Pattern of Lipid and Lipoproteins among Thyroid Dysfunction Patients Referred To Ethiopian Health and Nutrition Research Institute, Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Paulos Nigussie, entitled: *Pattern of Lipid and Lipoproteins among Thyroid Dysfunction Patients Referred to Ethiopian Health and Nutrition Research Institute*: and submitted in partial fulfillment of the requirements of the degree of masters of science (Clinical Laboratory Sciences with a specialty track of Clinical Chemistry) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Chair Department of Graduate Program Coordinator.

ABSTRACT

Introduction: The relationship between thyroid hormones and lipids has long been studied, having been first described more than 70 years ago. Since then, much new information has been discovered, which justifies a reevaluation of the relationship between thyroid pathology, dyslipidemia and further cardiovascular disease rick. Relations between thyroid function and lipid status remain incompletely understood in Ethiopia. Besides the emerging concern of stroke and hypertension, goiter is one of the highly endemic and prevalent noninfectious diseases in the country. Moreover, the country health policy and strategy mainly focus on prevention of communicable diseases and physicians may fail to manage lipid and lipoprotein abnormalities, and cardiovascular risks while treating their patients for thyroid dysfunctions.

Objective: To determine the relationship between thyroid dysfunction versus serum lipid profile and risk of cardiovascular disease among patients referred to the Ethiopian Health and Nutrition Research Institute.

Methods: Correctional with Control study was conducted from September, 2011 to May 2012. A total of 212 participants, 106 of them referred for thyroid function laboratory investigation or on follow-up and the rest 106 sex and age matched apparently healthy control group were consented, considered for exit interview and physical examination about their antropometrical**,** medication, nutritional status, and for sine and symptom of thyroid disfunction with trained nurses. The laboratory investigation includes lipid and lipoprotein panels, and thyroid functional test using COBAS Integra-400, and COBAS e-411analyzers. Data entry was done using Microsoft Excel 2007. Data analysis wsbe performed using Chi-square, Student-t-test and odds ratio, using SPSS version 19 and STATA version 8 were used to assess association between variables. p value <0.05 was considered as statistically significant.

Result: Of 212 study participants, 89.6% were females, mean age, mean age was 39.2 years; mean BMI was 23.4±4.9 among cases and 22.1±2.6 among control subjects. BP was statistically significantly higher among control than cases p<0.0001. Majority of the subjects were euthyroid (54.7%), followed by hyperthyroidism (23%) in which 9.4%, 6.1%, and 7.6% account for overt hyperthyroid, sub-clinical hyperthyroid, and T3 toxicosis, respectively. The rest 22.2% were hypothyroid in which 2.8%, 19.3%, account for overt hypothyroid and sub-clinical hypothyroid, respectively and 37% of the subjects have elevated thyrogloblin. However, sub-clinical hypothyroids 12.7%. Lipids and lipoproteins (TC, TRI, LDL, Apo-A, and Lp-A) were significantly elevated in hypothyroid condition than control subjects whereas HDL decreased significantly. And the reverse condition happens in hyperthyroid subjects. 47.3% of the case where taking medication for their thyroid dysfunction. Of this 21.1 % of them became euethyroid subjects after medication with L-thyroxin. And there lipid and lipoprotein profiles seem normal.

Conclusion: Thyroid dysfunction is associated with higher BP, higher BMI, and lipid and lipoprotein profile. Apparently healthy subjects may became sub-clinical hypothyroid and had higher thyrogloblins; this indicate below optimum iodine supplement. L-hhyroxine may help in the optimization of lipid and lipoproteins.

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I would like to express my gratitude to Addis Ababa University (AAU), College of Health Sciences, Department of Medical Laboratory Science and to Ethiopian Health and Nutrition Research Institute (EHNRI) for giving me the opportunity to do this research.

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Abbreviations

AAU	Addis Ababa University
apo B-48	apolipoprotein B-48
apo B-100	apolipoprotein B- 100
apo-AV	apolipoprotein AV
CVD	Cardiovascular disease
CHD	coronary heart disease
ECL	Electrochemiliminesence
EHNRI	Ethiopian Health and Nutrition Research Institute
FMoH	Federal Ministry of Health
HDL	high-density lipoprotein cholesterol
IBR	Institutional Review Board
IDA	Iodine deficient area
IDL	intermediate density lipoproteins
ISA	iodine sufficient area
LPL	lipoprotein lipase
Lp-a	Lipoprotein-a
LDL	low-density lipoprotein cholesterol
T_3	triiodothyronine
T_4	thyroxin
TG	thyrogloblin
TRI	triglyceride
TSH	thyroid-stimulating hormone
T-Up	T-Uptake
VLDL	very low density lipoproteins

Operational definition

Subclinical Hypothyroidism :-

Serum T_3, T_4 and TSH are in the reference range where as Free T_3 and/or Free T_4 lowered than reference range.

Or Serum T_3, T_4, Free T_3, and Free T_4 are in the reference range where as TSH is higher than reference range.

Clinical Hypothyroidism :-

Serum T_3, T_4, Free T_3, and Free T_4 lowered than reference range where as TSH higher than reference range.

Subclinical Hyperthyroidism:-

Serum T_3, T_4 and TSH are in the reference range where as Free T_3 and/or Free T_4 higher than reference range.

Or Serum T_3, T_4, Free T_3, and Free T_4 are in the reference range where as TSH is lower than reference range.

Clinical Hyperthyroidism :-

Serum T_3, T_4, Free T_3, and Free T_4 higher than reference range where as TSH is lower than reference range.

Euthyroid :-

All thyroid functional hormones T_3, T_4, Free T_3, Free T_4 and TSH in the reference range.

1. Introduction

1.1 Back Ground

The relationship between thyroid hormones and lipids has long been studied, having been first described more than 70 years ago. Since then, much new information has been discovered, which justifies a reevaluation of the relationship between thyroid pathology and dyslipidemia [1].

Plasma lipids, of which the most important are cholesterol, triglycerides and phospholipids, are transported in the plasma by water-soluble lipoproteins [2]. These are complex particles consisting of an outer layer of proteins and phospholipids, and a central core of neutral lipids. Various apolipoproteins are found in this structure; they act as intermediaries in the transport of triglycerides and exogenous and endogenous cholesterol [3]. Exogenous and endogenous lipoproteins can be distinguished by the presence in the former of apolipoprotein B-48 (apo B-48) and in the latter of apolipoprotein B- 100 (apo B-100). Lipoprotein-a (Lp-a), which is rich in cholesteryl esters, is also known to have atherogenic properties and can promote cholesterol deposition in endothelial lesions [4].

Thyroid hormones; triiodothyronine (T_3), and thyroxin (T_4) function regulates a wide array of metabolic parameters. These hormones significantly affect lipoprotein metabolism as well as some cardiovascular disease (CVD) risk factors, thus influencing overall CDV risk [5-7]. Indeed, even within the normal range of thyroid-stimulating hormone (TSH) values, a linear increase in total cholesterol, low-density lipoprotein cholesterol (LDL-C) and triglycerides and a linear decrease in high-density lipoprotein cholesterol (HDL-C) levels has been observed with increasing TSH [8].

Thyroid hormones induce the 3-hydroxy-3-methylglutarylcoenzyme-A reductase, which is the first step in cholesterol biosynthesis. Moreover, T_3 up regulates LDL receptors by controlling the LDL receptor gene activation. This T_3-mediated gene activation is done by the direct binding of T_3 to specific thyroid hormone responsive elements [9]. Furthermore, T_3 controls the sterol regulatory element-binding protein-2, which in turn regulates LDL receptor's gene expression [10]. T_3 has also been associated with protecting LDL from oxidation [11].

Approximately 3% of thyroxin (T_4) binds to lipoproteins, mainly HDL (92%), and to a lesser extent to LDL (6.7%) [12]. The T4-LDL complex is recognized by LDL receptors, and this is thus one way for T4 to enter cells [13]. The promoter of the LDL receptor gene contains a thyroid hormone responsive element, which enables triiodothyronine (T3) to enhance expression of LDL receptors and hence increase clearance of LDL and apo B, without affecting their synthesis [14].

Thyroid hormones can influence HDL metabolism by increasing cholesteryl ester transfer protein activity, which exchanges cholesteryl esters from HDL2 to the very low density lipoproteins (VLDL) and triglycerides to the opposite direction [15]. In addition, thyroid hormones stimulate the lipoprotein lipase (LPL), which catabolizes the triglyceride rich lipoproteins, and the hepatic lipase, which hydrolyzes HDL2 to HDL3 and contributes to the conversion of intermediate density lipoproteins (IDL) to LDL and in turn LDL to small dense LDL [16, 17]. Another effect of T_3 is the up-regulation of apolipoprotein AV (Apo-AV), which plays a major role in triglyceride regulation [18]. Indeed, increased levels of Apo-AV have been associated with decreased levels of triglycerides [19]. Proposed mechanisms for this effect include the decrease of hepatic VLDL-triglyceride production and the increase of plasma LPL levels and activity, resulting in increase of lipoprotein remnant generation due to enhanced LPL-mediated lipolysis of VLDL-triglyceride [19]. Moreover, a greater clearance of lipoprotein core remnants, caused by increased hepatic uptake due to an enhanced affinity for the LDL receptor, has also been ascribed to Apo-AV [19].

Thyroid dysfunction has well-characterized deleterious effects on the cardiovascular system; untreated thyrotoxicosis leads to a hyperdynamic state with increased heart rate, left ventricular contractility, and systolic hypertension and may be complicated by atrial fibrillation[20]. Hypothyroidism is associated with hypercholesterolemia, increased diastolic blood pressure, and heart failure. These changes were thought to be reversible on normalization of thyroid function, but there is indirect evidence that subclinical or treated thyroid disease remains associated with increased cardiovascular risk[20]. The cardiac consequences of subclinical hypothyroidism and hyperthyroidism are, in some respects, more concerning than overt disease, which is symptomatic and easily diagnosed. This absence of symptoms expectedly results in delayed diagnoses and therefore augments the risk of

complications. Studies show an increase in mortality from all causes as well as from circulatory diseases in individuals with subclinical hyperthyroidism [21]. Likewise, there are data suggesting an association of increased cardiovascular mortality with subclinical hypothyroidism[22].

1.2 Statement of the problem

Studies in the past decade have revealed that thyroid dysfunction especially goiter is endemic in several highlands of Ethiopia; most of these studies have concentrated on nutrition aspect of iodine deficiency disorder and general prevalence. In different studies conducted in immigrant children and adult show that the prevalence of goiter is up to 43.6% - 46.1% [23, 24]. In one study in Addis Ababa at Tikur Anbesa Hospital, the prevalence of hypothyroidism among adults with thyroid disorders were found to be 2.9% & 0.2% in women & men, respectively (25). Whereas hypothyroidism was found to be 24% among children with thyroid disorders were found in Ethio-Swedish children's hospital (26).

In another epidemiological study of thyroid disease in Ethiopia; the prevalence and incidence of hypothyroidism and hyperthyroidism varies, depending on whether overt and subclinical forms are included and whether newly or previously diagnosed dysfunction is considered. In an overview of the literature, the prevalence is 2 in 1000 for overt and 6 in 1000 for subclinical hyperthyroidism in iodine sufficient area (ISA). The values for hypothyroidism are 5 in 1000 and 15 in 1000, respectively. Change from iodine deficient area (IDA) to ISA: increases the percentage of hyperthyroidism up to 4 years after salt iodination. Whereas this effect is transient for Plummer's disease, a change from IDA to ISA seems to lead to a permanent increase in overt and subclinical Graves' disease. Thyroid cancer: most studies demonstrate that the histopathological types of thyroid cancer are different in IDA and ISA. There is a tendency toward an increase in differentiated and decrease of anaplastic cancer. The ratio of papillary to follicular thyroid cancer ranges from 6.5:1 to 3.4:1 in areas with high iodine intake, decreases 3.7:1 to 1.6:1 in areas with moderate iodine intake, and ranges from 1.7:1 to 0.19:1 in IDA [27].

Recently published article shows that adiposity (the fat found in adipose tissue) especially waist circumference is a vital tool in identifying cardiovascular disease risk among Ethiopian

adults [28]. In another article, high blood pressure is widely prevalent in Addis Ababa and may represent a silent epidemic in this population. Overweight, obesity and physical inactivity are found to be important determinants of high blood pressure [29]. Besides, stroke is not uncommon in our setting and associated with significant morbidity and mortality compounded by delayed diagnosis and possibly by less accurate etiological and clinical diagnosis [30].

Relations between thyroid function and lipid status remain incompletely understood in Ethiopia. Besides the emerging concern of stroke and hypertension, goiter is one of the highly endemic and prevalent noninfectious diseases in the country. Moreover, even though health policy and strategy mainly focus on prevention of communicable diseases and physicians may fail to manage lipid and lipoprotein abnormalities, and cardiovascular risks while treating their patients for thyroid dysfunctions. Hence, the present study will undertake physical examinations and laboratory investigation to assess the lipid and lipoproteins patterns and cardiovascular risk factor indicators in thyroid dysfunction patients.

2. Literature review

2.1 Thyroid hormone and lipid and lipoprotein metabolism

Studies were carried out by Abrams, J. J., and S. M. Grundy in California, U.S.A. on cholesterol metabolism in 11 non-obese patients and 16 obese patients with hypothyroidism and 13 with hyperthyroidism elucidate that: Hypothyroid patients usually had an increase in low density lipoprotein (LDL)-cholesterol. Treatment of hypothyroid patients produced the expected fall in LDL. On the other hand, the hormones appeared to increase the synthesis of cholesterol. Patients with hypothyroidism frequently had supersaturated bile. In contrast, the usually thin hyperthyroid patients did not have supersaturated bile. Other studies show that thyroid hormones a) influence LDL-cholesterol by an action on the catabolism of LDL independent of alterations in synthesis, catabolism, absorption, or excretion: b) Stimulate synthesis of cholesterol; and c) affect biliary lipid metabolism in large part by influencing energy balance and cholesterol synthesis [31].

Bjorn O Asvold and collages conducted a cross-sectional, population-based study with 30 656 individuals without no apparent thyroid disease to see if there exist any sort of associations with lipids. They found out that, within the reference range of TSH, there was a linear and significant (P for trend<0.001) increase in total serum cholesterol, LDL cholesterol, non-HDL cholesterol and triglycerides, and a linear decrease (P for trend < 0.001) in HDL cholesterol with increasing TSH. Subgroup analyses showed statistically significant associations for all lipids in men above 50 years of age, and for triglycerides in all age groups. For women, associations were statistically significant in all age groups except for HDL cholesterol in women below 50 years of age. The associations with triglycerides and HDL cholesterol were stronger among overweight than normal weight individuals [34].

Elizabeth et al, conducted prospective clinical study and cross-sectional cohort analysis in Bosten U.S.A. to determine whether lipoprotein sub-particle concentrations are associated with thyroid status. They found out that total cholesterol and LDL-C were increased during short-term overt hypothyroidism. Large LDL sub-particle concentrations increased during hypothyroidism (917±294 vs. 491±183 nmol/L; p<0.001), but more atherogenic small LDL

was unchanged. Triglycerides marginally increased during hypothyroidism, small VLDL particles significantly increased ($p<0.001$), whereas more atherogenic large VLDL was unchanged. Total HDL-C increased during hypothyroidism (76±13 mg/dL vs. 58±15 mg/dL; $p<0.001$). There was no change in large HDLC particle concentrations, whereas small ($p<0.001$) and medium ($p=0.002$) HDL-C particle concentrations decreased. Among Framingham women, adjusted total cholesterol and LDL-C were positively related to TSH categories ($p\leq0.003$). This was due to a positive correlation between adjusted large LDL sub-particle concentrations and log-TSH ($p<0.0001$); log small LDL sub-particle concentrations decreased slightly as log-TSH increased ($p=0.045$). Among Framingham men, the only significant association was a positive association between log-TSH and log large HDL sub-particle concentrations ($p=0.04$) [35].

Ali et al, conducted a correctional study to assess the prevalence of thyroid dysfunction and its correlation with serum hyperlipidemia among adult population of Murzok City, Libya. They have found out that the prevalence of thyroid dysfunction types were the following: overt hyperthyroidism (0.84%), subclinical hyperthyroidism (0.84%), overt hypothyroidism (1.12%), and subclinical hypothyroidism (6.18%). Also, these thyroid dysfunctions were more common in females (0.56%, 0.84%, 0.84%, and 4.21%) than in males (0.28%, 0.00%, 0.28%, and 1.97%) respectively. They found a higher prevalence of subclinical hypothyroidism (27%) among the subjects with hypercholesterolemia. They also found a significant negative correlation between subjects with normal T_3 and hypercholesterolemia ($P<0.05$), and a significant positive correlation between subjects with high T_4 and HDL ($P<0.05$) [33].

2.2 Thyroid function and cardiovascular risk factors

Park et al, assessed the relation of thyroid dysfunction to metabolic syndrome at an earlier stage in Korean population. Metabolic parameters such as body composition, blood pressure (BP), fasting glucose, total cholesterol, triglyceride (TG), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), thyroid-stimulating hormone (TSH) and free thyroxine FT_4 were measured. He followed up 5,998 Koreans aged over 18 yr for three years. And he found 694 cases of metabolic syndrome at follow-up. The mean age of the subjects was 45.6 ± 9.5 yr. Mean level of TSH was 2.02 ± 1.50 mIU/L, mean level of fT(4) was 1.23 ± 0.20 ρM/L. At

baseline, TSH levels and FT_4 levels were associated to waist circumference, BP, glucose and lipids in the subjects. Increase in systolic blood pressure, diastolic blood pressure (DBP), total cholesterol and TG were significantly associated with changes in TSH levels after adjustment. Changes in DBP, TG, HDL-C and fasting glucose were significantly associated with changes in FT_4 levels after adjustment. Increase in TSH levels even after further controlling for baseline TSH level predicted the metabolic syndrome over the study period. He concluded that, there is a relationship between thyroid function and cardiovascular risk factors, such as BP, total cholesterol, TG, HDL-C and fasting glucose. Also, higher levels of TSH may predict the metabolic syndrome in Korean [36].

Borge et al, conducted a research to critically evaluate lipoprotein(a) [Lp(a)] as a cardiovascular risk factor and, to advise on screening for elevated plasma Lp(a), on desirable levels, and on therapeutic strategies. They found out that the robust and specific association between elevated Lp(a) levels and increased cardiovascular disease (CVD)/coronary heart disease (CHD) risk, together with recent genetic findings, indicates that elevated Lp(a), like elevated LDL cholesterol, is causally related to premature CVD/CHD. The association is continuous without a threshold or dependence on LDL- or non-HDL-cholesterol levels. Mechanistically, elevated Lp(a) levels may either induce a prothrombotic/anti-fibrinolytic effect as apolipoprotein(a) resembles both plasminogen and plasmin but has no fibrinolytic activity, or may accelerate atherosclerosis because, like LDL, the Lp(a) particle is cholesterol-rich, or both. They advised that Lp(a) be measured once, using an isoform-insensitive assay, in subjects at intermediate or high CVD/CHD risk with premature CVD, familial hypercholesterolaemia, a family history of premature CVD and/or elevated Lp(a), recurrent CVD despite stain treatment, ≥3% 10-year risk of fatal CVD according to European guidelines, and/or ≥10% 10-year risk of fatal + non-fatal CHD according to US guidelines. As a secondary priority after LDL-cholesterol reduction, they recommended a desirable level for Lp(a) of 80^{th} percentile (less than 50 mg/dL) [37].

Moffat et al, carried out a research in Edinburgh, Scotland. to determine how far the extent of subclinical or treated thyroid disease is associated with increased vascular risk, and to explore whether the nature and/or treatment of thyroid disease are critical in this relationship, and found out that: Patients treated for Graves' disease had more hospitalizations from

cardiovascular disease than controls (relative risk, 1.42; 95% confidence interval, 1.20 to 1.67; $p < 0.001$). Toxic multinodular goiter was also associated with significantly higher rates of cardiovascular disease (relative risk, 1.50; 95% confidence interval, 1.11 to 2.02; $p = 0.008$). Patients with Hashimoto's thyroiditis aged over 50 years had a threefold increase in cardiovascular admissions compared to controls (23.5% and 6.5%, respectively; 95% confidence interval for difference, 6.0% to 27.9%; $p = 0.003$). Thus, different forms of thyroid disease were associated with increased long-term vascular risk despite restoration of euthyroidism [32].

Celestino et al, condacted a review of literatures published between 1980 and 2007 in Portugal and found out that increased cardiovascular risk in thyroid dysfunction is related to lipid profile, endothelial dysfunction, metabolic, hormonal and hemodynamic changes and coagulation disturbances. Because of its high prevalence in the population, hypothyroidism is the principal functional disorder. Lipid anomalies associated with hypothyroidism are at least partially responsible for the increase in coronary heart disease. The clinical benefit of treating subclinical hyperthyroidism is still the subject of debate, but treatment appears to be associated with beneficial effects on lipid profile and cardiovascular function [1].

3. Significance of the Study

Thyroid dysfunction has a great impact on lipids as well as a number of other cardiovascular risk factors [38]. On the other hand the emerging of hypertension and stroke is becoming public health trait in our country, Ethiopia [29, 30]. Since thyroid dysfunction is endemic in our country, the burden and complication of this non communicable disease is might be underestimated. Hence, the present study is designed to collect data and information on various types of thyroid dysfunction patients and will show the magnitude and complication of dislipidemia and risk factor for CVDs.

Ethiopian health policies and management give priority for communicable diseases. However, recent studies show that communicable diseases are silent killer in developing country including Ethiopia. Therefore, this study will provide information for policy makers and as a baseline data for further studies.

4. Objectives of the study

4.1. General objective

- To determine the pattern of lipids and lipoproteins, and risk cardiovascular disease among thyroid dysfunction patients referred to the Ethiopian Health and Nutrition Research Institute.

4.2. Specific objectives

- To determine the pattern of lipid and lipoproteins among study subjects.
- To determine the magnitude of thyroid abnormalities among study subjects.
- To determine the relation between thyroid abnormalities verses lipid profile.
- To determine the relation between thyroid abnormalities and cardiovascular disease risk factors.

4.3. Hypothesis

- Thyroid dysfunction can affect the lipid and lipoprotein profile.

5. Methodology

5.1. Study period

The study was conducted from September 2011- April 2012.

5.2. Study site:

The study was conducted in Addis Ababa at the Ethiopian Health & Nutrition Research Institute (EHNRI), which is the national referral diagnostic laboratory and research institute. The institute was established in 1948 E.C and an agency of the Federal Ministry of Health (FMoH).

5.3. Study design:

Institutional based, cross sectional with control study was conducted. In this study, a both quantitative and qualitative design form was used. To determine the possibility of inclusions in the study, laboratory clients were considered for exit interview and physical examination during which information on their medication was captured and nutritional parameters were recorded.

5.4. Source population:

The source populations were adolescent, adult, and elderly who visit EHNRI reception for referral laboratory diagnostic service from different part of the country.

5.5. Study population:

The study populations were the total number of Adolescents, Adults, and Elderly who were referred for the laboratory investigation of thyroid dysfunctions during the study period from different part of the country.

5.6. Inclusion and Exclusion Criteria:

Inclusion criteria:

- Patients equal or older than 18 years requested for thyroid function tests
- Patients both on or without treatment for thyroid dysfunction
- Respondents who willing to participate in this study

Exclusion criteria:

- Children and Infants
- Diabetic patients
- Patients requested for thyroid function tests who were on treatment for diabetics, anti-hypertension and known medication
- Patients who didn't want to participate in this study

5.7. Sample size:

To determine the sample size for two independent samples, the following formula is used.

$$n1 = n2 = \frac{\left[Z\alpha/2\sqrt{(r+1)pq} + Z\beta\sqrt{rp1q1 + p2q2}\right]^2}{r(p1-p2)^2}$$

Were using

- r = number of control from the previous study/number of cases from the previous study
- Study in Nepal (44); number of control = 100 and number of cases = 567
- r = 100/567 = 0.1764
- *p*1= The proportion of lipid profile of Thyroid Dysfunction patients = 39.72% = 0.3972
- *q*1= 1-*p*1 = 1- 0.3972= 0.6028
- *p*2= The proportion of lipid profile of non-Thyroid Dysfunction control groups = 9% = 0.09
- *q*2=1-*p*2= 1-0.09=0.91
- *p* = (*p*1 + r*p*2)/(r+1) =0.3972+ (0.1764 x 0.09) /(0.1764+1) = 0.351
- *q* =1-0.351= 0.649
- α (95%) = 0.05(2-sided) = Z 0.025 = 1.96
- power (80%) = 1-β = 0.80 = Z 0.2 =0.842

$$n1 = n2 = \frac{\left[Z\alpha/2\sqrt{(r+1)pq} + Z\beta\sqrt{rp1q1 + p2q2}\right]^2}{r(p1-p2)^2}$$

$$n1 = n2 = \frac{\left[1.96\sqrt{(0.1764+1)0.351*0.649} + 0.842\sqrt{(0.1764*0.3972*0.6028)+(0.09*0.91)}\right]^2}{0.1764(0.3972-0.09)^2} = 103.29$$

So with 5% contingency, calculated samples size were approximately 108 for thyroid dysfunction groups and 108 controls.

5.8. Study Variables

Dependent variables

1. Lipids and lipoproteins abnormalities,
2. Cardiovascular disease risk indicator

Independent variables

1. Socio-demographic characteristics
2. Physical examination for thyroid dysfunction
3. Nutritional status
4. Thyroid medication
5. Body Mass Index (BMI), Waist to Hip Ratio (WHR)
6. Systolic Blood Pressure, and Diastolic Blood Pressure

5.9. Data collection method

Structured data collection Questionnaire was used for collection of information on patient's demography, physical examination and confounding variables.

5.10. Laboratory Investigation

Specimen Collection:

For those included in this study, blood was drawn for their thyroid functional routine investigation testing. Appropriate amount (5-7ml) of blood sample was collected by venipuncture from fasting individuals using an evacuated tube system. Then the sample was transported to EHNRI Clinical Chemistry Laboratory with Ice Box for processing and analysis. Finally, serum was separated from the cells with one hour of sample collection. Leftover serum specimen was used for lipid and lipoprotein profile analysis, since they already provide blood for their thyroid hormonal tests.

Analytical Techniques and Procedures:

Laboratory analysis of thyroid functional test is based on Cobas-e-411 electrochemiluminisence (ECL) hormonal analyzer. And the levels of glucose, total cholesterol, HDL-C, triglycerides and lipoprotein A was estimated by COBAS INTEGRA 400 random access full automated auto analyzer and LDL-C concentration was calculated by Friedwald equation. Glucose was determined by Hexokinase (HK) method, triglycerides and total cholesterol was evaluated with enzymatic colorimetric method and HDL-C was analyzed by homogenous enzymatic colorimetric method (Annex III).

The cutoff point for the categorization of the glucose, total cholesterol, HDL-C, LDL-C and triglycerides levels was based on the guidelines of the American Diabetes Association and National Cholesterol Education Program (NCEP) III (42,43).

Thyroid functional tests: FreeT_3, FreeT_4, T_3, T_4, TSH, TG, T-Up take

Principle: Electrochemiluminisence (ECL) Immuno Assay

Procedure: Fully automated hormonal analyzer (ELCSYS 2010) will be used.

Interpretation: Referance range of the manufacturer was used

Lipids (Total cholesterol, HDL-Cholesterol, LDL-Cholesterol and Triglyceride) and **Glucose**:

Principle: Spectroscopic

Procedure: Fully automated clinical chemistry analyzer (Cobas Integra-400) was used.

Interpretation: Reference range of the manufacturer was used.

Lipoproteins: lipoprotein A,

Principle: Turbidometric

Procedure: Fully automated clinical chemistry analyzer (Cobas Integra-400) was used.

Interpretation: Reference range of the manufacturer was used.

5.11. Data Management and analysis

After having received a clear explanation of the objectives of the study and having signed the informed consent, the patients answered a questionnaire; gave information regarding Socio-demographic characteristics, Habits, Medication history, Sign and symptoms, and Nutritional

parameters. Height and weight were assessed for calculating the Body Mass Index. Wrist, Forearm, and Hip circumference were measured to calculate waist-to-hip ratio (WHR). Blood pressure and body temperature were measured. Laboratory investigation results for thyroid function, lipid and lipoprotein were collected from EHNRI clinical chemistry laboratory.

The completed data collection Questionnaire was checked for completeness, consistency and was coded by the principal investigator. Data entry was done using Microsoft Excel . Data cleanup was performed to check for accuracy, consistencies and values. Any error identified was corrected. Data analysis was performed using scores and odds ratio using SPSS version 19 software and STATA version 8 to look an association between independent variables and dependent variables. P values less than 0.05 was considered statistically significant.

5.12. Data quality assurance

The data collection Questionnaire was pre-tested before the actual data collection. All clinical and physical examination were filled and checked by the investigator. The Laboratory examination included internal quality controls (pathologic and normal) and external quality assessment scheme. The investigator double checked the correctness of data collection.

5.13 Ethical considerations

Before the research work, ethical clearance was obtained from the Institutional Review Board (IRB) of School of Medical Laboratory Science, Addis Ababa University. A formal letter of cooperation was requested to EHNRI**.** Nurses from the EHNRI laboratory collected patient data and information which may expose the identity of individual patients were not collected, which ensure confidentiality. The Patients received their results and those with highly elevated levels of glucose and lipid profile were advised to contact with clinicians for further diagnosis and treatment accordingly.

5.14 Dissemination of results

This study could serve as a reference material for researchers, experts or policy makers for intervention. To reach these bodies, the finalized paper was submitted to the SMLS, AAU. So

it can serve as a reference in the library. In addition, the result will also be disseminated through publication in peer reviewed local and international journals and through presenting it at relevant workshops and seminars.

5.15 Limitation

In this correctional with control study, this does not capture all physical examination at individual level. And didn’t include important laboratory examinations, such as Apo-B100. And this paper may include confounders for lipid abnormalities such as drugs.

6. Result

6.1 Demographic Characteristics

A total of 212 subjects were included in this study; 106 of them are suspected or confirmed subjects for thyroid dysfunction and the remaining 106 were age and sex matched apparently health subjects. 10.4% of study participants were males whereas 89.6% were females. The overall mean age in both cases and control subjects were 39.2± 15.2. 42.5% were in their 20's and 30's, followed by 40-59(%), 60-80(%) (older) and less than 20(%)(younger). The majority of our study subjects were (60.8%) married and 24.1% were single, and the rest 15% were either divorced or widowed. Amhara is the major ethnicity included with our study, accounting for 45.8% followed by Oromo 26.9%, Gurage 9.4%, Tigre 6.6%, and other 11.3%. Most of the study subjects live in Addis Ababa (64.6%) and the rest 35.4% were out of Addis Ababa. Most of the participants were merchants which account for 30.7% followed by government employee (20.3%), students (18.4%), private employee (11.8%) and the rest (19%) account for house wife or unemployed. The majority of study participant (33.5%) have completed grade 7-12, followed by college or university students or graduates (27.8%), grade 1-6 (15.6%), illiterate (13.2%), and the rest can only read and write (9.4%).

6.2 Cardiovascular disease risk indicators.

Almost all (95.3%) of the study subjects consume carbohydrate containing foods such as bread, Ingera, and others on regular basis. High protein diets, including milk and milk products, meat, poultry, eggs, and fish are occasionally consumed by the majority of the subject. In addition, more than 90% of the study subjects (in both control and cases) haven't changed their nutritional habits over the last one year. There is no statically significant difference between cases and controls with regard to nutritional parameters ($p>0.05$). (Table 1)

On the other hand, more than 88% of the subjects have never drunk alcohol, smoked cigarettes, and chewed chat. And almost 83% of the study participants do not perform physical exercise regularly. There is no statically significant different between in

drinking alcohol, smoking cigarette, chewing chat, and regular exercise between cases and control in our study participants. (Table 1)

Table 1. Distribution of study participants according to nutritional parameter and habits at EHNRI, Addis Ababa, Ethiopia, 2012.

Variable		Case	Control	P value	Total
Milk and milk products	≥1/day 1-4/week 1-2/month Occasionally Never	3 (2.8%) 4 (3.8%) 21(19.8%) 46 (43.4%) 32(30.2%)	2 (1.9%) 5 (4.7%) 15(14.2%) 53(50.0%) 31(29.2%)	χ^2= 1.9763 P= 0.740	5(2.3%) 7(3.3%) 36(16.9%) 99(46.7%) 65(30.6%)
Meat (including poultry, fish, etc…)	≥1/day 1-4/week 1-2/month Occasionally Never	1(0.9%) 9(8.5%) 25(23.6%) 52(49.1%) 19(17.9%)	2(1.9%) 6(5.7%) 23(21.7%) 57(53.7%) 18(16.9%)	χ^2= 1.2731 P= 0.866	3(1.4%) 15(7.1%) 48(22.6%) 109(51.4%) 37(17.4%)
Eggs	≥1/day 1-4/week 1-2/month Occasionally Never	1 (0.9%) 6 (5.7%) 20(18.9%) 49(46.2%) 30 (28.3%)	2(1.9%) 7(6.6%) 20(18.9%) 47(44.3%) 30(28.3%)	χ^2= 0.4519 P= 0.978	3(1.4%) 13(6.1%) 40(18.9%) 96(45.3%) 60(28.3%)
Carbohydrates	≥1/day 1-4/week 1-2/month Occasionally Never	104(98.1%) 0(0%) 0(0%) 2(1.9%) 0(0%)	98(92.5%) 0(0%) 0(0%) 8(7.5%) 0(0%)	χ^2= 3.7782 P= 0.052	202(95.3%) 10(4.7%)
Change in diet	Yes No	14(13.2%) 92(86.8%)	15(14.2%) 91(85.8%)	χ^2= 0.0399 P= 0.842	29(13.7%) 183(86.3)
Alcohol Drinking	Daily Weekend Occasionally Never	1(0.9%) 2(1.9%) 8(7.5%) 95(89.6%)	2(1.9%) 4 (3.8%) 8(7.5%) 92(86.8%)	χ^2= 2.1 P= 0.833	3(1.4%) 6(2.8%) 16(7.5%) 187(88.2%)
Cigarette Smoking	Chain Social Never	3 (2.8%) 3 (2.8%) 104(98.1%)	2(1.9%) 4(3.8%) 100 (94.3%)	χ^2= 2.1 P = 0.354	4(1.8%) 4(1.8%) 204(96.2%)
Chewing Chat	Daily Social Never	5(4.7%) 1(0.9) 100(94.3%)	2(1.9%) 2(1.9%) 102(96.2%)	χ^2= 2.3 P = 0.511	7(3.3%) 3(1.4%) 202(95.3%)
Exercise	Daily 2-3 days/week Occasionally Never	7(6.6%) 5(4.7%) 4(3.8%) 88(83.0%)	3(2.8%) 9(8.5%) 4(3.8%) 88(83.0%)	χ^2 = 2.9 P= 0.817	10(4.7%) 14(6.6%) 8(3.8%) 176(83.0%)

The average systolic BP of the subjects was 122 mHg, and diastolic BP was 79mmHg while the heart rate was 79/min. The average blood sugar was 87mg/dl were as body mass index and waist-hip ratio was 22.8 and 0.79 respectively. There is no statically difference in age and blood glucose level between cases and control in study subjects. Table 2.

Table 2. Distribution of study participants according to anthropometric measurements at EHNRI, Addis Ababa, Ethiopia, 2012.

Basic Characteristic	Case	Control	P value	Total
Age	39.3±15.1	38.9±14.8	P = 0.8655	38.1
Systolic BP	129.3±18.3	115.0±6.7	P < 0.0001	122.2
Diastolic BP	83.2±12.5	75.2±5.9	P < 0.0001	79.2
Heart Rate	81.4±16.6	77.1±8.6	P = 0.0164	79.2
BMI	23.4±4.9	22.1±2.6	P = 0.0165	22.8
WHR	0.94±0.22	0.64±0.16	P < 0.0001	0.79

6.3 Quality Control of Sample result

PreciControl Universal (PCU) level1 for normal, level2 for pathological is used to control the quality of laboratory investigation of thyrid function tests (i.e T3, T4, TSH, FT3, FT4, T-Up, and TG) on COBAS-e-411 immunochemitry analyzer. Precinorm Universal (PNU) normal level Precipath Universal (PPU) pathological level control sample were used for glucose, total cholesterol (TC), and triglyceride (TRI). Precipath HDL-C/LDL-C (PPHL) pathological level control was used for high density lipoprotein cholesterol (HDL) and low density lipoprotein cholesterol (LDL). All controls were run reputedly before the analysis of the sample and during each run of the sample daily. The calculated mean and standard deviation of repeatedly ran control samples by both analyzers were compared to the assigned mean and standard deviation of the kit insert for the thyroid function test, glucose, lipid, and lipoprotein reagents. These values and coefficient of variation (CV≤ 5%) were under the accepted range according to quality control rules. (Annex IV)

6.4 Magnitude of thyroid dysfunction

From the total of 212 subjects included in this investigation during the study period, majority of the subjects were euthyroid (54.7%), followed by hyperthyroidism (23%) in which 9.4%, 6.1%, and 7.6% account for overt hyperthyroid, sub-clinical hyperthyroid, and T3 toxicosis, respectively. The rest 22.2% were hypothyroid in which 2.8%, 19.3%, account for overt hypothyroid and sub-clinical hypothyroid, respectively. Thyroid dysfunctions in the form of overt hyperthyroid, sub-clinical hyperthyroid, and T3 toxicosis and overt hypothyroid was observed mainly in the cases than in controls. However, the number of sub-clinical hypothyroids greater in control subjects than the cases. In addition to the above condition, 37% of the subjects have elevated thyrogloblin in their blood (Table 4).

Thyroid dysfunctions were more frequent in women than male subjects. Of the thyroid dysfunctions sub-clinical were frequently observed among 20-39 whereas, overt thyroid dysfunction were prominent among 40-60 years of age(Table 4).

Table 4.Magnitude of thyroid dysfunction among age sex groups at EHNRI, Addis Ababa, Ethiopia, 2012.

Thyroid Disorder		**AGE**				**SEX**		**Total No (%)**
		<20	20-39	40-	>60	Male	Female	
Euthyroid	Case	2	22	7	10	6 (5.7)	35(33.0)	41(38.0)
	Control	9	29	28	9	4(3.8)	71(66.9)	75(57.5)
Overt hypothyroidism	Case	1	3	1	0	0(0)	5(4.7)	5(4.7)
	Control	0	1	0	0	0(0)	1(0.9)	1(0.9)
Subclinical hypothyroidism	Case	0	3	9	2	1(0.9)	13(12.3)	14(13.2)
	control	2	13	8	4	6(5.7)	21(19.8)	27(25.5)
Overt Hyperthyroid	Case	2	7	11	0	2(1.9)	18(16.9)	20(18.9)
	control	0	0	0	0	0	0	0(0)
Subclinical hypothyroidism	Case	3	3	5	0	0	11(10.4)	11(10.4)
	control	0	1	1	0	0	2(1.9)	2(1.9)
T3 Toxicosis	Case	4	5	4	2	2(1.9)	13(12.3)	15(14.2)
	control	0	0	1	0	1(0.9)	0	1((0.9)
Tyroglobulin	Case	6	23	23	6	4(3.8)	54(50.9)	58(54.7)
	control	1	9	6	5	3(2.8)	18(16.9)	21(19.8)
Total	Case	12	43	37	14	11(10.4)	95(89.6)	106(100)
	control	11	44	38	13	11(10.4)	95(89.6)	106(100)

6.5 Comparison of thyroid function tests and lipid and lipoprotein profile by mean.

Comparison of the mean of thyroid function tests between cases, and control shows; clear statistically significant means difference between case and control group in the level of T3, FT3, T4, TSH and thyroglobline in the serum ($p< 0.05$). However, there is statistically insignificant different between mean of FT4 and T-Uptake in both subject groups (Fig. 1.).

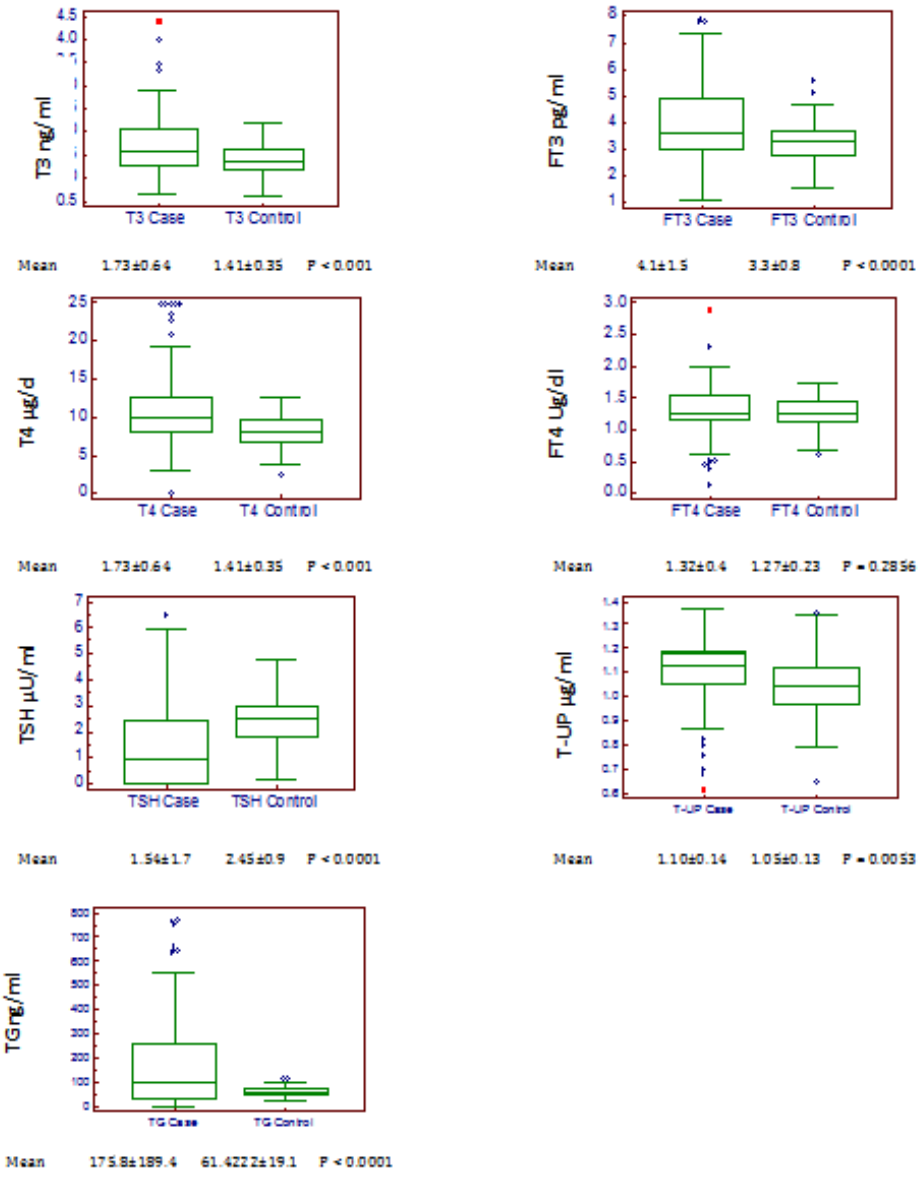

Figure 1. The comparison of tyroid function test among case and control subjects at EHNRI, Addis Ababa, Ethiopia, 2012.

Compare of the means of lipid and lipoprotein parameters showed clear and statistically significant difference between case and control group in there level of TC, TRI, HDL, LDL, Lp(A), Apo-A. ($p < 0.05$) (Fig 2).

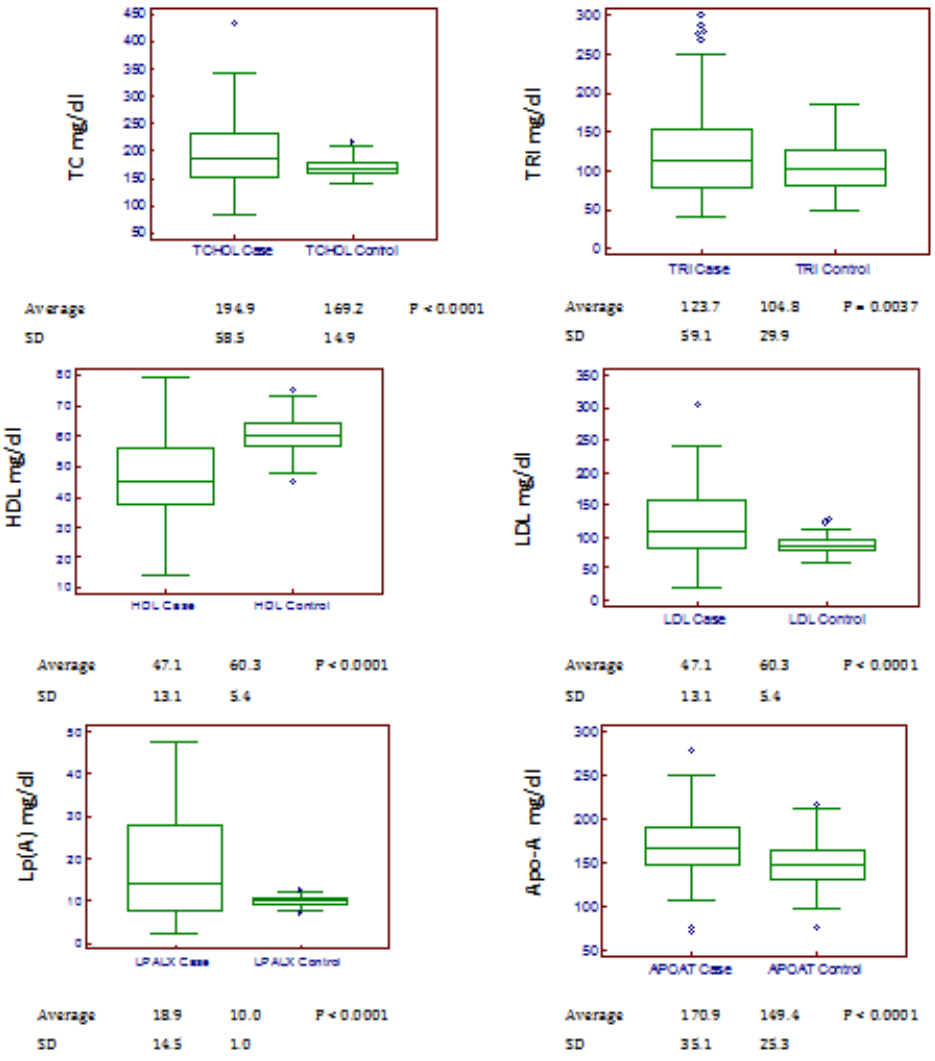

Figure 2. The comparison of lipid and lipoprotein profile among case and control subjects at EHNRI, Addis Ababa, Ethiopia, 2012.

6.6 Comparison of the means of lipid profile parameters between normal and thyroid dysfunction patients

Among case group having euethyroid state, there is no statistical difference with their lipid and lipoprotein profiles with controls. However, cases having overt and subclinical thyroid dysfunctional had significant statistical difference in almost all performed parameter for lipid and lipoproteins ($p<0.05$). From cases having hyperthyroid state: HDL and Lp(A) had significant statistical difference with the control. Among T3 Toxicosis, TC and LDL in sub-clinical hyperthyroid had significant statistical difference, where as HDL was the only lipid that have significant statistical difference with the control groups among overt hyperthyroid subjects. Table 5.

Table 5. The association of mean lipid and lipoprotein profile among different thyroid dysfunction at EHNRI, Addis Ababa, Ethiopia.

Lipids and Lipoproteins Profile	Thyroid Disorders							Normal
	Euthyroid	Overt Hypothyroid	Subclinical Hypothyroid	Overt Hyperthyroid	Subclinical Hyperthyroid	T_3 Toxicosis	Tyrogloblin	
TC mg/dl	189.0±48.0	197.2±56.2	229.7±76.7	169.1±35.9	217.5±67.9	176.0±50.2	197.9±60.9	169.2±14.9
	P = 0.3244	P = 0.0019	P = 0.0099	P = 0.9911	P = 0.0204	P = 0.7726	P = 0.0009	
TRI mg/dl	119.9±51.3	134.2±61.5	158.1±60.7	97.9±43.5	157.0±94.5	108.3±43.3	128.9±62.2	104.8±29.9
	P = 0.4777	P = 0.0477	P = 0.0035	P = 0.7261	P = 0.0766	P = 0.6644	P = 0.0033	
HDL mg/dl	48.0±12.7	43.4±15.8	45.8±11.4	46.7±12.1	48.6±17.2	46.3±14.8	47.9±12.9	60.3±5.4
	P = 0.0598	P < 0.0001	P = 0.0002	P < 0.0001	P = 0.0684	P = 0.0018	P < 0.0001	
LDL mg/dl	112.2±39.9	123.4±49.2	147.4±64.7	99.5±32.4	133.2±54.2	99.6±44.6	120.3±52.2	87.9±13.2
	P = 0.1863	P < 0.0001	P = 0.0040	P = 0.1880	P = 0.0104	P = 0.5089	P < 0.0001	
Lp(a) mg/dl	20.3±19.8	24.6±32.9	10.3±1.3	16.8±16.2	17.8±15.5	20.2±10.5	22.7±28.8	10.0±1
	P = 0.2586	P = 0.0059	P = 1.0000	P = 0.0769	P = 0.1139	P = 0.0011	P = 0.0010	
ApoA mg/dl	161.5±17.6	172.0±32.3	178.2±31.2	166.8±35.7	178.1±39.4	163.9±43.5	172.5±34.9	149.4±25.3
	P = 0.7300	P = 0.0019	P = 0.0165	P = 0.1056	P = 0.0668	P = 0.3386	P < 0.0001	

Table 6. Odd ratio for the association among thyroid dysfunction adjusted mainly for age group, lipid and lipoprotein profiles, and thyroid medication at EHNRI, Addis Ababa, Ethiopia, 2012.

Lipid and Lipoproteins	Euthyroid		Hypothyroid		Hyperthyroid		Tyoglobline	
	Odds Ratio	P value	Odds Ratio	P value	Odds Ratio	P value	Odds Ratio	P value
Sex	.8241867	0.819	1.143721	0.091	1.058232	0.946	1.202142	0.828
Age	.8446039	0.600	1.996076	0.120	.7891802	0.446	1.192984	0.583
TC	2.84318	0.203	1.613881	0.006	.2616647	0.037	7.621825	0.076
TRI	.7355031	0.542	1.992988	0.007	.8231995	0.095	2.071035	0.144
HDL	.6823867	0.459	0.59733	0.004	1.088338	0.067	.2923653	0.025
LDL	.8253518	0.814	1.5362291	0.081	0.890069	0.083	.1198661	0.065
Lp(A)	1.400359	0.444	1.6572645	0.025	1.90104	0.052	1.259712	0.601
Apo A	.438152	0.216	1.505429	0.055	1.69322	0.104	.2068355	0.022
Medication For thyroid dysfunction	.6225	0.042	1.29	0.565	1.31	0.497	0.735	0.70

7. Discussion

The present study looks in to the level of lipid and lipoprotein profile parameters among thyroid dysfunction confirmed or suspected subjects and compare them with apparently healthy individuals. A total of 212 subjects, 106 cases and 106 controls where enrolled and both sex and age matched. There was no statistically significant differences ($p<0.05$) with the mean and age in both case and control subjects, 39.3±15.1 and 38.9±14.8, mean respectively.

During physical examinations we undertook anthropometric measurements and interviewed for signs and symptoms of thyroid dysfunction, medication history, and nutritional parameters and other habits. On the base of our interviews and physical examinations, we found statistically significant difference between mean values in cases than controls with regard to Systolic BP, Diastolic BP, Heart Rate, BMI, and WHR ($p<0.05$). The means of these observations were generally higher in cases than control groups. Our finding is supported by other previously performed studies (39, 40). However, there were no statistically significant differences between cases and control in nutritional and other habits such as drinking alcohol, smoking cigarettes, chewing chat, and performing physical exercise.

Laboratory investigation of thyroid hormones shows that there is statistically significant variations between the means of cases and controls in T3, T4, TSH, FT3, and TG ($p<0.0001$). Conversely, no statistically differences were observed for FT4 and T-Up between both groups. This can explain by the presence of higher number of sub-clinical hypothyroids. This finding may be due to Ethiopia is a country that has mild iodine deficiency (41) that may contribute for sub-clinical hypothyroid conditions or the reference range for apparently healthy subjects in our country could be lower than available in the literature.

Thyrogloblin was found in 54.7% of the cases and 19.8% of control subjects above the reference value appear in the procedure. This observation explains the fact that morphological integrity change is three fold more common in cases than control subjects. And TG plays a decisive role in the synthesis of the peripheral thyroid hormones T3 and T4. The higher production of TG is stimulated by TSH, intrathyroidal iodine deficiency and the presence of thyroid-stimulation immunogloblins.(42, 43)

The majority, 70.8% of control and 38.7% cases were found to be euthyroid subjects. Whereas almost all hyper thyroid (overt, subclinical, and T3 toxicity) and overt hypothyroidism is found in cases than control subjects. The most affected age group is among 20-39 among control. Age had a positive correlation in that thyroid dysfunction in which thyroid dysfunction is more prevalent with increasing age in both sexes. Thyroid disfunction was found more frequent in women than in male subjects. A multivariate statistical analysis shows that, being a women predispose to a 1.45 times higher risk for having thyroid dysfunction. The major thyroid dysfunction among women is hypothyroid. Sub-clinical hypothyroid is more frequent at reproductive ages of 20-39 and overt hypothyroid is relatively frequent at ages of near menopause (above 40years). This can be explained by the fact that those undetected subclinical thyroid dysfunctions commonly seen in young ages mostly are caused by the increased demand for thyroid hormones for growth and sexual maturity would clinically manifest after or near menopause. In another similar study conducted in Nepal reflect similar situation however being women is 1.34 time risk for thyroid dysfunction(44).

The laboratory investigation of lipid and lipoprotein profile parameters shows that there are statistically significant variation between cases and controls mean level of TC, TRI, HDL, LDL, Lp(A), and ApoA. We also found significant positive correlation between Overt hypothyroidism and TC >200mg/dl, TRI >150mg/dl, LDL> 130mg/dl, HDL<50mg/dl, Lp(A)> 11mg/dl, ApoA>150mg/dl. Although overt hypothyroidism has always been associated with hypercholesterolemia, there is much controversy on its associations with subclinical hypothyroidism and hypercholesterolemia(45, 46). In this study, all the parameters of lipid profile except HDL i.e, TC, , LDL, Lp(A) TG, and ApoA, were found to be elevated and HDL was decreased in subclinical hypothyroidism and the differences were statistically significant. The increase in total cholesterol and LDL can be attributed to the effect of thyroid hormones on the expression of LDL receptors and CYP7A, a rate limiting enzyme in bile acid synthesis(47). Decreased thyroid function does not only increase the number of LDL particles but also promotes LDL oxidation, thereby increasing the risk of atherosclerosis(47). TG levels are also increased in both overt and subclinical hypothyroidism which is attributable to the decreased activity of lipoprotein lipase that is responsible for the clearance of triglyceride rich lipoprotein(46, 48).

We also found majority of lipid (TC, TRI, and LDL) and lipoprotein (Apo-A, Lp(A)) decreased in overt hypothyroid condition. However, the increase was significant only in case of subclinical hyperthyroidism. Our finding is reflected in another research conducted by Ali his collages (33). On the other hand HDL was increased in all of hypothyroid ($p<0.005$). Elevation in HDL cholesterol could be due to decreased activity of cholesteryl ester transfer protein and hepatic lipase (48). In subclinical hyperthyroidism, however, TC and LDL levels were increased significantly. Despite the increased activity of HMG-CoA reductase, the cholesterol levels tend to be increased in hyperthyroidism due to augmented excretion of cholesterol by bile (47) together with enhanced receptor mediated catabolism of LDL particles. Variations, generally, not very marked, observed in TRI levels could be due to the action of thyroid hormone on VLDL (49).

In our study, 47.3% of the cases were taking medication for their thyroid dysfunction. Of these 21.1 % of them became euethyroid subjects after medication with L-thyronoxin and their lipid and lipoprotein profiles returned to normal. This may be the effect of treatment, especially for hypothyroid subjects on follow-up. In addition, this phenomenon is also reflected in our multi-variance analysis. The fact that thyroxin treatment has a potential to normalize lipid and lipoprotein levels is supported by a other literatures(50).

Multi-variance statistical analysis shows that being hypothyroid has a potential increase in lipid and lipoproteins profile parameter by 1.6, 1.9, 1.5, 1.5, and 1.7 of TC, TRI, LDL, Apo-A, and Lp(A) respectively and decrease in HDL by 0.41 times' than apparently healthy individuals. Whereas being hyperthyroid is an odd for decreasing lipid and lipoproteins such as TC, TRI, LDL, and Lp(A) by 0.74, 0.18, 0.11, and 0.09 respectively. And hyperthyroidism can boost HDL level by 1.09 times than apparently healthy individuals. Our find is also in line with previously performed researches (31, 34, 35).

8. Conclusion

This correctional, with control study is the first of its kind in Ethiopia, to explain the effect of thyroid abnormality on the metabolism of lipid and lipoproteins, and risk for cardiovascular disease. In general we found hypothyroidism is an agent for the increment of lipids such as TC, Triglyceride, LDL, and decrease HDL were as hyperthyroidism have had a reverse effect. The existence of frequent sub-clinical hypothyroid and thymoglobulin in apparently health subjects and reflect slight iodine iodine deficiency. Cardiovascular disease risks explain in the literature such as nutritional parameters and habits didn't have such contribution.

9. Recommendation

All thyroid abnormalities having patients should undergo laboratory investigation of lipid and lipoprotein profile. Since apparently healthy individuals in our community have high prevalence of subclinical hypothyroidism duo to slight deficiency of Iodine, Iodine supplement is highly recommended especially in younger individuals at reproductive age. In addition, screening for thyroid dysfunction is critically important to reduce the impact and complication of hydroid dysfunction

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Annexes:

Annex I: English Version Information Sheet, Consent Form, and Questionnaire

INFORMATION SHEET

ADDIS ABABA UNIVERSITY

SCHOOL OF MEDICAL LABORATORY SCIENCES

Hello, how are you? My name is Paulos Nigussie. I am currently a student of Addis Ababa University, School of Medical laboratory Sciences going to conduct a survey. I would like to interview you few questions about your Socio-demographic characteristics, Antropometrical measurements, Nutritional information, Habits, Medication history, Sign and symptoms of thyroid and related disease.

The objective of the study is to assess the relationship between thyroid statuses and serum lipid and lipoproteins which intern relate to cardiovascular disease. Your cooperation and willingness for interview will be very helpful in identifying the problems related to this issue.

Your name will not be written in the form and I assure you all the information you give will be kept strictly confidential. Your participation is voluntary and you are not obliged to answer any questions that you do not want to answer. If you are not comfortable with the interview, please feel free to stop any time you like. Do I have your permission to continue?

If yes, continue to the next page for the consent form and interview.

If no, continue to the next patient.

CONSENT FORM

I have read the information sheet above and clearly understood the purpose and anticipated benefit of the research. I hereby need to assure with my signature below that I, without any coercion or forceful act by the research team, have decided to voluntarily participate in the study to contribute my part in the effort being made

Study participant's identification | | | |

Signature _____

Date_____

Interviewer's name_____
Signature_____

Date of interview_____Time started_____ Time finished_____

Supervisor's Name_____ Signature_____

I thank you for your cooperation

QUESTIONNAIRE

Study participant's identification | | | |

Date: DD\MM\YY | | | |

1. **Socio-demographic characteristics of the study participants**

No	Questions	Response classification	Code
101	Sex of the respondents	Male = 1 Female = 2	
102	Age in years	Age in completed years___ 1<20, 2 20-40, 3 40-60, 4 60-80 Do not Know = 99 No response = 909	
103	What is your marital status?	Single = 1 Marries = 2 Divorced = 3 Widowed = 4 Other specify = 5 No response = 909	
104	What is your ethnicity?	Oromo = 1 Amhara =2 Tigray = 3 Gurage =4 Other specify……	
105	To which religion are you belonging?	Orthodox = 1 Muslim = 2 Protestant =3 Other specify…….	
106	Where is your place of residence?	Addis Ababa = 1 Outside Addis Ababa Region _____	
107	What is your last level of education?	Illiterate = 1 Read and write = 2 Grade 1-6 = 3 Grade 7-12 College = 5 No response = 909	

108	What is your current occupation ?	Student = 1 Government employee = 2 Private enterprise employee = 3 Daily laborer = 5 Merchant = 6 Housewife = 7 No job =8 Other specify…….	
109	How much income you earn monthly?	…… Ethiopian Birr Do not Know = 99 No response = 909	

2. Antropometrical measurements

No	Questions	Response classification		Code
201	Current weight in Kg			
202	Current height in m			
203	Current height in m^2			
204	Body Mass Index (BMI) in kg/m^2		Underweight = <18.5 = 1 Normal weight = 18.5–24.9 = 2 Overweight = 25–29.9 = 3 Obesity = BMI of 30 or greater =4	
205	Wrist circumference in cm			
206	Forearm circumference in cm			
207	Hip circumference in cm			
208	Waist circumference in cm			
209	waist-to-hip ratio (WHR)		male < 0.9, Female < 0.8 acceptable = 1 male > 0.9, Female > 0.8 unacceptable= 2	
210	Systolic Blood Pressure in mm/hg		< 120 = Normal = 1 120-139 = Pre-hypertension = 2 >140 = High blood pressure = 3	
211	Diastolic Blood Pressure in mm/hg		< 80 = Normal = 1 80-89 = Pre-hypertension = 2 >90 = High blood pressure = 3	
	Heart rate		55-85, 1<55, 2 55-85, 3 >85	
212	Body temperature in °C			

3. Nutritional information

No	Questions	Response classification	Code
301	How often do you consume the following foods?	- Never =1 - Occasionally (holiday, wedding, ceremony etc…) = 2 - Once or twice/month = 3 - 1-4/week = 4 - Once or more than once/day = 5	
	- Milk and milk products (cheese, butter, etc..)		
	- Meat (including poultery, fish, etc…)		
	- Eggs		
	- Carbohydrates (bread, pasta, etc…)		
302	Do you employ change in diet to mange your fat distribution?	Yes = 1 No = 2	

4. Habits

No	Questions	Response classification	Code
401	Do you drink?	Yes = 1 No = 2	
	If yes how often do you drink?	Daily = 1 Every weekend = 2 Occasionally = 3	
402	Do you Smoke?	Yes = 1 No = 2	
	If yes how often do you Smoke?	Chain smoker = 1 Social smoker = 2	
403	Do you exercise?	Yes = 1 No = 2	
	If yes how often do you exercise?	Daily = 1 2-3 days/week = 2 Occasionally = 3	
402	Do you chewing chat?	Yes = 1 No = 2	
	If yes how often do you Chewing?	Daily = 1 Social chewing = 2	

5. Medication history

No	Questions	Response classification	Code
501	Do you take any medication for your thyroid?	Yes = 1 No = 2	
	If yes what type of therapy do you take?	Levothyroxine (L-thyroxine) = 3 Methimazole (Tapazole) = 4 Propylthiouracil (PTU) = 5 Other Specify = 6_____	
	If yes what dose do you take?		
	If yes how long do you take?		
502	Do you take any medication for your lipid abnormality?	Yes = 1 No = 2	
	If yes what type of therapy do you take?	Specify = _____	
	If yes what dose do you take?		
	If yes how long do you take?		
503	Do you take any medication for your CVD?	Yes = 1 No = 2	
	If yes what type of therapy do you take?	Specify = _____	
	If yes what dose do you take?		
	If yes how long do you take?		

6. Sign and symptoms

No	Questions	Response classification		Code
601	**Thyroid dysfunction symptom** In the past three months, have you noticed any of the following?	Yes = 1	No = 2	
	fatigue, exhaustion	1	2	
	feeling run down and sluggish	1	2	
	depression	1	2	
	difficulty concentrating,	1	2	
	unexplained or excessive weight gain	1	2	
	dry, coarse and/or itchy skin	1	2	
	dry, coarse and/or thinning hair	1	2	
	feeling cold, especially in the extremities	1	2	
	constipation	1	2	
	muscle cramps	1	2	
	increased menstrual flow	1	2	
	more frequent periods	1	2	
	infertility/miscarriage (not applicable = 0) (menopause 3)	1	2	
	nervousness	1	2	
	irritability	1	2	
602	**Lipodystrophy symptom** In the past three months, have you noticed any of the following?	Yes = 1 No = 2	If yes, Increased = 3 Decreased =4	
	To be filled from interview:			
	Facial fat	1 2	3 4	
	Arm fat	1 2	3 4	
	Leg fat	1 2	3 4	
	Buttock fat	1 2	3 4	
	Abdominal fat	1 2	3 4	
	Back neck fat	1 2	3 4	
	Breast fat	1 2	3 4	
	Hip fat	1 2	3 4	
	To be filled from observation:			
	Facial fat	1 2		
	Arm fat	1 2		
	Leg fat	1 2		
	Buttock fat	1 2		
	Abdominal fat	1 2		
	Back neck fat	1 2		
	Breast fat	1 2		
	Hip fat	1 2		

Annex II: Amharic Version Information Sheet, Consent Form, and Questionnaire

□መረ□ □ፅ

አዲስ አበባ ዩኒቨርሲቲ

የህክምና ላቦራቶሪ ሳይንስ ት/ቤት

ጤናስጥልኝ! ስሜ ጳውሎስ ንጉሴ እባላለሁ፡፡ የአዲስ አበባ ዩኒቨርሲቲ የህክምና ላቦራቶሪ ሳይንስ ት/ቤት ተማሪ ነኝ፡፡ ስለ እርሶ ማህበራዊ ህይወት፣ አመጋገብ፣ ባህሪይ፣ የህክምና ታሪክ እና ስለ ህመሞ በታይሮይድ ዙሪያ አንዳነድ መጠይቅ ማድረግ እፈልጋለሁ፡፡

የጥናቱ አላማ በታይሮይድ እና በሰውነታችን ውስጥ ባለው ጮማ ብሎም ስለሚመጡ ተያያዝ ችግሮች ሲሆን፡፡ የእርሶ ተሳትፎ ማድረግ ለጥናቱ እጅግ በጣም ጠቃሚ ነው፡፡

የእርሶ ስም በመጠይቁ ላይ አይፃፍም በተጨማሪም ማንኛውም መረጃ በሚስጥር የቀመጣል፡ የእርሶ ተሳትፎ በፈቃደኝነት ላይ የተመሰረተ ነው፡፡ በጥናቱ ያለመሳተፍ መሉመብት አሎት፡፡ ጥናቱ ከተጀመረ በኻላ በማንኛውም ሰአት ራስዎን ከጥናቱ ማግለል ይችላሉ፡፡ ይህን በማድረጎ ምንም አይነት የእንክብካቤ መ1ደል አያስከትሎቦትም፡፡ ለሚወስኑት ውሳኔ ማንም ሰው ምክንያት እነዲገልጹ አያስገረድዶትም፡፡ ፈቃደኛ ኖት?

ከሆነ በሚቀጥለው ገጽ ላይ የፈቃደኝነት መረጃ እና መጠይቅ ያገኛሉ፡፡

ካልሆነ ወደሚቀጥለው ህመምተኛ

የፈቃደኝነት መረጃ

ከላይ የተጻፈውን የመረጃ ቅፅ አንብ□ የጥናቱን □ላማና ጥቅም በግልፅ ተረድቻለሁ፡፡ በዚህም መሰረት ያለ ጥናት ቡድኑ □ባላት ተ□እኖ በሙሉ ፈቃደኝነት በዚሁ ጥናት ውስጥ የሚጠበቅብኝን አስተዋ□ኡ ለማበርከት መወሰኔን በፊርማዬ አረጋግጣለሁ፡፡

የታካሚው መለያ ቁጥር | | | |

ፊርማ _____

ቀን_____

የመረጃ ሰብሳቢ ሥም _____ ፊርማ _____

መረጃ የተሰበሰበበት ቀን_____የተጀመረበት ሠዓት _____ ያለቀበት ሰዓት _____

የተቆጣጣሪ ሥም _____ ፊርማ _____

ለተባባሪነቶ እናመሰግናለን፡፡

መጠይቅ

1. ማህበራዊ ህይወት

ተ.ቁ	ጥያቄ	ምላሽ	ኮድ
101	የመጠይቁ መላሽ ጾታ ምንድን ነው?	ወንድ= 1 ሴት= 2	
102	ዕድሜዎ ከላፈው ልደት ጀምሮ ስንት ነው?	---------- ዓመት አላውቅም=99 ምላሽ የለም=909	
103	የጋብቻዎ ሁኔታ ምን ይመስላል ?	ያላገባ/ች =1 ባለትዳር እና አሁን በትዳር ላይ=2 የተፋታ/ች=3 የትዳር ጓደኛ የሞተበት/ባት=4 ሌላ ሁኔታ =5 ምላሽ የለም =909	
104	ብሔር ?	ኦሮሞ =1 አማራ = 2 ትግራይ = 3 ጉራጌ =4 ሌላ ብሔር ይግለጹ ----------	
105	የየትኛው ሐይማኖት ተከታይ ነዎት ?	ኦርቶዶክስ=1 እስልምና=2 ኘሮቴስታንት=3 ምላሽ የለም=909 ሌላ ሐይማኖት ይግለጹ----------	

106	የመኖሪያ አድራሻዎ የት ነው ?	አዲስ አበባ = 1 ከአዲስ አበባ ውጪ =2	
107	የትምህርት ደረጃዎ ሁኔታ ምን ይመስላል ?	ያልተማረ/ች =1 ማንበብና መጻፍ እችላለሁ=2 ከ1-6 ክፍል =3 ከ7-12 ክፍል/10+2 =4 ኮሌጅ የተማረ/ች = 5 መልስ የለም = 909	
108	በአሁኑ ጊዜ የሚሠሩት ስራ ምን ዐይነት ነው?	ተማሪ =1 የመንግሥት ሰራተኛ=2 የግል ድርጅት ሰራተኛ=3 የቀን ሰራተኛ=4 ነጋዴ = 5 የቤት እመቤት = 6 ሥራ አጥ = 7 ሌላ አይነት ሥራ ይገለጽ ---------	
109	የወር ገቢዎ ምን ያህል ነው ?	---------- ብር አይታወቅም = 99 ምላሽ የለም =909	

2. መሰረታዊ የሰውነት የጤና አቋም

ተ.ቁ	ጥያቄ	ምላሽ		ኮድ
201	ክብደት በ ኪ/ግ			
202	ቁመት በ ሜትር			
203	ቁመት በ ሜትር ስኩዌር			
204	ክብደት በ ኪ/ግ /ቁመት በ ሜትር ስኩዌር አካፋይ		ከመጠን በታች= <18.5 = 1 ትክክለኛ መጠን = 18.5–24.9 = 2 ከመጠን በላይ = 25–29.9 = 3 ከመጠን በላይ ውፍረት = BMI of 30 ወይም ከዛበላይ =4	
205	የእጅ አልቦ ዙሪያ በ ሴ/ሜ			
206	የክንድ ዙሪያ በ ሴ/ሜ			
207	የዳሌ ዙሪያ በ ሴ/ሜ			
208	የወገብ ዙሪያ በ ሴ/ሜ			
209	የዳሌ ዙሪያ /የወገብ ዙሪያ አካፋይ		ወንድ < 0.9, ሴት < 0.8 ተቀባይነት ያለው =1 ወንድ > 0.9, ሴት > 0.8 ተቀባይነት የሌለው= 2	
210	የላይኛው የደም ግፊት		< 120 = ትክክለኛ መጠን = 1 120-139 = ቅድመ-የደም ግፊት = 2 >140 = ከፍተኛ የደም ግፊት = 3	
211	የታችኛው የደም ግፊት		< 80 = ትክክለኛ መጠን = 1 80-89 = ቅድመ-የደም ግፊት = 2 >90 = ከፍተኛ የደም ግፊት = 3	
212	የሰውነት የሙቀት መጠን በ ዲ/ሴ			

3. የአመጋገብ ሁኔታ መግለጫ

ተ.ቁ	ጥያቄ	ምላሽ	ኮድ
301	ከዚህ በታች የተዘረዘሩትን ምግቦች ምን ያህል ይመገባሉ?	- አልጠቀምም =1 - አንዳንዴ (ለበአል፣ለሰርግ፣ለክብረበአሎች ወዘተ…) = 2 - በወር አንድ ወይም ሁለት ግዜ = 3 - በሳምንት ከ1-4 ግዜ = 4 - በቀን አንዴ ወይም ከዛበላይ = 5	
	- ወተት እና የወተት ውጤቶች(አይብ፣ቅቤ፣ወዘተ…)		
	- ስጋ (የዶሮ፣የአሳ፣ወዘተ…)		
	- እንቁላል		
	- ሀይል ሰጪ ምግቦች(ዳቦ፣ፓስታ፣ወዘተ…)		
302	የሰውነቶን የስብ መጠን ለማስተካከል አመጋገቦን ይለዋወጣሉ?	አዎ = 1 አይ = 2	

4. ልምዶች

ተ.ቁ	ጥያቄ	ምላሽ	ኮድ
401	የአልኮል መጠጥ ይጠጣሉ?	አዎ = 1 አይ = 2	
	መልሶ አዎ ከሆነ ምን ያህል ግዜ ይጠጣሉ?	በየቀኑ = 1 በሳምንቱ መጨረሻ ሁል ግዜ = 2 አንዳንድ ግዜ = 3	
402	ያጨሳሉ?	አዎ = 1 አይ = 2	
	መልሶ አዎ ከሆነ ምን ያህል ያጨሳሉ?	ሁል ዚዜ,=1 አንዳንዴ = 2	
403	የአካል ብቃት እንቅስቃሴ ያደርጋሉ?	አዎ = 1 አይ = 2	
	መልሶ አዎ ከሆነ ምን ያህል ግዜ ያደርጋሉ?	በየቀኑ = 1 በሳምንት ከ2-3 ቀን = 2 አንዳንድ ግዜ = 3	
402	ጫት ይቅማሉ?	አዎ = 1 አይ = 2	
	መልሶ አዎ ከሆነ ምን ያህል ግዜ ይቅማሉ?	በየቀኑ = 1 አንዳንዴ = 2	

5. የህክምና ሁኔታ

ተ.ቁ	ጥያቄ	ምላሽ	ኮድ
501	ለታይሮይድ የሚሆን ህክምና ወስደው ያውቃሉ?	አዎ = 1 አይ = 2	
	መልሶ አዎ ከሆነ ምን አይነት ህክምና ነው የወሰዱት?	ሌቮቲሮክሲን = 1 ሜቲማዞል = 2 ሜፕሮፒለቴራሲል(ፒቲዩ) = 3 ሌላ ካለ ይግለጹ= 4 _____	
	መልሶ አዎ ከሆነ ምን ያህል መጠን ነው የወሰዱት?		
	መልሶ አዎ ከሆነ ምን ያህል ግዜ ነው የወሰዱት?		
502	ከውፍረት ጋር ለተያያዘ ችግር ህክምና ወስደው ያውቃሉ?	አዎ = 1 አይ = 2	
	መልሶ አዎ ከሆነ ምን አይነት ህክምና ነው የወሰዱት?	በዝርዝር ይግለጹ= ----------	
	መልሶ አዎ ከሆነ ምን ያህል መጠን ነው የወሰዱት?		
	መልሶ አዎ ከሆነ ምን ያህል ግዜ ነው የወሰዱት?		
503	ለCVD ህክምና ወስደው ያውቃሉ?	አዎ = 1 አይ = 2	
	መልሶ አዎ ከሆነ ምን አይነት ህክምና ነው የወሰዱት?	በዝርዝር ይግለጹ = _____	
	መልሶ አዎ ከሆነ ምን ያህል መጠን ነው የወሰዱት?		
	መልሶ አዎ ከሆነ ምን ያህል ግዜ ነው የወሰዱት?		

6. ምልክቶች እና የህመሞች ስሜት

ተ.ቁ	ጥያቄ	ምላሽ		ኮድ
601	**የታይሮይድ በሽታ ህመሞች ባለፉት ሶስት ወራት ውስጥ የሚከተሉትን ምልክቶች አስተውለዋል**?	አዎ = 1	አይ = 2	
	መዛል፣ መጫጫን	1	2	
	ድካም ድካም ማለት	1	2	
	ድብርት	1	2	
	ትኩረትን ለመሰብሰብ መቸገር	1	2	
	ከመጠን በላይ የሆነ ምክንያቱ ያልታወቀ ውፍረት	1	2	
	ደረቅ፣ የሚያሳክክ ቆዳ	1	2	
	ደረቅ፣ የሳሳ ፀጉር	1	2	
	ብርድብርድ ማለት፤በተለይም በመገጣጠሚያ አካባቢ	1	2	
	ድርቀት	1	2	
	የጡንቻ መሸማቀቅ	1	2	
	የወር አበባ መብዛት	1	2	
	የወር አበባ ቶሎቶሎ መምጣት	1	2	
	መካንነት/ውርጃ	1	2	
	መቅበጥበጥ	1	2	
	መቆጥቆጥ	1	2	
602	**የ Lipodystrophy በሽታ ህመሞች ባለፉት ሶስት ወራት ውስጥ የሚከተሉትን ምልክቶች አስተውለዋል**?	አዎ = 1 አይ = 2	መልሶ አዎ ከሆነ፣ ጨምሯል = 3 ቀንሷል =4	
	ከመጠይቁ የሚሞላ:			
	የፊት ውፍረት(ስብ)	1 2	3 4	
	የእጅ ውፍረት(ስብ)	1 2	3 4	
	የእግር ውፍረት(ስብ)	1 2	3 4	
	የመቀመጫ ውፍረት(ስብ)	1 2	3 4	
	የሆድ ውፍረት(ስብ)	1 2	3 4	
	የማንጅራት ውፍረት(ስብ)	1 2	3 4	
	የጡት ውፍረት(ስብ)	1 2	3 4	
	የዳሌ ውፍረት(ስብ)	1 2	3 4	
	በእይታ የሚሞላ:			
	የፊት ውፍረት(ስብ)	1 2		
	የእጅ ውፍረት(ስብ)	1 2		
	የእግር ውፍረት(ስብ)	1 2		
	የመቀመጫ ውፍረት(ስብ)	1 2		
	የሆድ ውፍረት(ስብ)	1 2		
	የማንጅራት ውፍረት(ስብ)	1 2		
	የጡት ውፍረት(ስብ)	1 2		
	የዳሌ ውፍረት(ስብ)	1 2		

Annex-III Standard operating procedure

Measurement of HDL-C((HDL-Cholesterol, no pretreatment Cobas)

(Homogeneous enzymatic Colorimetric method)

Principle: HDL cholesterol is first separated by precipitation apoprotein B-containing lipoproteins from serum by using a combination of a polyanion and a divalent cation, such as dextran sulfate/magnesium chloride or phosphotungstate/magnesium chloride. In the first step LDL, VLDL and chylomicrons are eliminated and transfomated to nonreaction compounds and specific condition for the reaction. By the second reagent, only the HDL-cholesterol is subject to color reaction:

Cholesterol + H_2O $\xrightarrow{\text{Cholesterol Esterase}}$ ▶Cholesterol + fatty acid

Cholesterol + O_2 $\xrightarrow{\text{Cholesterol Oxidase}}$ ▶Cholesten-3-on + H_2O

H_2O_2 + Phenol + 4-Amino-antipyrine $\xrightarrow{\text{Peroxidase}}$ ▶Quinoneimine dye + $4H_2O$

Reagents

Reagent

R1: good's buffer, pH 7,0	100mmol/l
Cholesterol oxidase	>0.8KU/l
Cholesterol esterase	>1.0KU/l
Catalase	>500KU/l
HDCBS	0.5mmol/l
R2: Peroxidase	30KU/l
4-Aminoantipyrine	4mmol/l
R4: LDL cholesterol	see lable

Procedure

Materials provided

Working solutions, calibrator, control and 0.9% NaCl

	Reagent Blank	Sample/calib./stand.
R1	600µl	600 µl
Sample/calib/stan.		6 µl
Mix well and incubate at 37^0c for 5min		
R2	200 µl	200 µl

Incubate at 37^0c, Read the initial absorbance A1 after exactly 30 sec. and after 5 min A2 for calibrator and sample calculate ΔA

Calculation :

$\frac{\Delta A \text{ sample}}{\Delta A \text{ calib/stand}}$ X Calib/Stand/ Conce/ = HDL cholesterol conc.

Reference values

	No risk	Moderate risk	High risk
Men			
mg/dl	>55	33-55	<35
mmol/l	>1.45	0.90-1.45	<0.90
Women			
mg/dl	>65	45-65	<45
mmol/l	>1.68	1.15-1.68	<1.15

Measurement of LDL-Cholesterol **(LDL-Cholesterol, no pretreatment Cobas)**

Homogeneous enzymatic assay

Principle: In the first step HDL,VLDL and chylomicrons are eliminated and transformed to nonreactive components under specific conditions for the reaction. By the second reagent only the LDL-Cholesterol is subject to color reaction:

Cholesterol ester h + H2O $\xrightarrow{\text{Cholesterol esterase}}$ cholesterol ester + fatty acid

Cholesterol + O2 $\xrightarrow{\text{cholesterol oxidase}}$ cholesten-3-on + H_2O_2

$2H_2O_2$ + 4-aminoantipyrine + phenol $\xrightarrow{\text{peroxidase}}$ quinoneimine dye + 4 H2O

Reagent

R1: good's buffer, ph 7,0 50mmol/l

Cholesterol oxidase 500U/l

Cholesterol esterase 600U/l

Catalase 600KU/l

Ascorbate oxidase 3KU/l

TOOS 2mmol/l

R2: Peroxidase 4KU/l

4-Aminoantipyrine 4mmol/l

R4: LDL cholesterol see lable

Procedure: materials provided: calibrators and controls and 0.9% NaCl

	Reagent blank	Sampl/calib/stand
R1	600 µl	600 µl
Sample/calib/stand.		6 µl
Mix well and incubate at 37^0C for 5 minutes then add:		
R2	200 µl	200 µl

Incubate at 37^0C. read the initial absorbance A1 after exactly 30 sec. and after 5 min A2 for calibarator and sample. Calculate ΔA

Calculation :

$$\frac{\Delta A \text{ sample}}{\Delta A \text{ calib/stand}} \times \text{Calib/Stand/ Conce/} = \text{LDL cholesterol conc.}$$

Reference values

	Recommended (desirable)	Moderate risk	High risk
mg/dl	< 130	130-159	≥ 160
mmol/l	<3.37	3.37-4.12	≥ 4.14

Total Cholesterol(CHOD-PAP Cobas)

(enzymatic colorimetric method)

Principle: Cholesterol is measured enzymaticaly in serum or plasma in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction by products, H_2O_2 is measured quantitatively in a peroxidase catalyzed reaction that produces a color. Absorbance is measured at 500 nm. The color intensity is proportional to cholesterol concentration. The reaction sequence is as follows:

Cholesteryl ester hydrolase + Cholesteryl ester + H_2O ---------->cholesterol + fatty acid

Cholesterol oxidase + Cholesterol + O_2 ----------> cholest-4-en-3-one + H_2O_2

peroxidase + $2H_2O_2$ + 4-aminophenazone + phenol ----------> 4-(p-benzoquinone-monoimino)-phenazone + 4 H_2O

Reagent:

Reagent (R1)

Pipes buffer, pH=7.00	50 mmol/l
ADPS	1 mmol/l
Cholesterol esterase	180 U/l
Peroxidase	1000 U/l
Ascorbate oxidase	≥3000 U/l

Reagent (R2)

4-Aminoantipyrine	0.9 mmol/l
Cholesterol oxidase	200 U/l

Standard

Cholesterol	See label for exact value.

Procedure:

	Blank	Standard	Sample
R1	ml	ml	ml
Distilled water	15ml		

Standard		15ml	
Sample			15ml
Mix and wait 1minute and add			
R2	500ml	500ml	500ml

Mix and read the absorbance (A) after 5-minute incubation.

Calculation using calibration

Absorbance of sample X Concentration of standard = Concentration of sample

Absorbance of standard

Reference range: Cholesterol Conc (mg/dL) Interpretation: < 200 Desirable, 200-239 Borderline-High > 240 High and below 170 mg/dL in children.

Triglycerides (Tiglyceride GPO-PAP Cobas)

Enzymatic colorimetric method method

Test principle

Triglycerides are measured enzymatically in serum or plasma using a series of coupled reactions in which triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H_2O_2, one of the reaction products, is measured as described above for cholesterol. Absorbance is measured at 500 nm. The reaction sequence is as follows:

Lipase Triglycerides + 3H2O ----------> glycerol + fatty acids

Glycerokinase Glycerol + ATP ----------> glycerol-3-phosphate + ADP

Glycerophosphate oxidase Glycerol-3-phosphate + O2 ----> dihydroxyacetone phosphate + H_2O_2

peroxidase

H2O2 + 4-aminophenazone + 4-chlorophenol ----------> 4-(p-benzoquinone-monoimino)-phenazone + 2H2O + HCl.

Desirable fasting TGlevels are considered to be those below 200 mg/dL, and are further categorized as Borderline, 200-400 mg/dL; High, 400-1,000 mg/dL; and Very High (> 1000 mg/dL).

Lipoprotein(a)

Lipoprotein

Lipoprotein (a) [Lp(a)] is a low-density lipoprotein LDL-like particle with a cholesterol rich core and a molecule of apolipoprotein B-100 linked by a disulphide bridge to the glycoprotein Apolipoprotein (a) [apo(a)]. The Lp(a) component varies in size ranging from 200 to 700kD and has structural similarities with plasminogen. Lp(a) competes with plasminogen for binding sites on the cell surface, decreasing plasminogen activation and inhibiting clot lysis, therefore promoting wound healing.

Clinical Significance

Lipoprotein (a) determination is intended for use in conjunction with clinical evaluation, patient risk assessment and other lipid tests to evaluate disorders of lipid metabolism and to assess coronary heart disease in specific populations. Elevated Lp(a) concentration in plasma is an independant genetic marker correlating with increased risk of atherosclerotic disorders including myocardial and cerebral infarction. Levels are also elevated in nephritic syndrome, patients undergoing renal dialysis, patients with uncontrolled diabetes mellitus and hypothyroidism.

How is Lp(a) measured

Cobas integra 400 has developed an immunoturbidimetric end-point method for the determination of Lp(a) on automated analysers. A specific anti-Lp(a) antibody agglutinates with Lp(a) in the test sample due to an antigen-antibody reaction. This agglutination is detected as an absorbance change at 700 nm proportional to the concentration of Lp(a) in the sample.
High Performance Reagents
Range - 2.1-90 mg/dl.

Precision - The following coefficients of variation were obtained on a Hitachi™ 717 analyser.

Sensitivity - 2.1 mg/dl.

Stability - Reagents are liquid stable to expiry.

Correlation - _ A correlation coefficient of 0.995 was obtained with an alternate commercially available method.

Antigen Excess (Prozone) - _was not observed up to a level of 341 mg/dl.

Interference - The following analyte concentrations were not found to affect the assay.

Ascorbic Acid	50 mg/dl
Bilirubin	35 mg/dl
Haemoglobin	1040 mg/dl
Intralipid®	5%
Triglycerides	493 mg/dl
Plasminogen	200 mg/dl
Apolipoprotein B	200 mg/dl

Completely Automated Protocols - are available for a range of analysers.

	Lipoprotein (a) Mean (mg/dl)	Mean % CV	n
Intra-assay precision	21.0	1.63	20
	51.5	1.53	20
	83.05	2.40	20
Inter-assay precision	24.19	3.11	20
	32.58	3.52	20
	50.48	2.83	20

Glucose

INTRODUCTION

Glucose is the major carbohydrate present in the peripheral blood. The oxidation of glucose is the major source of cellular energy in the body. Glucose determinations are run primarily to aid in the diagnosis and treatment of diabetes mellitus. Elevated glucose levels are mainly associated with insulinemia or insulin-induced hypoglycemia.21 A number of secondary factors also can contribute to elevated blood glucose levels. These include pancreatitis, pituitary or thyroid dysfunction, renal failure and liver disease. An enzymatic approach for glucose determination involves hexokinase coupled with glucose-6-phosphate dehydrogenase.4 A revision of this approach is proposed by the U.S. Center For Disease Control as the reference method for glucose and forms the basis of the reagent for glucose.

PRINCIPLE

Glucose + ATP HK > G-6-P + ADP 3

G-6-P + NAD G6PDH > 6-Phosphogluconate + NADH

The enzymatic hexokinase (HK) catalyzes the reaction between glucose and adenosine triphosphate (ATP) to form glucose 6phosphate and adenosine diphosphate (ADP). In the presence of NAD, the enzyme glucose-6-phosphate dehydrogenase (G6PDH), oxidizes glucose-6-phosphate to 6-phosphogluconate. The increase in NADH concentration is directly proportional to the glucose concentration and can be measured spectrophotometrically at 340 nm.

REAGENT COMPOSITION

When reconstituted with distilled water as directed, the reagent contains the following:
Hexokinase 1,000 U/L;
G6PDH 1,000 U/L; ATP 1.0 nm;
NAD 1.0 mM;
Buffer 100 mM pH = 7.5 + 0.1 (30°C);
nonreactive stabilizers and preservatives have been added.

PRECAUTIONS Reagent is for "in vitro" diagnostic use only.

REAGENT PREPARATION

Reconstitute reagent vials with volume of distilled water stated on vial label. Swirl gently to dissolve.

REAGENT STORAGE

1. The dry reagent and standard should be stored refrigerated at 2-8°C.
2. Reconstituted reagent is stable for 48 hours at room temperature and for 30 days refrigerated at 2 - 8°C.

REAGENT DETERIORATION

Do not use if:
1. Reagent has an absorbance greater than 0.30 when measured against water at 340 nm.
2. The reagent fails to recover stated control values or meet stated linearity.
3. The reconstituted reagent develops turbidity, indicating contamination.

SPECIMEN COLLECTION

1. Either serum or plasma may be used.
2. Plasma or serum samples without preservatives should be separated from the cells or clot within a half hour of being drawn. 3.Glucose in separated unhemolyzed serum is generally stable up to eight hours at 25°C and up to 72 hours at 4°C.5
4. Glycolysis can be inhibited by collecting the specimen in sodium fluoride. Glucose in a sodium fluoride-oxalate mixture is reported to be stable up to 24 hours at 25°C.65

MATERIALS PROVIDED

1. Glucose hexokinase reagent.
2. Glucose standard (100 mg/dl).

PROCEDURE (AUTOMATED)

Refer to specific instrument application instructions.

PROCEDURE (MANUAL)

1. Appropriately label tubes: reagent blank, standard, control, sample, etc.
2. Pipette 1.0 ml of reagent into all tubes.
3. Add 0.005 ml (5 µl) of sample to respective tubes. Mix well.Incubate all tubes at 37°C for five (5) minutes.
4. After incubation, zero the spectrophotometer with the reagent blank at 340 nm.
5. Read and record the absorbance of all tubes.

CALCULATIONS

Abs. = absorbance at 340 nm Sample Abs. × Conc. of standard = Conc. of glucose (mg/dl)
Standard Abs.

Note: To convert the results into SI units (mmol/L), multiply the result (mg/dl) by 0.0556.

LIMITATIONS 1. A "Sample Blank" should be prepared if the sample is moderately

Chemiluminescence

Chemiluminescence (CL) is the emission of light by the electronically excited product of a chemical reaction when it relaxes to the ground state. The efficiency of a chemiluminescent reaction is given by the quantum yield, which is a measure of the fraction of reacting molecules that actually produce light.

Electrochemiluminescence or electrogenerated chemiluminescence(ECL) is a form of chemiluminescence(CL) in which the light emitting chemiluminescent reaction is preceded by an electrochemical reaction.

The ECL Reaction at the Electrode Surface

Two electrochemically active substances, the ruthenium complex and tripropylamine (TPA), are involved in the reactions that lead to the emission of light. Both substances remain

stable, as long as a voltage is not applied.

The ECL reaction of ruthenium tris(bipyridyl)$^{2+}$ and tripropylamine occurs at the surface of a platinum electrode. The applied voltage creates an electrical field, which causes all the materials in this field to react. Tripropylamine is oxidized at the electrode, releases an electron and forms an intermediate tripropylamine radical-cation, which further reacts by releasing a proton (H^+) to form a TPA radical (TPA•).

In turn, the ruthenium complex also releases an electron at the surface of the electrode thus oxidizing to form the $Ru(bpy)_3^{3+}$ cation. This ruthenium cation is the second reaction component for the following chemiluminescent reaction with the TPA radical.

TPA• and $Ru(bpy)_3^{3+}$ react with one another, whereby $Ru(bpy)_3^{3+}$ is reduced to $Ru(bpy)_3^{2+}$ and at the same time forms an excited state via energy transfer. This excited state is unstable and decays with emission of a photon at 620 nm to its original state. The reaction cycle can now start again. The tripropylamine radical reduces to by-products which do not affect the chemiluminescent process. TPA is used up and therefore must be present in excess. The reaction is controlled by diffusion of the TPA and the amount of ruthenium complex present. As TPA in the electrical field is depleted, the signal strength (light) is slowly reduced once the maximum is reached.

Although during measurement, TPA is used up, the ruthenium ground state complex is continually regenerated. This means that the ruthenium complex can perform many light-generating cycles during the measurement process, therefore showing an inherent amplification effect which contributes to the technology's sensitivity. Many photons can be created from one antigen-antibody complex.

ECL Signal Generation

The graph displays a typical ECL signal generation. Viewed from an electrical perspective, the reaction can be explained as follows: When a voltage is applied to the detection cell electrode, a peak of light emission occurs over a short time interval and can be detected as the resulting ECL signal. A defined area under the curve is measured around the intensity maximum.

The dotted line indicates the voltage at the electrode used to generate the ECL signal. The solid line is the actual light output measured by the photomultiplier detector.

ECL Measuring Cell

The core of the system is the ECL detection cell, which is designed as a flow- through cell. Essentially, three operating steps are performed in the measuring cell:

- **Bound/Free Separation**

Using a magnet, the streptavidin microparticles that are coated with antigen-antibody complexes, are uniformly deposited on the working electrode. A system buffer (ProCell) is used to wash the particles on the working electrode and to flush out the excess reagent and sample materials from the measuring cell.

- **ECL Reaction**

The magnet is removed and a voltage is then applied to the electrode on which the microparticles, coated with antigen-antibody complexes, are deposited to initiate the ECL reaction. The light emission is measured with a photomultiplier. The system then uses the corresponding signals for the calculation of results.

- **Release of Microparticles and Cell Cleaning**

Once the measurement is completed, the paramagnetic microparticles are washed away

from the electrode surface with a special cleaning solution (CleanCell). The surface of the measuring cell is regenerated by varying the potential on the electrode. The cell is then ready for another measurement.

Test Principle

Four test principles are available on the Immunoassay System: 1st Competitive principle for extremely small analytes such as Free-T3 and Free-T4, 2nd sandwich principle (one or two steps) for larger analytes such as T3, T4, and TSH, 3rd a bridging principle to detect antibodies in the sample such as anti thyroidal antibodies, and 4th DNA/RNA probe assay which is not used applicable of thyroid functional tests.

Competitive Principle

This principle is applied to analytes of low molecular weight, such as FT3.

- In the first step, sample and a specific anti-T3 antibody labeled with a ruthenium complex are combined in an assay cup.
- After the first incubation, biotinylated T3 and streptavidin-coated paramagnetic microparticles are added. The still free binding sites of the labeled antibody become occupied, with formation of anantibody-hapten complex. The entire complex is bound to the microparticle via interaction of biotin and streptavidin.
- After the second incubation, the reaction mixture containing the immune complexes is transported into the measuring cell. The immune complexes are magnetically entrapped on the working electrode, but unbound reagent and sample are washed away by ProCell.
- In the ECL reaction, the conjugate is a ruthenium based derivative and the chemiluminescent reaction is electrically stimulated to produce light. The amount of light produced is indirectly proportional to the amount of antigen in the patient sample.

Evaluation and calculation of concentration of the antigen are carried out by means of a calibration curve that was established using standards of known antigen concentration.

Sandwich Principle

The sandwich principle is applied to higher molecular weight analytes, such as thyroid-stimulating hormone (TSH).

- In the first step, patient sample is combined with a reagent containing biotinylated TSH antibody and a ruthenium-labeled TSH-specific antibody in an assay cup. During a nine-minute incubation step, antibodies capture the TSH present in the sample.
- In the second step, streptavidin-coated paramagnetic microparticles are added. During a second nine-minute incubation, the biotinylated antibody attaches to the streptavidin-coated surface of the microparticles.
- After the second incubation, the reaction mixture containing the immune complexes is transported into the measuring cell; the immune complexes are magnetically entrapped on the working electrode, but unbound reagent and sample are washed away by ProCell.
- In the ECL reaction, the conjugate is a ruthenium based derivative and the chemiluminescent reaction is electrically stimulated to produce light. The amount of light produced is directly proportional to the amount of TSH in the sample.

Evaluation and calculation of concentration of the antigen or analyte are carried out by means of a calibration curve that was established using standards of known antigen concentration.

Annex-IV Quality Control Sample Result

Table 3.The comparison of repeatedly done quality control sample values by COBAS-e-411 immuno-chemistry analyzer, and COBAS INTEGRA 400 with kit inserts' assigned values for glucose and lipid profile reagents.

Analyte	Control	Assigned mean	Assigned SD	N	Calculated mean	Calculated SD	Calculated %CV
T3	PCU1	1.54	0.11	20	1.44	0.20	4.07
	PCU2	3.39	0.27	18	3.63	0.51	4.09
T4	PCU1	8.23	0.58	17	8.12	1.14	3.83
	PCU2	12.35	0.87	17	12.97	1.14	3.83
TSH	PCU1	1.66	0.1	82	1.5	0.244	4.25
	PCU2	9.80	0.49	85	9.13	1.54	4.87
FT3	PCU1	3.78	0.30	48	3.73	0.599	3.05
	PCU2	13.02	0.88	25	12.2	0.68	4.57
FT4	PCU1	1.26	0.06	60	1.27	0.073	4.79
	PCU2	2.95	0.14	63	2.93	0.256	3.73
T-UP	PCU1	1.1	0.077	10	1.2	1.54	1.2
TG	PCU1	26.6	2.13	10	21.92	3.5	2.19
Glucose	PNU	94	5	20	91.4	4.57	5
	PPU	254	13	20	257	9.65	3.7
TC	PNU	97	5	20	97	2.9	2.99
	PPU	193	10	20	197	4.6	2.37
TRI	PNU	98	5	20	99.1	4.4	3.75
	PPU	213	11	20	216	8.7	4.44
HDL	PPHL	30	2	20	26,8	1.03	3.85
LDL	PPHL	200	6	10	206	3.01	1.46
Apo-A	PNL	150	5	10	147.1	5.4	3.75
Lp(A)	PNL	10	1.8	10	10.5	1.3	1.32