3 to T2, both inactive metabolites; it also converts rT3 torT2.The patients

with cirrhosis have low total and fT3 and an elevated rT3, probably reflecting a reduced deiodinase type 1 activity, resulting in reduced conversion of T4 to T3. This results in an increase in conversion of T4 to rT3 by the deiodinase type 3 systems, and an increase in the rT3 to T3 ratio [7]. The level of thyroid hormones is also important for normal hepatic function and bilirubin metabolism. Thyroid hormones regulate the basal metabolic rate of hepatocytes, and thereby modulate hepatic function; low total and free T3 levels may be regarded as an adaptive hypothyroid state that serves to reduce the basal metabolic rate within hepatocytes and preserve liver function and total body protein stores [8]. In experimental animal, thyrotoxicosis is associated with an increase bilirubin output in bile, which may result from increase degradation of hepatic heme [9]. Thyroid induced alterations in hepatic metabolism of bilirubin, specifically a decrease in glucronyltransferase, may be responsible for clinical occurrence of unconjugated hyperbilirubinemia, possibly by masking previously unorganized Gilbert's syndrome. Thyroid hormone decreases bile acid production and total bile acid pool size [10]. Liver on the other hand metabolizes the thyroid hormones and regulates their systemic endocrine effects. Conceivably, the disorders of these two organs would interact or influence each other. The derangements in thyroid profile in liver patients have been well documented in English literature. The plasma T3**:**rT3 ratio has a negative correlation with the severity of cirrhosis when assessed in non-alcoholic cirrhosis. In patients with acute hepatitis of mild or moderate severity, there is elevated serum levels of total T4, due to increased thyroid-binding globulin, which is synthesized as an acute-phase reactant, but normal levels of free T4. In cirrhosis total and fT3 are found to be low, probably reflecting a reduced deiodinase type 1 activity, resulting in reduced conversion of T4 to T3 [10].

The human immunodeficiency virus (HIV) has thus far infected over 22.4 million people in sub-Saharan Africa and Nigeria remains the country with the third highest number of HIV-infected subjects in the world [11]. Since HAART was introduced in the mid-1990s, as a treatment for HIV/AIDS, the morbidity and mortality associated with HIV/AIDS has reduced considerably. According to the 2015 HIV Sentinel Survey and National HIV Prevalence AIDS Estimates Reports, an estimated 645,810 HIV-infected Nigerians would have been requiring ART by 2010 [12]. HAART regimens typically include a combination of at least three drugs, such as different association of protease inhibitors (PI), non-nucleoside reverse transcriptase inhibitors (NNRTI) and nucleoside reverse transcriptase inhibitors (NRTI). HAART however, has been reported to be associated with a number of side effects in HIV/AIDS subjects among which dyslipidaemia and lipodystrophy are common metabolic disorders with increased risk of cardiovascular diseases and diabetes in the HIV-infected subjects [13-16]. Studies have also shown that HAART treatment, especially those including protease inhibitors, is associated with hypertriglyceridaemia, hypercholesterolaemia, hypo HDL-cholesterolaemia and hyperinsulinaemia [17-20].

Although thyroid function tests are often abnormal in HIV patients, the prevalence of overt thyroid disorder is not significantly different from that of the general population. Most asymptomatic patients with HIV infection have normal thyroid function. Some, however, exhibit increased serum T4 and T3 concentrations. These increases are as a result of increases in serum thyroxine-binding globulin, the cause of which is unknown. However, with progression of HIV infection and as the patients become more ill, serum T4 and T3 concentrations decline, as is obtained in most if not all chronically ill patients; serum thyrotropin concentrations however remain normal or slightly depressed. These changes are as a result of reduction in serum binding proteins, decreased extra thyroidal conversion of T4 to T3, and decreased secretion of thyrotropin. Cytokines may be involved in some of these; especially the reduction in the peripheral conversion of T4 to T3.

The abnormalities of thyroid function that have been identified among patients infected with HIV include: overt hypothyroidism, subclinical hypothyroidism, Graves' disease, subclinical hyperthyroidism, Isolated

low free T4 (FT4), Isolated low free T3 (FT3), acute suppurative thyroiditis, and sick euthyroid syndrome (non-thyroidal illness). Thyroid hormones are crucial for optimal function of the immune system [21]. A study conducted in southern Nigeria found that seropositive subjects who progressed to clinical AIDS, were more likely to be hypothyroid while HIV negative subjects were more likely to have normal or abnormally high thyroid hormone levels [22]. This was similar to a finding of lower thyroid hormone levels in HIV positive patients compared to HIV negative controls in another study [23]. A study carried out by Unachukwu et al. [24] in southeast Nigeria, found the prevalence of sick euthyroid syndrome and subclinical hypothyroidism to be 48% and 3.5-12.2% respectively among patients with advanced AIDS.

Thyroid dysfunction reduces the quality of life of patients infected with HIV. Changes in pituitary-thyroid function occur in patients with virtually all illnesses and those undergoing major surgical procedures. Although such changes are referred to as the euthyroid sick syndrome, the key changes namely; decreases in serum triiodothyronine and thyroxine concentrations have multiple causes, vary considerably in different patients, and very likely have different effects on different tissues [25]. Although it is generally assumed that the decreases have no pathophysiologic consequences, it is by no means clear that the patients are in fact euthyroid.

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. Overt hyperthyroidism is characterized by irritability, heat intolerance, sweating, warm moist skin, palpitations, tachycardia, fatigue, weight loss with increased appetite, diarrhea, tremor, muscle weakness, hyperreflexia, and lid retraction. 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 hyperfunction [26]. Furthermore, subclinical hyperthyroidism may precede overt hyperthyroidism [27]. It is unclear why HIV-infected patients are susceptible to thyroid dysfunction, but HIV infection is regarded as a crucial factor. Furthermore, the influence of HIV infection on thyroid function changes with the course of the disease. Asymptomatic, subtle abnormalities of thyroid function tests have been described in a small minority of patients with stable HIV infection [28-30]. With the progression of the disease, a pattern of sick euthyroid syndrome may develop. The most frequent abnormalities in thyroid function tests are those associated with subclinical hypothyroidism [26-28, 31, 32]. An increasing number of patients taking anti-HIV drugs are presenting with thyroid disorders as a result of improved immune function (immune reconstitution syndrome). Graves' disease is the commonest among immune reconstitution syndromes; others include Hashimoto's thyroiditis and hypothyroidism. Autoimmune Thyroid disease (AITD) occurs in 3% of women and 0.2% of men. Goddard & Shoenfeld [33] proposed a staging of autoimmune manifestations related to HIV/AIDS.

Asymptomatic subtle abnormalities of thyroid function tests, such as low serum levels of only T4, have been described in a small percentage of patients with stable HIV infection by some authors [29, 30] but most asymptomatic patients with early HIV infection and stable body weight have had normal T3 and T4 levels, as well as low reverse triiodothyronine (rT3) and often high thyroid binding globulin (TBG) values [28]. Moreover, low T3 and FT3 levels associated with low rT3 and high TBG levels have been found in other groups of patients in relation to the worsening of HIV illness when anorexia and weight loss occur, as happens in a classic sick euthyroid syndrome which, unlike the HIV condition, is usually characterised by high rT3 levels as a result of its decreased clearance [34].

The most common change is a decrease in extrathyroidal conversion of thyroxine (T4) to triiodothyronine (T3), the active form of thyroid hormone. This reaction is responsible for the production of 75 to 80 percent

of the circulating triiodothyronine in normal subjects and probably more of the intracellular triiodothyronine [35]. In illness, the production and serum concentrations of triiodothyronine decrease as a result either of decreased delivery of thyroxine to the widely distributed intracellular deiodinases that catalyze the conversion or of decreases in the activity of the enzymes [36]. A second change is a decrease in thyrotropin releasing hormone (TRH) secretion, which causes decreased TSH secretion and, in time, decreases serum thyroxine concentrations and further decrease in serum triiodothyronine concentrations; the latter due both to decreased thyroid secretion of triiodothyronine and decreased availability of thyroxine for peripheral conversion to triiodothyronine [37, 38]. We hereby perform this research to evaluate the thyroid profile (TSH, T3 and T4) of HIV positive subjects in Benin City, Edo State.

Introduction:

Human Immunodeficiency Virus (HIV) infection, caused by a lentivirus within the Retroviridae family, leads to progressive impairment of the cellular immune system, increasing susceptibility to infections and tumors, and culminating in Acquired Immune Deficiency Syndrome (AIDS) (Prescott, Harley, & Klein, 2019) [1]. Recognized as an emerging disease in the early 1980s, AIDS rapidly established itself globally, evolving from a mysterious illness to a pandemic infecting tens of millions within two decades [2]. According to UNAIDS 2018 data, 37.9 million people, including 2.1 million children under 15, live with HIV/AIDS, predominantly in low- and middle-income countries, with only 24.5 million accessing antiretroviral therapy (ART) [3]. India, with the third-largest HIV epidemic, had 2.1 million HIV-positive individuals in 2015, with the epidemic shifting from high-risk groups to the general population. By 2017, 79% of HIV-positive individuals were aware of their status, with 56% on ART [3]. HIV-related endocrine disorders, particularly thyroid dysfunction, are increasingly recognized, with cross-sectional studies reporting high prevalence of thyroid abnormalities in HIV patients. Lambert et al. [4] described progressive elevation in serum thyroxin-binding globulin (sr.TBG) without corresponding increases in other binding proteins, correlating with CD4+ count decline. Feldt-Rasmussen et al. [5] reported elevated sr.TSH and s.TBG concentrations with low FT-4, correlating with CD4+ cell depletion and immunosuppression. Subtle thyroid function test (TFT) alterations are common early in HIV infection, with proposed mechanisms including direct thyroid infection by opportunistic organisms, tumors, cytokine effects, drug side effects, and direct HIV infection [6]. Thyroid hormone metabolism is regulated by three deiodinase enzymes: Type 1 (D1), Type 2 (D2), and Type 3 (D3), responsible for T4 to T3 activation, T4 to rT3 inactivation, and rT3 and T3 to T2 conversion. D1 is mainly in the liver and kidney, contributing to 30–40% of extra-thyroidal T3 production, while D3, found in the liver, skin, and CNS, primarily converts T4 to rT3 and T3 to T2. Cirrhotic patients exhibit low total and free T3 with elevated rT3 due to reduced D1 activity, increasing T4 to rT3 conversion by D3 and rT3 to T3 ratio [7]. Thyroid hormones regulate hepatic function and bilirubin metabolism, with low T3 levels reducing hepatocyte basal metabolic rate and preserving liver function [8]. Thyrotoxicosis in experimental animals increases bile bilirubin output, possibly from increased hepatic heme degradation [9]. Thyroid hormone-induced alterations in bilirubin metabolism, specifically decreased glucuronyltransferase activity, may cause unconjugated hyperbilirubinemia, potentially masking Gilbert's syndrome. Thyroid hormones also decrease bile acid production and pool size [10]. Liver

disorders impact thyroid hormone metabolism and systemic effects, with derangements in thyroid profiles documented in liver patients. The T3:rT3 ratio negatively correlates with cirrhosis severity, and acute hepatitis elevates total T4 with normal free T4 due to increased thyroid-binding globulin as an acute-phase reactant. Cirrhosis reduces total and free T3, reflecting decreased D1 activity and T4 to T3 conversion [10]. Human Immunodeficiency Virus (HIV) infection is caused by human immunodeficiency virus, a lentivirus within the family Retroviridae. It leads to progressive impairment of the body's cellular immune system, increasing susceptibility to infection, tumors, and the fatal condition Acquired Immune Deficiency Syndrome (AIDS) [1]. Initially recognized as an emerging disease in the early 1980s, AIDS has rapidly become a global pandemic, infecting tens of millions of people worldwide within a short period [2]. According to UNAIDS 2018 data, 37.9 million people, including 2.1 million children under 15 years, are living with HIV/AIDS globally, with the majority in low- and middle-income countries, and only a portion accessing antiretroviral therapy [3]. India has the third largest HIV epidemic globally, with 2.1 million people living with HIV in 2015. The epidemic in India has transitioned from high-risk groups to the general population, with increasing awareness and ART coverage over the years [3]. Thyroid function abnormalities are prevalent in HIV-infected individuals, including unique alterations such as elevated serum thyroxin-binding globulin (sr.TBG) and thyroid-stimulating hormone (sr.TSH) concentrations, often correlating with CD4+ cell depletion and disease progression [4-6]. Proposed mechanisms for these abnormalities include direct infection of the thyroid gland by opportunistic organisms, tumor infiltration, effects of humoral factors, and side effects of medications used in HIV treatment [6]. The liver plays a crucial role in thyroid hormone metabolism, and alterations in thyroid function can affect hepatic function and bilirubin metabolism. For instance, cirrhosis is associated with low total and free T3 levels and elevated reverse T3 (rT3), reflecting reduced deiodinase type 1 activity and impaired T4 to T3 conversion [7]. Thyroid hormones also regulate basal metabolic rate in hepatocytes, impacting hepatic function and total body protein stores [8]. Experimental evidence suggests thyrotoxicosis increases bilirubin output and decreases bile acid production, highlighting the intricate relationship between thyroid and liver function [9-10]. HIV infection in sub-Saharan Africa, including Nigeria, remains a significant public health concern, with HAART significantly reducing morbidity and mortality associated with the disease. However, HAART is linked to metabolic disorders such as dyslipidemia and lipodystrophy, increasing cardiovascular disease and diabetes risk [11-16]. Despite the prevalence of abnormal thyroid function tests in HIV patients, overt thyroid disorders are not significantly different from the general population. Most asymptomatic HIV patients have normal thyroid function, while some exhibit altered thyroid hormone levels as the disease progresses [17-20]. Thyroid abnormalities in HIV patients include hypothyroidism, hyperthyroidism, subclinical thyroid dysfunction, and sick euthyroid syndrome. These abnormalities impact immune function and significantly reduce patients' quality of life [21-24]. The mechanisms underlying thyroid dysfunction in HIV patients involve reductions in peripheral T4 to T3 conversion, decreased thyrotropin-releasing hormone (TRH) secretion, and alterations in thyroid-binding proteins [25-28]. Asymptomatic thyroid function test abnormalities, such as low serum T4 levels, are observed in some stable HIV patients, while others exhibit classic sick euthyroid syndrome patterns with low T3 and FT3 levels correlated with

HIV illness worsening [29-30]. These changes occur due to reduced conversion of T4 to T3 and decreased TRH secretion, impacting thyroid hormone production and peripheral conversion [35-38]. This study aims to evaluate thyroid profiles in HIV-positive individuals in Benin City, Edo State, contributing to understanding thyroid dysfunction in HIV/AIDS.

2. MATERIALS AND METHODS

Area of Study

This study was carried out in Benin-City, Edo State. Benin City is a city with year 2006 estimated population of 1,147,188 and the capital of Edo State in southern Nigeria. It is a city approximately 25 miles north of the Benin River. It is situated 200 miles by road east of Lagos. Benin is the centre of Nigeria's rubber industry, but processing palm nuts for oil is also an important traditional industry. Benin City is situated at 6.34^{o} North latitude, 5.63^{o} East longitude and 80 meters elevation above the sea level [39].

Study Population

The subjects in this study comprise of HIV positive volunteers aged between 18 to 50 years attending Central Hospital Ekpoma, Edo State, Nigeria. A total of one hundred (100) subjects were recruited for this study. The sample size (N) is calculated from the formula below using prevalence from previous studies. The prevalence of 3.4% of HIV subjects in Edo state was from the study conducted by Onovo *et al*., [40]

Samples size (**N**) = $\frac{Z^2Pq}{d^2}$, where

N = the desired size
Z = 1.96 (standard score)
P = Prevalence (3.4%) (0.034)
q. = 1- P (0.966)
d = sample error tolerated (0.05)

N=$\frac{\mathbf{1.96^2\times0.034\times0.966}}{\mathbf{0.05^2}}$**=50.5 approximately= 51**

The sample size was upgraded to 80 due to 50% attrition of the original sample size. The study was conducted on 120 subjects comprising of forty (40) apparently healthy subjects (controls) and eighty (80) HIV positive subjects (test samples).

Research Design

This study was designed as a prospective and cross-sectional study to evaluate the thyroid profile (TSH, T3 and T4) of HIV positive subjects in Benin city, Edo State, Nigeria. A complete record of medical history (age, gender and other important medical information) was obtained for each subject from the patient's medical records. This study was carried out within six (6) months. Furthermore, in order to ensure HIV status validity, both confirmed and negative cases were re-tested by the researcher with HIV test strips using standard Laboratory procedures. It is on the basis of this validity that HIV positive and control subjects were selected and grouped for the study. The results of the thyroid profile (TSH, T3 and T4) obtained for the HIV positive subjects obtained were compared with that of the control using statistical methods.

Sample Collection

Five millilitres (5mls) of venous blood sample were collected from each subject from the ante-cubital vein using sterile disposable syringe. The blood samples were immediately placed in a labelled lithium heparin and EDTA containers for both subjects and control individuals for the estimation of thyroid profile (TSH, T3 and T4) and some micronutrients (Selenium, Copper, and Zinc). The blood was spun for 10 minutes at 5000 rpm. The serum was separated from the red cells using a dry clean Pasteur pipette into dry clean plain specimen containers which was labelled corresponding to the initial blood samples containers. The serum was then stored at -20°C pending the analysis of the samples.

Sample Analysis

HIV Serology Test

HIV serostatus was determined according to centre for disease and prevention (CDC – UMD). HIV rapid testing serial algorithm II guideline [41]. Determine HIV – ½ kit, an immunochromatographic technique, was the kit used. Positive results by "Determine HIVI/II" kit (Alere Medical Co., Ltd., Chiba, Japan) was confirmed with "Unigold HIVI/II" test kit (Trinity Biotech Plc., Bray, Ireland) while negative results by "Determine HIVI/II" ended testing. Discordant results are repeated and finally tested with a tie-breaker kit-statpak. Final results were considered positive or negative on the basis of tie-breaker result.

Determine HIVI/II Test

Principle: Alere Determine HIVI/II is an immunochromatographic test for the qualitative detection of antibodies to HIV I/II. Sample is added to the sample pad. As the sample migrates through the conjugate pad, it reconstitutes and mixes with the selenium colloid-antigen conjugate. This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the patient window site. If antibodies to HIV -1 and/or HIVII are present in the sample, the antibodies bind to the antigen-selenium colloid and to the antigen at the patient window, forming a red line at the patient window site. If antibodies to HIVI/II and /or HIVII are absent, the antigen-selenium colloid flows past the patient window, and no red line is formed at the patient window site. To ensure assay validity, a procedural control bar is incorporated in the assay device [41].

UNIGOLD HIVI/II Test

Principle: HIV is a rapid immunoassay based on the immunochromatographic sandwich principle. Recombinant proteins representing the immunodominant regions of the envelope proteins of HIVI/II and HIVII, glycoprotein gp41, gp120 (HIVI/II) and glycoprotein gp36 (HIVII) respectively, are immobilized at the test region of the nitrocellulose strip. These proteins are also linked to colloidal gold and impregnated below the test region of the device. A narrow band of the nitrocellulose membrane is also sensitized as a control region [41].

Determination of Total T_3 Concentration: Plasma total T_3 was quantitatively determined using enzyme immunoassay [42].

Principle: In the T3 EIA, a second antibody (goat anti-mouse IgG) is coated on microtiter wells. measured amount of patient serum, a certain amount of mouse monoclonal anti-T3 antibody, and a constant amount of T3 conjugated with horseradish peroxidase are added to the microtiter wells. During incubation, the mouse anti-T3 antibody is bound to the second antibody on the wells, and T3 and conjugated T3 compete for the limited binding sites on the anti-T3 antibody. After a 60-minute incubation at room temperature, the wells are washed 5 times by water to remove unbound T3 conjugate. A solution of TMB Reagent is then added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of Stop Solution, and the absorbance is measured spectrophotometrically at 450nm. The intensity of the color formed is proportional to the amount of enzyme present and is inversely

related to the amount of unlabeled T3 standards assayed in the same way, the concentration of T3 in the unknown sample is then calculated [42].

Determination of Total T_4 Concentration: Plasma Thyroxin (T_4) level was determined using Enzyme immuno assay (EIA) [42].
Principle: To measure T4 by competitive immunoassay techniques, a sample of serum or plasma containing the T4 to be quantified is mixed with labeled T4 and T4 antibody. The labeled T4 contains 8-anilino-1-napthalene sulfonic acid (ANS) to inhibit binding of T4 to serum proteins, which would otherwise interfere with the assay. During incubation, a fixed amount of labeled T4 competes with the unlabeled T4 in the sample, standard, or quality control serum for a fixed number of binding sites on the specific T4 antibody. Separation of the unbound T4 from antibody-bound T4 and the subsequent measurement of the labeled fraction of the bound phase completes the test. By comparing results of the unknown sample with those obtained from a series of T4 calibrators, an accurate measurement of the T4 concentration in the sample can be obtained [42].

Determination of Thyroid Stimulating Hormone: Plasma Thyroid stimulating Hormone (TSH) was quantitatively determined using ELISA.
Principle: The TSH ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact TSH molecule. Mouse monoclonal anti-TSH antibody is used for solid phase immobilization (on the microtiter wells). A goat anti-TSH antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 60-minute incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution, changing the color to yellow. The concentration of TSH is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

Guidelines for detection of thyroid dysfunction

i. Normal function: T3, T4 and TSH within normal range.
ii. Primary hypothyroidism: TSH more than 8.1MIU/ml; T3 and T4 less than the normal values.
iii. Secondary hypothyroidism: T3 and T4 less than normal values; TSH is within normal.
iv. Subclinical hypothyroidism: TSH more than 8.1MIU/ml; T3 and T4 within normal range.
v. Primary hyperthyroidism: TSH less than or equal 0.2; T3 and T4 more than normal values.
vi. Secondary hyperthyroidism: T3 and T4 more than the normal value; TSH within normal.
vii. Subclinical hyperthyroidism: TSH less than the normal values; T3 and T4 within normal [43].

Data Analysis
The Mean and standard deviation of the results obtained was calculated. ANOVA (LSD) was used for the analysis using SPSS package version 21. Values with $p< 0.05$ shall be considered statistically significant in this study.

Materials and Methods:

The study was conducted in Benin City, Edo State, Nigeria, which boasts an estimated population of 1,147,188 as of 2006. Positioned at 6.34o North latitude and 5.63o East longitude, Benin City serves as the

capital of Edo State and holds significance for its rubber industry and traditional palm oil processing. With its geographical coordinates placing it 80 meters above sea level, the city is approximately 25 miles north of the Benin River and 200 miles east of Lagos by road [39]. The research subjects comprised HIV-positive volunteers aged between 18 to 50 years, drawn from Central Hospital Ekpoma, Edo State. A total of one hundred participants were recruited, including forty apparently healthy individuals as controls and eighty HIV-positive individuals as test samples. The sample size determination was based on a prevalence rate of 3.4% for HIV in Edo State, as reported by Onovo et al. [40]. Considering a 50% attrition rate, the initial sample size was upgraded to 80 subjects. The study adopted a prospective and cross-sectional design, aiming to assess the thyroid profile (TSH, T3, and T4) of HIV-positive subjects in Benin City, Edo State, Nigeria. Medical history, including age, gender, and other pertinent medical information, was collected from each participant's medical records. The study spanned a duration of six months [39-42]. To ensure the validity of HIV status, both confirmed positive and negative cases were re-tested using HIV test strips and standard laboratory procedures. Based on this validation, HIV-positive and control subjects were selected and grouped for the study. Thyroid profile analysis, including TSH, T3, and T4, was conducted using venous blood samples collected from the ante-cubital vein. Blood samples were processed to separate serum from red cells and stored at -20°C pending analysis. HIV serostatus was determined according to the CDC – UMD HIV rapid testing serial algorithm II guideline, utilizing the Determine HIV – ½ kit for initial testing. Positive results were confirmed using the Unigold HIVI/II test kit, with discordant results resolved using a tie-breaker kit (statpak) [41]. Furthermore, plasma total T3 and T4 levels were quantitatively determined using enzyme immunoassay techniques, while plasma TSH levels were quantified using ELISA. The immunoassay techniques involved competitive binding of labeled and unlabeled hormones to specific antibodies, with subsequent measurement of the labeled fraction to calculate hormone concentrations. Thyroid function categories were defined based on established criteria for hypothyroidism and hyperthyroidism, including primary, secondary, and subclinical forms of both conditions. Statistical analysis of the obtained results was performed using ANOVA (LSD) with significance set at $p < 0.05$ [42, 43].

3. RESULTS

Socio-Demographic Characteristics of the Study Population

Table 1 revealed the socio-demographic characteristics of the study population. The subjects were categorized into five age groups; 16-25 years; 26-35 years; 36-45 years; 46-55 years and 56-65 years. The result for age showed that majority of the subjects were within the age range of 36-45 years accounting for 33.8%, followed by 46-55 years 23.8%, 26-35 years 18.8%, 16-25 years 15.0% and 56-65 years being the least accounted for 8.8%. The Age (Mean ±SD) of the subjects was (39.34±11.76). With respect to gender, 27.5% of the subjects were male and 72.5% of the subjects were female. Based on duration of drugs, 8.8% of the subjects have been on drugs within the period of 0-11months, 1-5years 33.8%, 6-10years 32.5%, 11-15years 18.8% and 16-20years 6.3%.

Table .1: Socio-Demographic Characteristics of the Study Population (n=120)

Variables		Frequency	Percentage (%)
Age (Years)	16-25	12	15.0
	26-35	15	18.8
	36-45	27	33.8
	46-55	19	23.8
	56-65	7	8.8

	Age (Mean ±SD)	39.34±11.76		
Gender	Male	22	27.5	
	Female	58	72.5	
Duration of drugs	0-11months	7	8.8	
	1-5years	27	33.8	
	6-10years	26	32.5	
	11-15years	15	18.8	
	16-20years	5	6.3	

Parameters	**Control (n=40)**	**Subjects (n=80)**	**t value**	**P value**
TSH (miu/L)	1.92±1.11	2.75±1.59	2.967	0.004
T_3 (ng/L)	1.49±0.35	1.32±0.63	1.560	0.121
T_4 (ng/L)	7.09±1.78	6.06±1.83	2.913	0.004

TSH, T_3 and T_4 levels between Subjects and Control

The results in table 2 showed the comparison of TSH, T_3 and T_4 levels between the subjects and control. The result showed that TSH levels were significantly higher ($p<0.05$) in subjects (2.75±1.59 miu/L) when compared with the control (1.92±1.11 miu/L). On the contrary, T_3 levels were also not significantly lower ($p>0.05$) in subjects (1.32±0.63 ng/L) when compared with the control (1.49±0.35 ng/L). T_4 levels were significantly lower ($p<0.05$) in subjects (6.06±1.83 ng/L) when compared with the control (7.09±1.78 ng/L).

Table 2: TSH, T_3 and T_4 levels between subjects and control

KEY: n=Sample size ; p>0.05= Not significant; p<0.05= Significant

Parameters	**Male Control (n=21)**	**Male Subjects (n=22)**	**t value**	**P value**
TSH (miu/L)	2.32±1.23	2.59±1.58	0.619	0.539
T_3 (ng/L)	1.54±0.38	1.33±0.65	1.279	0.208
T_4 (ng/L)	6.58±1.70	6.27±1.88	0.571	0.571

TSH, T_3 and T_4 levels between Male Subjects and Male Control

The results in table 3 showed the comparison of TSH, T_3 and T_4 levels between the male subjects and control. The result showed that TSH levels were not significantly higher ($p>0.05$) in male subjects (2.59±1.58 miu/L) when compared with the control (2.32±1.23 miu/ L). T_3 levels were not significantly lower ($p>0.05$) in male subjects (1.33±0.65 ng/L) when compared with the control (1.54±0.38 ng/L). T_4 levels were also not significantly lower ($p>0.05$) in male subjects (6.27±1.88 ng/L) when compared with the control (6.58±1.70 ng/L).

Table 3: TSH, T_3 and T_4 levels between male subjects and male control

KEY: n=Sample size ; p>0.05= Not significant; p<0.05= Significant

TSH, T_3 and T_4 levels between Female Subjects and Female Control

The results in table 4 showed the comparison of TSH, T_3 and T_4 levels between the female subjects and control. The result showed that TSH levels were significantly higher ($p<0.05$) in female subjects (2.81±1.60 miu/L) when compared with the control (1.47±0.76 miu/ L). On the contrary, T_3 levels were not significantly lower ($p>0.05$) in female subjects (1.32±0.64 ng/L) when compared with the control

(1.44±0.32 ng/ L). T_4 levels were significantly lower ($p<0.05$) in female subjects (5.98±1.83 ng/L) when compared with the control (7.65±1.75 ng/L).

Table 4: TSH, T_3 and T_4 between female subjects and female control
KEY: n=Sample size ; p>0.05= Not significant; p<0.05= Significant

TSH, T_3 and T_4 levels of the Subjects with respect to Gender

Parameters	Male Subjects (n=22)	Female Subjects (n=58)	t value	P value
TSH (miu/L)	2.59±1.58	2.81±1.60	0.557	0.579
T_3 (ng/L)	1.33±0.65	1.32±0.64	0.063	0.950
T_4 (ng/L)	6.27±1.88	5.98±1.83	0.615	0.540

The results in table 5 showed the comparison of TSH, T_3 and T_4 levels of the subjects with respect to gender. The result showed that TSH levels were not significantly lower ($p>0.05$) in male subjects (2.59±1.58 miu/ L) when compared with the female subjects (2.81±1.60 miu/ L). T_3 levels were not significantly higher ($p>0.05$) in male subjects (1.33±0.65 ng/ L) when compared with the female subjects (1.32±0.64 ng/ L). T_4 levels were also not significantly higher ($p>0.05$) in male subjects (6.27±1.88 ng/ L) when compared with the female subjects (5.98±1.83 ng/ L).

Table 5: TSH, T_3 and T_4 levels of subjects with respect to gender
KEY: n=Sample size; p>0.05= Not significant; p<0.05= Significant

TSH, T_3 and T_4 levels of the Subjects according to Age

The results in table 6 showed the comparison of TSH, T_3 and T_4 levels of the subjects according to age. The results showed a significant non-increased in TSH ($p>0.05$) in subjects aged 16-25 years (3.04±1.26 miu/L) when compared with subjects within the age range of 46-55 years (3.01±1.92 miu/L), 36-45 years (2.95±1.69 miu/L), 56-65 years (2.30±0.49 miu/L) and 26-35 years (2.03±1.39 miu/L). T_3 levels were higher ($p>0.05$) within the age range of 26-35 years (1.69±0.73 ng/L) when compared with 56-65 years (1.31±0.68 ng/L), 16-25 years (1.25±0.55 ng/L), 46-55 years (1.24±0.67 ng/L) and 36-45 years (1.21±0.53 ng/L). T_4 levels were higher within the age range of 26-35 years (7.10±1.80 ng/L) when compared with 56-65 years (6.13±2.01 ng/L), 45-55 years (6.04±1.88 ng/L), 36-45 years (5.83±1.83 ng/L) and 16-25 years (5.22±1.24 ng/L).

Parameters	16-25 Years (n=21)	26-35 Years (n=29)	36-45 Years (n=26)	46-55 Years (n=23)	56-65 Years (n=11)	F value	P value
TSH (miu/L)	3.04±1.26 [a]	2.03±1.39 [a]	2.95±1.69 [a]	3.01±1.92 [a]	2.30±0.49 [a]	1.255	0.295
T_3 (ng/L)	1.25±0.55 [a]	1.69±0.73 [a]	1.21±0.53 [ab]	1.24±0.67 [ab]	1.31±0.68 [a]	1.645	0.172
T_4 (ng/L)	5.22±1.24 [a]	7.10±1.80 [b]	5.83±1.83 [a]	6.04±1.88 [ab]	6.13±2.01 [ab]	2.226	0.074

Parameters	Female Control (n=19)	Female Subjects (n=58)	t value	P value
TSH (miu/L)	1.47±0.76	2.81±1.60	3.502	0.001
T_3 (ng/L)	1.44±0.32	1.32±0.64	0.771	0.443
T_4 (ng/L)	7.65±1.75	5.98±1.83	3.481	0.001

Table 6: TSH, T3 and T4 levels of the subjects according to age

Parameters	0-11 Months (n=21)	1-5 Years (n=29)	6-10 Years (n=26)	11-15 Years (n=23)	16-20 Years (n=11)	F value	P value
TSH (miu/L)	2.75±1.25 [a]	2.83±1.73 [a]	2.56±1.37 [a]	3.33±1.86 [a]	1.53±0.78 [ab]	1.370	0.253
T_3 (ng/L)	1.36±0.63 [a]	1.20±0.71 [a]	1.39±0.58 [a]	1.27±0.65 [a]	1.74±0.31 [a]	0.884	0.478
T_4 (ng/L)	5.34±2.61 [a]	5.83±1.74 [a]	6.26±1.65 [a]	6.28±1.73 [a]	6.60±2.63 [a]	0.609	0.658

KEY: n=Sample size; p>0.05= Not significant; p<0.05= Significant
Values in a row with the same superscript are not significantly different at p<0.05

TSH, T_3 and T_4 levels of the Subjects according to Duration of Drugs.

The results in table 7 showed the comparison of TSH, T_3 and T_4 levels of the subjects according to duration of drugs. The results showed a significant non increased in TSH (p>0.05) in subjects who had been on drugs within 11-15years, when compared with subjects who have been on drugs for a period of 0-5 months, 1-5 years, 6-10 years and 16-20 years. T_3 levels were higher (p>0.05) in subjects who had been on drugs within 16-20 years, when compared with subjects who have been on drugs for a period of 0-5 months, 1-5 years, 6-10 years and 11-15 years. T_4 levels were higher (p>0.05) in subjects who had been on drugs within 16-20 years, when compared with subjects who have been on drugs for a period of 0-5 months, 1-5 years, 6-10 years and 11-15 years.

Table 7: TSH, T3 and T4 levels of the subjects according to duration of drugs

KEY: n=Sample size; p>0.05= Not significant; p<0.05= Significant
Values in a row with the same superscript are not significantly different at p<0.05

4. DISCUSSION

The study aims at evaluating the thyroid profile (TSH, T3 and T4) of HIV positive subjects visiting Central Hospital in Benin City, Edo State. In our study majority of the subjects were within the age range of 36-45 years accounting for 33.8%, followed by 46-55 years 23.8%, 26-35 years 18.8%, 16-25 years 15.0% and 56-65 years being the least accounted for 8.8%. The Age (Mean ±SD) of the subjects was (39.34±11.76). This is in agreement with the study of Shukla et al [44] in which majority of the cases (81.45%) were between 20-45 years and Michèle et al [45] in which mean age was 40.8 years (SD = 9.54). The mean age of participants were similar to that observed in previous studies [23, 46, 47].

With respect to gender, 27.5% of the subjects were male and 72.5% of the subjects were female. Based on duration of drugs, 8.8% of the subjects have been on drugs within the period of 0-11months, 1-5years 33.8%, 6-10years 32.5%, 11-15years 18.8% and 16-20years 6.3%. The result from this study showed that TSH levels were significantly higher (p<0.05) in subjects (2.75±1.59 miu/L) when compared with the control (1.92±1.11 miu/L). On the contrary, T_3 levels were also not significantly lower (p>0.05) in subjects (1.32±0.63 ng/L) when compared with the control (1.49±0.35 ng/L). T_4 levels were significantly lower (p<0.05) in subjects (6.06±1.83 ng/L) when compared with the control (7.09±1.78 ng/L). Palanisamy *et al* [48] concluded that thyroid dysfunction is frequent in HIV infection and that with progression of disease there is a primary hypothyroid like stage that occurs in patients with HIV infection [49]. Therefore, free T3, free T4 and serum TSH can be used as a surrogate marker of progression of the disease. Thongam *et al* [32]

reported that thyroid dysfunction may be a marker of severity or progression of HIV. In a study by Panamonta *et al.*, [50] 14% of Thai children with HIV had low serum T3 and normal TSH, and FT4 levels consistent with sick-euthyroid and all were clinically euthyroid.

The cause of thyroid dysfunction is unclear, but hypotheses include autoimmune disease, concurrent infections, destruction by opportunistic infections, and drug reactions [51]. Thyroid abnormalities are associated with disease progression, including severe immunosuppression and high viral load [52, 53]. Serum levels of TBG progressively increase with the progression of the disease [54, 55]. The reason for the increase in TBG is unknown but seems unrelated to serum estrogen levels or clearance of the protein [56].

On the duration of HAART, the results showed a significant non-increased in TSH ($p>0.05$) in subjects who had been on drugs within 11-15years, when compared with subjects who have been on drugs for a period of 0-5 months, 1-5 years, 6-10 years and 16-20 years. T_3 levels were higher ($p>0.05$) in subjects who had been on drugs within 16-20 years, when compared with subjects who have been on drugs for a period of 0-5 months, 1-5 years, 6-10 years and 11-15 years. T_4 levels were higher ($p>0.05$) in subjects who had been on drugs within 16-20 years, when compared with subjects who have been on drugs for a period of 0-5 months, 1-5 years, 6-10 years and 11-15 years. A previous study ascribed the elevated levels of thyroid autoantibodies to some non-specific consequences of increased B-cell activation seen in the clinical course of HIV infection as opposed to the Immune Reconstitution syndrome [57].

During HIV infection abnormalities in thyroid include both pathological changes and disturbances in its function [6, 53]. Our present study shows that thyroid dysfunction is frequent in HIV infection and with progression of disease there is a subclinical hypothyroid like stage that occurs in patients with advancing HIV infection [58]. Various thyroid function tests such as FT3/FT4 /serum TSH can be used as a surrogate marker as these correlate with the progression of the disease. One should not start upon the replacement therapy for hypothyroid like state in HIV infection, as the state may be responsive to highly active antiretroviral therapy. Our present study may give the true picture of thyroid abnormality in HIV-AIDS patients. To conclude, abnormal TFTs are encountered often in HIV infection and AIDS patient's individuals. Management guidelines exist for overt dysfunction as described above. However, larger studies are needed to evaluate the prevalence and outcomes of mild thyroid dysfunction in HIV-infected patients and to formulate screening and treatment guidelines.

Thyroid function testing is appropriate for the diagnoses of thyroid disorders in patients with thyroid-related symptoms or with nonspecific systemic symptoms. However, thyroid function screening of asymptomatic individuals is an area of controversy, both for HIV-infected patients and for the general population. Regardless of HIV status, screening of older patients may be justified by the high prevalence of subclincal hypothyroidism and by the potential benefit of levothyroxine therapy in this population [59]. Although cross-sectional studies have reported a higher prevalence of subclincal hypothyroidism than normally observed in the general population, the pathophysiology of subclincal hypothyroidism may differ in HIV-infected patients; at this point, there is insufficient evidence to support routine screening of all HIV-infected individuals. Similarly, although common, the finding of an isolated low FT4 level has unclear consequences and should not be the target of routine screening.

Measurement of the TSH level is appropriate for patients with symptoms suggestive of thyroid dysfunction, reduced bone mineral density, dyslipidemia, depression, or atrial fibrillation. The finding of an elevated TSH level should prompt the health care provider to measure the FT4 level, whereas both the FT4 level and the T3 level should be measured in patients with a low TSH level (to rule out T3 toxicosis). When testing

is performed, nonthyroidal illness should be considered in the differential diagnosis of abnormal thyroid function test results, particularly for patients with advanced AIDS or uncontrolled HIV infection.

Stress of advanced disease or concomitant morbidities may manifest as the classic sick euthyroid syndrome probably due to hypothalamic-pituitary deficit related to the progress of immunodeficiency and cachexia [60]. Stavudine used for treatment of HIV infection may impair thyroid function but as the number of patients on this drug is limited this association cannot be established in the present study.

And finally, as a conclusive remarks, abnormal thyroid function test results are common among HIV-infected individuals. This is especially true during HAART, when Graves' disease may be triggered by immune reconstitution and the presence of subclinical hypothyroidism, and when isolated low FT4 levels appear to be more common. Currently, there is insufficient evidence in favor of screening for thyroid abnormalities among asymptomatic HIV-infected individuals. In conclusion, this study highlights that TSH levels were significantly higher ($p<0.05$) in subjects when compared with the control, and T_4 levels were significantly lower ($p<0.05$) in subjects when compared with the control. However is recommended further studies with reasonanle sample size to investigate the epidemiology and health consequences of mild thyroid dysfunction in HIV-infected subjects and to better inform screening and treatment guidelines.

CONSENT

Only already diagnosed and confirmed HIV positive subjects and apparently healthy subjects who had no known medical condition were recruited for this study. This study specifically included only subjects between 18-50 years who gave consent for the study.

Individuals who are not within the age range of 18-50 years whose HIV status has not been confirmed were excluded. Control subjects who show any sign of visible ailments, would not give consent and have any underlying illness such as diabetes, cardiovascular, sickle cell, pregnant and renal diseases were also excluded from this study.

ETHICAL APPROVAL

Ethical permission for this study was obtained from the Edo State Hospital Management Board, Benin City, Edo State, Nigeria. Informed consent was obtained from the patients prior to the collection of samples for this study. The purpose of the study was exhaustively explained to the patients and assured of the confidentiality of the information obtained from them.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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