STUDY OF ASSOCIATION BETWEEN SUBCLINICAL HYPOTHYROIDISM AND RISK FACTORS OF CARDIOVASCULAR DISEASES.

By

Dr. SMITA. KOTTAGI. **MBBS**

Dissertation submitted to the

BLDE UNIVERSITY, BIJAPUR, KARNATAKA

In partial fulfillment of the requirements for the degree of

DOCTOR OF MEDICINE

In

BIOCHEMISTRY

Under the guidance of

Dr. DILEEP. RATHI. **M.D**
PROFESSOR AND HEAD
DEPARTMENT OF BIOCHEMISTRY
B.L.D.E.U'S SHRI B.M.PATIL MEDICAL COLLEGE
HOSPITAL & RESEARCH CENTRE, BIJAPUR
KARNATAKA **2014**

B.L.D.E UNIVERSITY'S

SHRI B.M.PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, BIJAPUR

DECLARATION BY THE CANDIDATE

I, **Dr. SMITA. KOTTAGI,** hereby declare that this dissertation entitled **"STUDY OF ASSOCIATION BETWEEN SUBCLINICAL HYPOTHYROIDISM AND RISK FACTORS OF CARDIOVASCULAR DISEASES"** is a bonafide and genuine research work carried out by me under the guidance of **Dr. DILEEP. RATHI.$_{M.D}$** Professor and Head, Department of Biochemistry, B.L.D.E.U's Shri B M Patil Medical College and Research Centre, Bijapur.

Date:

Place: Bijapur

Dr. SMITA. KOTTAGI. MBBS

Post Graduate Student,

Department of Biochemistry,

BLDEU's Shri B. M. Patil Medical College,

Hospital & Research Centre, Bijapur.

B.L.D.E UNIVERSITY'S
SHRI B.M.PATIL MEDICAL COLLEGE, HOSPITAL &
RESEARCH CENTRE, BIJAPUR

CERTIFICATE BY THE GUIDE

This to certify that the dissertation entitled "**STUDY OF ASSOCIATION BETWEEN SUBCLINICAL HYPOTHYROIDISM AND RISK FACTORS OF CARDIOVASCULAR DISEASES**" is a bonafide research work done by **Dr. SMITA. KOTTAGI**. Under my overall supervision and guidance, in partial fulfillment of the requirements for the degree of M.D. in Biochemistry.

Date:

Place: Bijapur

Dr. DILEEP. RATHI. M.D
Professor and Head
Department of Biochemistry,
BLDEU's Shri B. M. Patil Medical College,
Hospital & Research Centre, Bijapur

B.L.D.E UNIVERSITY'S
SHRI B.M.PATIL MEDICAL COLLEGE, HOSPITAL &
RESEARCH CENTRE, BIJAPUR

ENDORSEMENT BY THE HEAD OF DEPARTMENT

This to certify that the dissertation entitled "**STUDY OF ASSOCIATION BETWEEN SUBCLINICAL HYPOTHYROIDISM AND RISK FACTORS OF CARDIOVASCULAR DISEASES**" is a bonafide research work done by **Dr. SMITA. KOTTAGI.** under the guidance of **Dr. DILEEP. RATHI. M.D** Professor and Head , Department of Biochemistry at B.L.D.E.U's Shri. B M Patil Medical College Hospital and Research Centre, Bijapur.

Date:

Place: BIJAPUR

DR. DILEEP. RATHI. M.D
Professor and Head
Department of Biochemistry,
BLDEU's Shri B. M. Patil Medical College,
Hospital & Research Centre, Bijapur.

B.L.D.E UNIVERSITY'S
SHRI B.M.PATIL MEDICAL COLLEGE, HOSPITAL &
RESEARCH CENTRE, BIJAPUR

ENDORSEMENT BY THE PRINCIPAL

This to certify that the dissertation entitled "**STUDY OF ASSOCIATION BETWEEN SUBCLINICAL HYPOTHYROIDISM AND RISK FACTORS OF CARDIOVASCULAR DISEASES**" is a bonafide research work done by **Dr. SMITA. KOTTAGI.** under the guidance of **Dr. DILEEP. RATHI.M.D** Professor and Head, Department of Biochemistry B.L.D.E.U's Shri. B M Patil Medical College Hospital and Research Centre, Bijapur.

Date**:** **DR. M. S. BIRADAR. M.D.**

Place: Bijapur. Principal,

B.L.D.E.U.'s Shri B. M. Patil Medical College,

Hospital & Research Centre, Bijapur.

DECLARATION BY THE CANDIDATE

COPY RIGHT

I hereby declare that the BLDE UNIVERSITY, BIJAPUR, Karnataka shall have the rights to preserve, use and disseminate this dissertation/thesis in print or electronic format for academic/research purposes.

Date: **Dr. SMITA. KOTTAGI. MBBS**

Place: Bijapur

ACKNOWLEDGEMENT

With regard to the dissertation titled "**STUDY OF ASSOCIATION BETWEEN SUBCLINICAL HYPOTHYROIDISM AND RISK FACTORS OF CARDIO VASCULAR DISEASES"**. I consider it a privilege to express a few words of gratitude and respect to all those who guided and inspired me in completing the dissertation.

I take this opportunity to express my profound sense of gratitude and indebtedness to my guide **Dr. DILEEP. RATHI** M.D. Professor & Head, Department of Biochemistry, Shri B M Patil Medical College, Bijapur, who has been the source of propelling force, complete guidance and inspiration for this dissertation / research work. He has guided me by his constant encouragement and valuable suggestions to carry out and complete this dissertation.

My sincere thanks are due to **Dr. M S Biradar** M.D. Principal, Shri B M Patil Medical College, Bijapur, for his support and encouragement.

I want to thank all the department staff Dr. J.G. Ambekar, Dr. B. B. Devaranavadagi, Dr. B. S. Aski, Dr. Nilima. Dongre, Dr. I. A. Hundekari, Dr. Parinita. S. K, Dr. Anand. K. P, Dr. Deepa S.S, Dr. Shivaleela. M. B. for their help, support and advice. I also want to thank Mr. S. S. Walvekar, for his help in laboratory sample collection and analysis.

I am thankful to all the Technical and non-teaching Staff of the Department of Biochemisrty, Shri. B M Patil Medical College, Bijapur for their co-operation.

I thank Mrs. Vijaya Soraganvi of Community Medicine Department for her valuable help in statistical analysis of data.

I sincerely acknowledge the support and kindness shown towards me by all the staff of Central Library, Shri B M Patil Medical College, Bijapur, at all times.

I would like to thank my parents, my husband, my daughter and my parents in-law for their constant encouragement, love, sacrifice and support in all the endeavours of my life and work.

I am grateful to all the people who have helped in the editing, printing and binding of this thesis work.

Last but not the least, I would like to acknowledge all the participants (patients as well as controls) for providing with necessary data and forming the backbone of this study, without whom this study would not have been possible.

I thank **GOD**, for all his blessings.

Date: **Dr. SMITA. KOTTAGI.**

Place: Bijapur

LIST OF ABBREVIATIONS

TSH	–	Thyroid Stimulating Hormone
T3	–	Tri-iodothyronine
T4	–	Tetra-iodothyronine
ELISA	–	Enzyme Linked Immunosorbent Assay
TC	–	Total Cholesterol
TG	-	Triglycerides
HDL-C	–	High Density Lipoprotein Cholesterol
LDL-C	-	Low Density Lipoprotein Cholesterol
CHOD PAP	-	Cholesterol Oxidase Phenol 4-Aminoantipyrine Peroxidase
GPO POD	-	Glycerol Phosphate Oxidase Peroxidase
GOD POD	-	Glucose Oxidase Peroxidase

ABSTRACT

Background and objectives:

Thyroid hormones play an important role in regulating lipid and glucose metabolism. Overt hypothyroidism is associated with atherosclerosis and coronary heart disease. Whether subclinical hypothyroidism (SCH) is associated with increased cardiovascular risk is controversial. Data on the risk of cardiovascular disease are conflicting. This study was done to investigate subclinical hypothyroidism and its associations with cardiovascular diseases.

Materials and Methods:

60 SCH cases were compared with 30 euthyroid controls. Serums T3, T4, TSH were estimated by ELISA method, Serum Total-Cholesterol, HDL-Cholesterol and LDL-Cholesterol by enzymatic CHOD-PAP method, Triglycerides by GPO-POD method and Fasting blood glucose by GOD-POD method.

Results and Interpretation:

Significant increase was found in the mean serum levels of TSH ($P<0.001$), Total cholesterol ($p<0.001$), Triglycerides ($P<0.001$), LDL Cholesterol ($P<0.001$), systolic blood pressure ($p<0.001$) and diastolic blood pressure ($P<0.001$). No significant change was observed in levels of serum T4, HDL-Cholesterol, Fasting blood glucose. Percentage of subjects with increased Total Cholesterol , Triglycerides, LDL-C, systolic blood pressure and diastolic blood pressure and decreased HDL-C were more in subclinical hypothyroidism as compared to euthyroid controls indicating the dyslipidemic and hypertensive changes in subclinical hypothyroidism. A weak positive correlation was

observed between TSH and Total Cholesterol , Triglycerides , LDL-C and systolic blood pressure and a weak negative correlation was obtained between TSH and HDL-C and Fasting blood glucose and diastolic blood pressure showing that these risk factors of cardiovascular diseases are associated with subclinical hypothyroidism

Conclusion:

The Study revealed that hypertension and dyslipidemic state is seen in subjects with subclinical hypothyroidism leading to increased risk for cardiovascular disease and pointing towards the association between subclinical hypothyroidism and risk factors of cardiovascular disease. This indicates the importance of screening the patients for subclinical hypothyroidism and weigh in favor of treating the patients with subclinical hypothyroidism.

Keywords: Overt hypothyroidism Subclinical hypothyroidism (SCH), cardiovascular risk, dyslipidemia.

TABLE OF CONTENTS

ANNEXURE

LIST OF TABLES

LIST OF CHARTS

LIST OF FIGURES

INTRODUCTION:

Subclinical hypothyroidism is defined as a serum TSH concentration above the statistically defined upper limit of the reference range when serum T3 and T4 concentrations are within reference range [1].

A 2-fold change in free thyroxine will produce a 100-fold change in TSH. Therefore, a slight reduction of T4 within the normal range will result in elevation of serum TSH above the normal range. Thus, serum TSH measurement is the necessary test for diagnosis of mild thyroid failure when the peripheral thyroid hormone levels are within normal laboratory range [2].

Subclinical hypothyroidism or mild thyroid failure is a common problem, with a prevalence of 3% to 8% in the population without known thyroid disease. The prevalence increases with age and is higher in women. After the sixth decade of life, the prevalence in men approaches to that of women, with a combined prevalence of 10%. Antithyroid antibodies can be detected in 80% of patients with SCH, and 80% of patients with SCH have a serum TSH of less than 10 mIU/L [3].

Subclinical thyroid disease is a laboratory diagnosis. Patients with subclinical disease have few or no definitive clinical signs or symptoms of thyroid dysfunction [1]. Before diagnosis of Subclinical hypothyroidism, other causes of an elevated TSH level, such as recovery from nonthyroidal illness, assay variability, presence of heterophile antibodies interfering with the TSH assay, and certain cases of central hypothyroidism with biologically inactive TSH and thyroid hormone resistance, should be excluded. However, the most common cause of elevated TSH is autoimmune thyroid disease.

Previous radioiodine therapy, thyroid surgery, and external radiation therapy can also result in mild thyroid failure. Transient SCH may occur after episodes of postpartum, silent, and granulomatous thyroiditis [4].

The clinical importance of and therapy for mild elevation of serum TSH (<10 mIU/L) [1] and the exact upper limit of normal for the serum TSH level remain subjects of debate [5]. When the TSH level is above 10 mIU/L, levothyroxine therapy is generally agreed to be appropriate [6]. However, management of patients with a serum TSH level of less than 10mIU/L is controversial. Some authors argue for routine and some for selective therapy [7].

Subclinical hypothyroidism has been associated with higher levels of some cardiovascular risk factors. Despite some conflicting results, many studies found that subjects with subclinical hypothyroidism have higher total cholesterol and low density lipoprotein cholesterol levels than euthyroid subjects. Few studies have showed that subjects with subclinical hypothyroidism have increased C-reactive protein values. Subclinical hypothyroidism has been associated with increased risk for atherosclerosis. However, data on coronary heart disease (CHD) in subjects with subclinical hypothyroidism are conflicting [8].

Small percentage of these patients advance to overt hypothyroidism each year, Lipid abnormalities are reported to be more common in patients with overt hypothyroidism and are thought to contribute to the disproportionate increase in cardiovascular risk in these persons.

Controversy continues over whether elderly individuals should be screened for subclinical hypothyroidism.

The decision about whether to screen patients for this disorder is clouded by inconsistent evidence of association of dyslipidemia and other risk factors of cardiovascular disease with SCH and also any benefit from early treatment. A few trials have found that persons with subclinical hypothyroidism who are given L-thyroxine experience some improvements in their energy level and feelings of well-being [9].

Cardiovascular diseases (CVDs) are the most common cause of mortality, primarily affecting older adults. Heart disease causes nearly 700 000 deaths annually in the United States. Although established risk factors explain most cardiac risks, significant attention has been focused on alternative biochemical markers to assist in identifying those at risk of a clinical cardiac event. Previous studies have suggested that abnormal levels of thyroid stimulating hormone (TSH) may represent a novel cardiac risk factor [10].

If one can prove clearly that SCH is definitely associated with lipid abnormalities, then one can go for general screening and treatment of patients with SCH with levothyroxine and thereby preventing the overt hypothyroidism and thereby the cardiovascular complications at a very early stage.

There are few population-based studies that have compared lipid levels in patients who have subclinical hypothyroidism with lipid levels in euthyroid persons. So the purpose of this study is to determine whether the known risk factors for the CAD such as hypertension, increase in fasting blood glucose and lipid abnormalities are more

significant in patients with subclinical hypothyroidism when compared with those in euthyroid individuals.

AIM OF STUDY:

To study the association between subclinical hypothyroidism and risk factors of cardio vascular diseases.

OBJECTIVES:

1. To quantitatively detect the levels of fasting blood glucose, lipid profile and range of blood pressure, in subclinical hypothyroid and euthyroid subjects.

2. To detect the association between subclinical hypothyroidism and risk factors of cardiovascular diseases.

REVIEW OF LITERATURE:

Subclinical hypothyroidism, defined as an asymptomatic state characterized by normal serum concentrations of free thyroxine and elevated serum concentrations of thyroid-stimulating hormone (TSH) [11], is highly prevalent in elderly women[12]. Whether subclinical hypothyroidism is related to risk for cardiovascular disease is controversial. Case-control and cross-sectional studies on the association between subclinical hypothyroidism and cardiovascular disease have been done. Results from these studies are not consistent.

In the year 1998 Georgia Michalopoulou and co-workers studied High serum cholesterol levels in persons with 'high-normal' TSH levels, they observed that Subjects with high-normal TSH levels combined with Thyroid antibodies have subclinical hypothyroidism presenting with elevated cholesterol levels. They further gave the thyroxine replacement and concluded that patients might benefit from thyroxine administration [38].

In the year 2000, A. Elisabeth Hak and co-workers investigated whether subclinical hypothyroidism and thyroid autoimmunity are associated with aortic atherosclerosis and myocardial infarction in postmenopausal women. Subclinical hypothyroidism was present in 10.8% of participants and was associated with a greater age-adjusted prevalence of aortic atherosclerosis (odds ratio, 1.7 [95% CI, 1.1 to 2.6]) and myocardial infarction (odds ratio, 2.3 [CI, 1.3 to 4.0]). Additional adjustment for body mass index, total and high-density lipoprotein cholesterol level, blood pressure, and smoking status, as well as exclusion of women, who took β-blockers, did not affect these

estimates. Associations were slightly stronger in women who had subclinical hypothyroidism and antibodies to thyroid peroxidase (odds ratio for aortic atherosclerosis, (1.9 [CI, 1.1 to 3.6]); odds ratio for myocardial infarction,(3.1[CI,1.5to6.3]). They concluded that Subclinical hypothyroidism is a strong indicator of risk for atherosclerosis and myocardial infarction in elderly women[13].

In the year 2001, Zoe Efstathiadou and co-workers aimed to assess the association of Subclinical hypothyroidism with lipid abnormalities and to quantify the effect of L-thyroxine therapy on serum lipid profiles. They found that, Patients with Subclinical hypothyroidism had higher total cholesterol (TC) (222±45 (S.D.) vs 190±32mg/dl); low-density lipoprotein cholesterol (LDL-C) (139±28 vs 118±39mg/dl); apolipoprotein B (149±21 vs 139±18mg/dl) and lipoprotein (a) (Lp(a)) [median 12.5 (0.8–101) mg/dl vs 7 (0.8–44) mg/dl] levels compared with euthyroid controls (P <0:05 for all comparisons). In a follow-up study they included 37 patients with Subclinical Hypothyroidism, and repeated all measurements after restoration of a euthyroid state with incremental doses of L-thyroxine. No significant changes in serum lipid profiles were observed except for a decrease in high-density lipoprotein cholesterol (59±15 to 55±14mg/dl; P < 0:05): However, patients with high pre-treatment TC (≥240mg/dl) showed a significant reduction in both TC (278±28 vs 257±36mg/dl; P < 0:05) and LDL-C (192±23 vs 173±28mg/dl; P < 0:01) levels. They concluded that although patients with subclinical hypothyroidism exhibited increased levels of the atherogenic parameters (mainly LDL-C and Lp(a)), thyroid substitution therapy did not seem to significantly improve dyslipidemia in the whole group of patients[14.]

In the year 2002, Nadia Caraccio and co-workers evaluated the lipoprotein profile in a group of rigorously selected patients with stable SCH and positive antithyroid antibody titers. A possible genetic influence was also investigated by recording familial disposition to diabetes mellitus and/or premature coronary heart disease. SCH patients showed significantly higher TC ($P < 0.01$), LDL-C ($P = 0.01$), and apolipoprotein B ($P = 0.001$) levels than controls, positively correlated with baseline TSH levels ($P =0.003$, $P= 0.01$, and $P= 0.03$, respectively). Elevated Lp(a) levels were significantly more frequent in SCH ($P < 0.05$) and associated with familial diabetes mellitus and/or coronary heart disease ($P < 0.01$). To verify the potential beneficial effect of L-T4 therapy, the patients were then randomized to a placebo-controlled, L-T4 treatment course. Levothyroxine treatment resulted in a significant decrease of both TC and LDL-C concentrations ($P=0.003$), in direct proportion to the respective baseline values ($P<0.05$ and $P<0.01$, respectively), whereas no change in Lp(a) level was observed. They concluded that, only serum LDL-C levels are increased significantly and reversibly in association with SCH. Altered Lp(a) values reflect a genetic influence rather than a reduced thyroid hormone action[15].

In the year 2002, Rafael Luboshitzky and co-workers studied women with subclinical hypothyroidism and compared their blood pressure, plasma homocysteine, and serum lipid profiles with those obtained in age-matched euthyroid controls. Mean values of TC, HDL-C, LDL-C, triglycerides, TC/HDL-C, and LDL-C/HDL-C were not different in patients with SCH compared with controls. Individual analysis revealed that the percentage of patients with subclinical hypothyroidism having hypertension (20%), hypertriglyceridemia (26.9%), elevated TC/HDL-C (11.5%), and LDL-C/HDL-C (4%)

ratios were higher than the percentages in controls. Hyperhomocysteinemia (>/= 10.98 mmol/L) was observed in 29.4% of SH and was not significantly different from the percentage in controls (21.4%). No significant correlation between TSH and biochemical parameters was detected. They conclude that subclinical hypothyroidism in middle-aged women is associated with hypertension, hypertriglyceridemia, and elevated TC/HDL-C ratio. This may increase the risk of accelerated atherosclerosis and premature coronary artery disease in some patients[16].

In the year 2004, Misa Imaizumi and co-workers investigated possible associations between subclinical hypothyroidism and atherosclerotic diseases (ischemic heart disease and cerebrovascular disease) and mortality. According to their study, subclinical hypothyroidism was associated with ischemic heart disease independent of age, systolic blood pressure, body mass index, cholesterol, smoking, or presence of diabetes mellitus [odds ratio (OR), 2.5; 95% confidence interval (95% CI), 1.1–5.4 in total subjects and OR, 4.0; 95% CI, 1.4–11.5 in men] but not in women. In a 10-yr follow-up study until 1998, increased mortalities from all causes in year 3–6 after baseline measurement were apparent in men with subclinical hypothyroidism (hazard ratio, 1.9–2.1) but not in women, although specific causes of death were not determined. Results indicated that subclinical hypothyroidism is associated with ischemic heart disease and might affect all-cause mortality in men[17].

In the year 2004, Rafael Luboshitzky and Paula Herer conducted a study to determine whether CRP, total homocysteine and conventional risk for CHD already exist in untreated patients with Subclinical hypothyroidism. Thus, they studied middle-aged women with Subclinical hypothyroidism and compared their plasma total homocysteine,

CRP, lipid profiles and blood pressure with data obtained in age-matched euthyroid control women and overt hypothyroidism. They found that in Subclinical hypothyroidism, tHct and CRP levels were not as augmented as compared to controls. Their mean systolic and diastolic blood pressure values were increased vs. controls ($p<0.04$; $p<0.01$, respectively). Mean values of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, TC/HDL-C and LDL-C/HDL-C were not different in patients with Subclinical hypothyroidism compared to controls. Individual analysis revealed that the percentage of patients with Subclinical hypothyroidism having hypertension, hypertriglyceridemia, hypercholesterolemia, elevated TC/HDL-C and LDL-C/HDL-C ratios were higher than the percentage in controls. Their findings suggest that subclinical hypothyroidism in middle-aged women is associated with hypertension and dyslipidemia. CRP and tHct do not appear to contribute to the increased risk for CHD in these patients[18].

In the same year, William J. Hueston and William S. Pearson conducted a study to determine whether lipid abnormalities are more common in patients with subclinical hypothyroidism when compared lipid levels in euthyroid individuals using data from the Third National Health and Nutritional Examination Survey (NHANES III). They found that subclinical hypothyroidism had higher mean cholesterol levels (226 vs 217 mg/dL, $P = 0.003$) and rates of elevated cholesterol levels (74.2% vs 63.9%, $P = 0.02$) than the euthyroid control group, but there were no significant differences in low-density lipoprotein cholesterol(LDL-C) or high- density lipoprotein cholesterol (HDL-C) levels. When adjusted for age, race, sex, and the use of lipid-lowering drugs, however,

subclinical hypothyroidism was not related to elevations in cholesterol levels (adjusted odds ratio [OR] = 1.06, 95% confidence interval [CI], 0.57–1.97), LDL-C levels (adjusted OR = 0.89; 95% CI, 0.59–1.35), or triglyceride levels (adjusted OR = 1.83; 95% CI, 0.87–3.85) or to a low HDL-C level (adjusted OR = 0.94; 95% CI, 0.36–2.48). They concluded that, Subclinical hypothyroidism does not appear to be associated with abnormalities in serum cholesterol or triglyceride levels when adjusted for confounding variables in this population-based study[9].

Because data on cardiovascular outcomes in subjects with subclinical hypothyroidism are limited, Nicolas Rodondi and coworkers in the year 2005 performed a prospective analysis in a longitudinal cohort study of older adults to examine rates of CHF, CHD, stroke, peripheral arterial disease (PAD), and cardiovascular-related and total mortality in relationship to TSH levels. They found that, CHF events occurred more frequently among those with a TSH level of 7.0 mIU/L or greater (35.0 vs 16.5 per 1000person-years; P=0.006), but not among those with TSH levels between 4.5 and 6.9 mIU/L. In mult-variate analyses, they found that the risk of CHF was higher among those with high TSH levels (TSH of 7.0-9.9 mIU/L: hazard ratio, 2.58 [95% confidence interval, 1.19-5.60]; and TSH of 10.0 mIU/L: hazard ratio, 3.26 [95% confidence interval, 1.37-7.77]). Among the 2555 participants without CHF at baseline, the hazard ratio for incident CHF events was 2.33 (95% confidence interval, 1.10-4.96; P=.03) in those with a TSH of 7.0mIU/L or greater. Sub clinical hypothyroidism was not associated with increased risk for coronary heart disease, stroke, peripheral arterial disease, or cardiovascular related or total mortality. Subclinical hypothyroidism is associated with an increased risk of CHF among older adults with a TSH level of 7.0 mIU/L or greater, but

not with other cardiovascular events and mortality. Further investigation is warranted to assess whether subclinical hypothyroidism causes or worsens pre-existing heart failure[8].

Anne R. Cappola and coworkers in the year 2006 studied Thyroid Status, Cardiovascular Risk, and Mortality in Older Adults. They studied cardiovascular risk in subclinical hyperthyroidism, overt hypothyroidism and euthyroid cases. They concluded that there were no differences between the subclinical hypothyroidism or overt hypothyroidism groups and the euthyroidism group for cardiovascular outcomes or mortality[19].

In the year 2010, Stephan Ruhla and co-workers investigated the associations between TSH and the features and prevalence of the metabolic syndrome in euthyroid German subjects. Their study revealed that, in euthyroidism, subjects with a TSH in the upper normal range (2.5–4.5 mU/l) were more obese, had higher triglycerides, and had an increased likeliness for the metabolic syndrome. They found that TSH was weakly correlated with BMI (R =0.061, P =0 .025). This association remained significant after adjustment for sex, age, and impaired glucose metabolism (P =0.002). Subjects with a TSH in the upper normal range (2.5–4.5 mU/l, n = 119) had a significantly higher BMI (30.47 ± 0.57 vs. 28.74 ± 0.18 kg/m2, P =0 .001) and higher fasting triglycerides (1.583 ± 0.082 vs.1 .422 ± 0.024 mmol/l, P =0 .023). Therefore, a TSH below 2.5 mU/l is associated with a favorable metabolic profile. Whether lowering TSH to levels below 2.5 mU/l improves metabolism needs to be investigated in intervention trials[20].

In the year 2011, Sharma R and coworkers studied subclinical hypothyroidism and its association with cardiovascular risk factors. They observed that patients with

subclinical hypothyroidism had significantly higher levels of serum hs-CRP, total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) when compared to same parameters of controls. They found a significant positive correlation between TSH and hs-CRP, LDL-C and TC in subjects with subclinical hypothyroidism. However, TG levels showed no significant correlation with TSH levels. They concluded that the SCH patients presented increased Cardiovascular Risk factors[21].

David Nanchen and coworkers in the year 2012 studied Subclinical Thyroid Dysfunction and the Risk of Heart Failure in Older Persons at High Cardiovascular Risk. They found that there was no strong evidence of an association between subclinical thyroid dysfunction and cardiovascular events or mortality, except in those with TSH above 10 mIU/liter. Cases with TSH levels more than 10mIU / liter were associated with cardiovascular risk[22].

In the year 2013 Gencer B and his coworkers studied Subclinical thyroid dysfunction and cardiovascular outcomes among prospective cohort studies. They concluded that subclinical hypothyroidism is associated with an increased risk of coronary heart disease (CHD) events, CHD mortality and heart failure (HF) events in individuals with higher TSH levels, particularly in those with TSH levels ≥10.0 mIU/L [23].

ANATOMY OF THYRIOD GLAND

The thyroid gland is a butterfly-shaped organ and is composed of two lobes, connected via the isthmus. The organ is situated on the anterior side of the neck, lying against and around the larynx and trachea, reaching posteriorly the oesophagus and carotid sheath. It starts cranially at the oblique line on the thyroid cartilage (just below the laryngeal prominence), and extends inferiorly to approximately the fifth or sixth tracheal ring. It is difficult to demarcate the gland's upper and lower border with vertebral levels because it moves position in relation to these during swallowing.

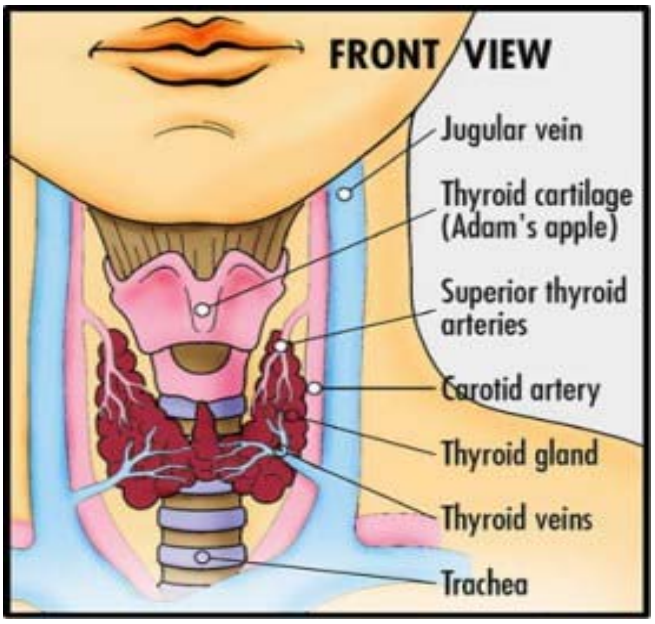
The thyroid gland is covered by a thin fibrous sheath, the capsula glandulae thyroidea, composed of an internal and external layer. The external layer is anteriorly continuous with the lamina pretrachealis fasciae cervicalis and posteriorolaterally continuous with the carotid sheath. The gland is covered anteriorly with infrahyoid muscles and laterally with the sternocleidomastoid muscle. On the posterior side, the gland is fixed to the cricoid and tracheal cartilage and cricopharyngeus muscle by a thickening of the fascia to form the posterior suspensory ligament of Berry. The thyroid glands firm attachment to the underlying trachea is the reason behind its movement with swallowing. A pyramidal extension of the thyroid lobe, is present at the most anterior side of the lobe. In this region, the recurrent laryngeal nerve and the inferior thyroid artery pass next to or in the ligament and tubercle. Between the two layers of the capsule and on the posterior side of the lobes, there are on each side two parathyroid glands.

The thyroid isthmus is variable in presence and size, and can encompass a cranially extending pyramid lobe. The thyroid is one of the larger endocrine glands, weighing 2-3 grams in neonates and 18-60 grams in adults, and is increased in pregnancy.

The thyroid is supplied with arterial blood from the superior thyroid artery, a branch of the external carotid artery, and the inferior thyroid artery, a branch of the thyrocervical trunk, and sometimes by the thyroid ima artery, branching directly from the brachiocephalic trunk. The venous blood is drained via superior thyroid veins, draining in the internal jugular vein, and via inferior thyroid veins, draining via the plexus thyroideus impar in the left brachiocephalic vein.

Lymphatic drainage passes frequently the lateral deep cervical lymph nodes and the pre and parathracheal lymph nodes. The gland is supplied by parasympathetic nerve input from the superior laryngeal nerve and the recurrent laryngeal nerve.

FIG 1: SHOWS ANATOMY OF THYROID GLAND

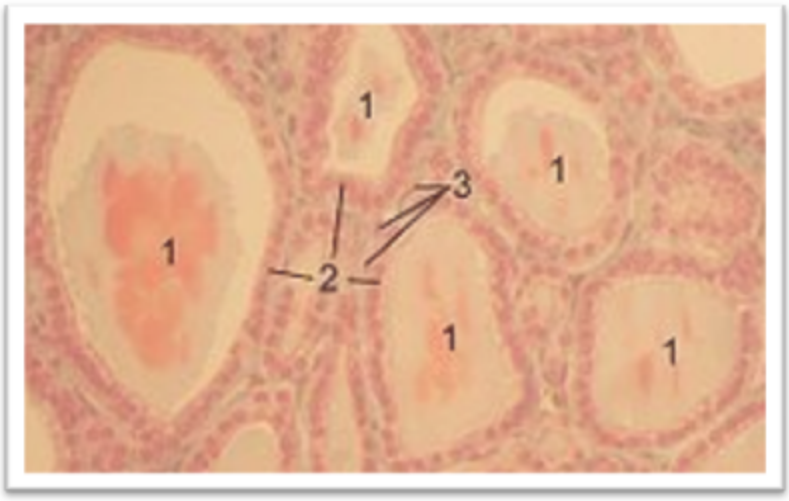


HISTOLOGY

At the microscopic level, there are three primary features of the thyroid. 1 follicles, 2 follicular epithelial cells, 3 endothelial cells.

1. Follicles - The thyroid is composed of spherical follicles that selectively absorb iodine (as iodide ions, I^-) from the blood for production of thyroid hormones, and also for storage of iodine in thyroglobulin. Twenty-five percent of all the body's iodide ions are in the thyroid gland. Inside the follicles, in a region called the follicular lumen, colloid serves as a reservoir of materials for thyroid hormone production and, to a lesser extent, acts as a reservoir for the hormones themselves. Colloid is rich in a protein called thyroglobulin.
2. Thyroid epithelial cells - The follicles are surrounded by a single layer of thyroid epithelial cells, which secrete T_3 and T_4. When the gland is not secreting T_3/T_4 (inactive), the epithelial cells range from low columnar to cuboidal cells. When active, the epithelial cells become tall columnar cells.
3. Parafollicular cells - Scattered among follicular cells and in spaces between the spherical follicles are another type of thyroid cell, parafollicular cells, which secrete calcitonin.

FIG 2: SHOWS MICROSCOPIC PICTURE OF THYROID GLAND



1. Follicles 2. Thyroid epithelial cells 3. Parafollicular cells

BIOCHEMISTRY OF HORMONES RELATED TO THYROID GLAND[49]

1. Thyrotropin Releasing Hormone (TRH): It is a tripeptide produced in the hypothalamus that stimulates the synthesis and release of TSH from anterior pituitary.
2. Thyroid stimulating hormone (TSH) : A polypeptide hormone synthesized by the anterior pituitary gland that promotes the growth of thyroid gland and stimulates the synthesis and releases of thyroid hormones by thyroid gland . It is also called as thyrotropin.

3. Thyroxine (T4) : The major hormone synthesized and released by the thyroid gland that contains four iodine molecules (L-3,5,3',5'-tetraiodothyronine).
4. Triiodothyronine (T3): The biologically active form of thyroid hormone formed outside the thyroid gland by peripheral deiodination of thyroxine (T4). It has three iodine molecules attached to its molecular structure (L-3,5,3',triiodothyronine). Reverse T3 is a biologically inert metabolite of thyroxine (T4), with three iodine molecules attached to its molecular structure (L-3,3',5'-triiodothyronine).

Thyroid hormones: Thyroid gland secrets two hormones: thyroxine and triiodothyronine. Commonly known as T4 and T3.

FIG 3: SHOWS STRUCTURE OF T3 AND T4

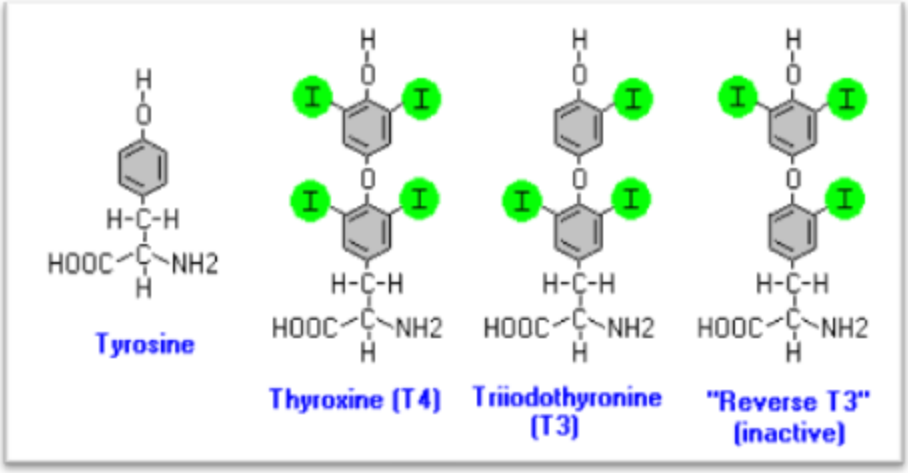


FIG 4: SHOWS STRUCTURE OF TSH

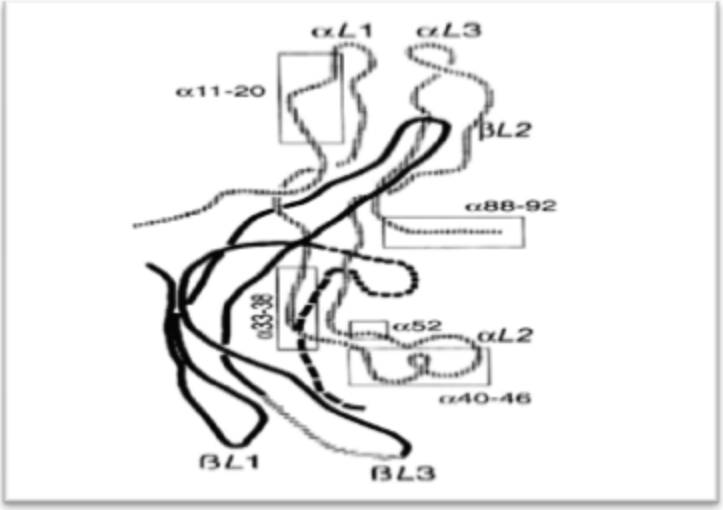
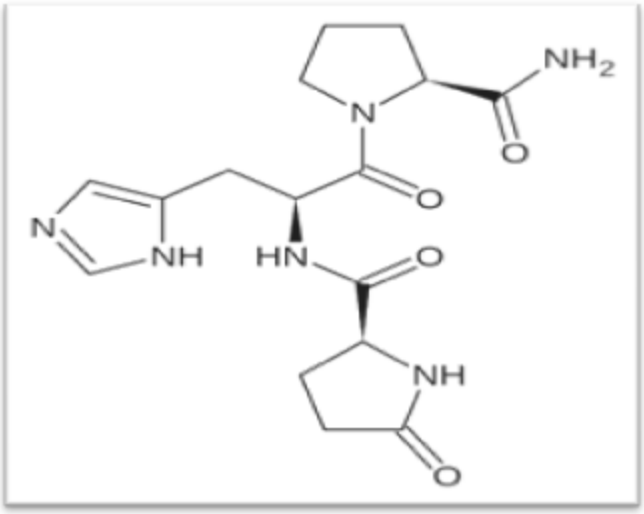


FIG. 4; The schematic drawing of TSH showing domains important for bioactivity. For clarity, the carbohydrate chains are not shown. The α -subunit backbone is shown as a gray line, and the β -subunit chain is shown as a black line. The functionally critical domains are marked directly within the line drawings. The peripheral β-hairpin loops are marked as follows: α L1, α L3 in the α -subunit; β L1, βL3 in the β -subunit. Two long loops are α L2 with α -helical structure and β L2, a loop analogous to the "Keutmann loop" in the human chorionic gonadotropin β -subunit.

FIG 5: SHOWS STRUCTURE OF TRH

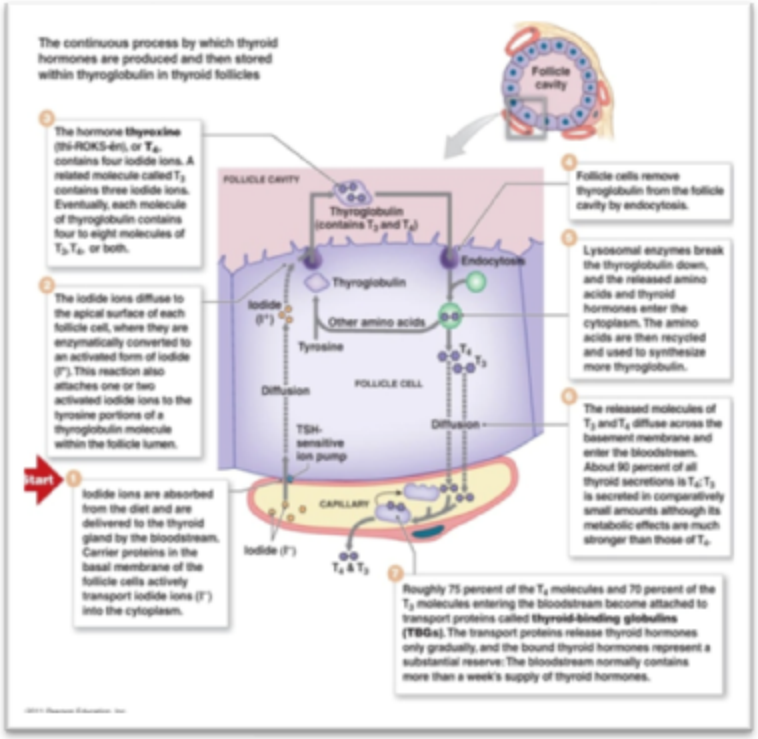


BIOSYNTHESIS OF THYROID HORMONES :

Biosynthesis of thyroid hormones involves

1. Trapping of circulating iodide by the thyroid gland
2. Incorporation of iodine in tyrosine
3. Coupling of iodinated tyrosil residues to form thyronines (T4 and T3)

FIG 6 : SYNTHESIS OF THYROID HORMONES



1. Trapping of circulating iodide by the thyroid gland :

   Thyroid gland takes up iodine. This step is stimulated by TSH and is inhibited by thyocynate and perchlorate. The iodide taken up by the thyroid cells is oxidized to active iodine . This reaction is catalysed by the enzyme thyroperoxidase. This step is stimulated by TSH and is inhibited by antithyroid drugs such as thiourea ,thiouracil , methimazole.

2. Incorporation of iodine in tyrosine :

   Thyroglobulin is synthesized by thyroid follicular cells. There are 115 tyrosine residues in thyroglobulin out of which 35 residues can be iodinated. Iodination of tyrosine takes place on the intact tyroglobulin molecule in the follicular space. Thus mono-iodo and di-iodo tyrosine are produced.

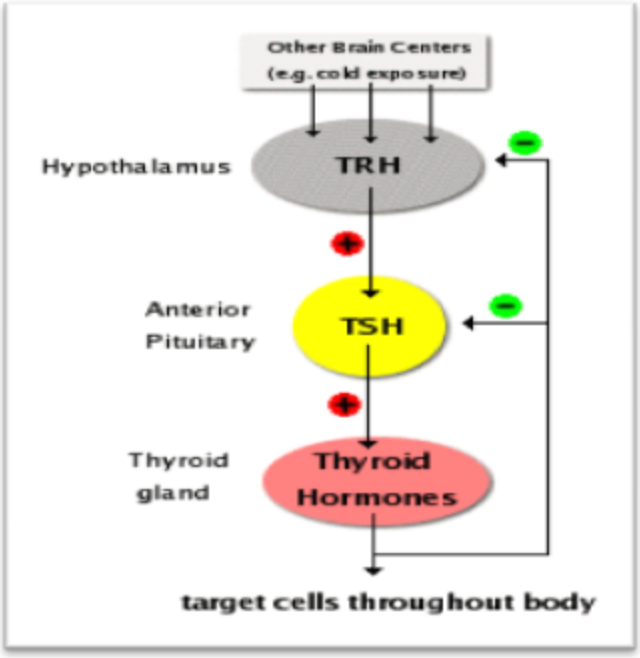
3. Coupling of iodinated tyrosil residues to form thyronines (T4 and T3):

   Coupling takes place in the borders of epithelial cells . Triiodothyronine (T3) and Tetraiodothyronine (T4) are formed by coupling of iodotyrosil residues present on thyroglobulin. Once synthesized T3 and T4 are stored in colloid follicles Release of thyroid hormones occur by fusion of colloid particles with lysosomes and proteolytic degradation of thyroglobulin.

TRANSPORT: The released hormones are transported in plasma. Most of the T4 is bound to thyroid binding globulin (TBG). It is also transported as pre-albumin-albumin complex. Only small amounts are present in free form (less than 0.03%).

PERIPHERAL CONVERSION OF T4 TO T3 : Approximately 40% of secreted T4 is deiodinated in peripheral tissue by enzyme deiodinase to yield T3 , and about 45% is deiodinated to yield rT3 ,a biologically inactive metabolite.

FIG 7: REGULATION OF THYROID HORMONE SYNYHESIS



Synthesis of thyroid hormones is stimulated by the secretion of TSH from pituitary. TSH secretion is stimulated by TRH produced from hypothalamus. The negative control (feedback regulation) occurs by the thyroid hormones through inhibition of TSH or TRH.

METHODS OF ESTIMATION OF THYROID HORMONES:

1. Enzyme Linked Immunosorbent Assay (ELISA)
2. Enzyme Linked FluroscentAssay (ELFA)
3. Chemiluminescence Assay

CELLULAR MECHANISMS OF THYROID HORMONE ACTION

T4 and T3 are synthesized by the thyroid gland in response to TSH. The thyroid gland primarily secretes T4 (nearly 85%), which is converted to T3 by 5'-monodeiodination in the liver, kidney, and skeletal muscle. The heart relies mainly on serum T3 because no significant myocyte intracellular deiodinase activity takes place, and it appears that T3, and not T4, is transported into the myocyte (Figure 8)T3 exerts its cellular actions through binding to thyroid hormone nuclear receptors (TRs). These receptor proteins mediate the induction of transcription by binding to thyroid hormone response elements (TREs) in the promoter regions of positively regulated genes. TRs belong to the super family of steroid hormone receptors, but unlike other steroid hormone receptors, TRs bind to TREs in the absence as well as in the presence of ligand. TRs bind to TREs as homodimers or, more commonly, as heterodimers with 1 of 3 isoforms of retinoid X receptor (RXR a , RXR b , or RXR gamma). While bound to T3, TRs induce transcription, and in the absence of T3 they repress transcription. Negatively regulated cardiac genes such as beta-myosin heavy chain and phospholamban are induced in the absence of T3 and repressed in the presence of T3

FIG 8: SHOWS CELLULAR MECHANISMS OF THYROID HORMONE ACTION

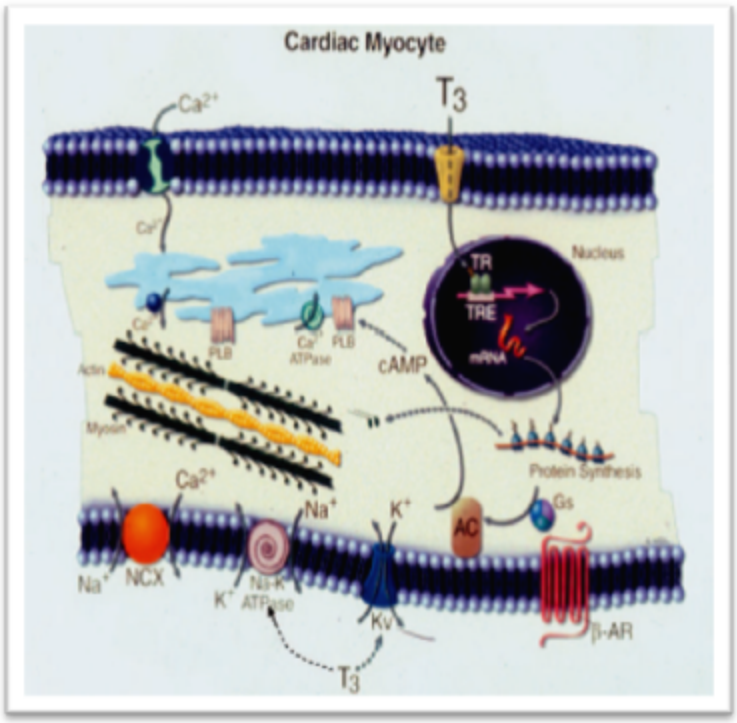


Figure 8. T3 effects on the cardiac myocyte. T3 has both genomic and nongenomic effects on the cardiac myocyte. Genomic mechanisms involve T3 binding to TRs, which regulate transcription of specific cardiac genes. Nongenomic mechanisms include direct modulation of membrane ion channels as indicated by the dashed arrows. AC indicates adenylyl cyclase; β –AR = β adrenergic receptor; Gs = guanine nucleotide binding protein; Kv = voltage-gated potassium channels; NCX = sodium calcium exchanger; and PLB= phospholamban

EFFECT OF THYROD HORMONE ON CARDIAC GENE EXPRESSION

Positively Regulated	**Negatively Regulated**
Alpha-Myosin heavy chain	beta-Myosin heavy chain
Sarcoplasmic reticulum Ca-ATPase	Phospholamban
Na/K-ATPase	Adenylyl cyclase catalytic subunits
beta1-Adrenergic receptor	Thyroid hormone receptor alpha 1
Atrial natriuretic hormone	Na/Ca exchanger
Voltage-gated potassium channels	

Effects of Thyroid Hormone on Cardiovascular Hemodynamics

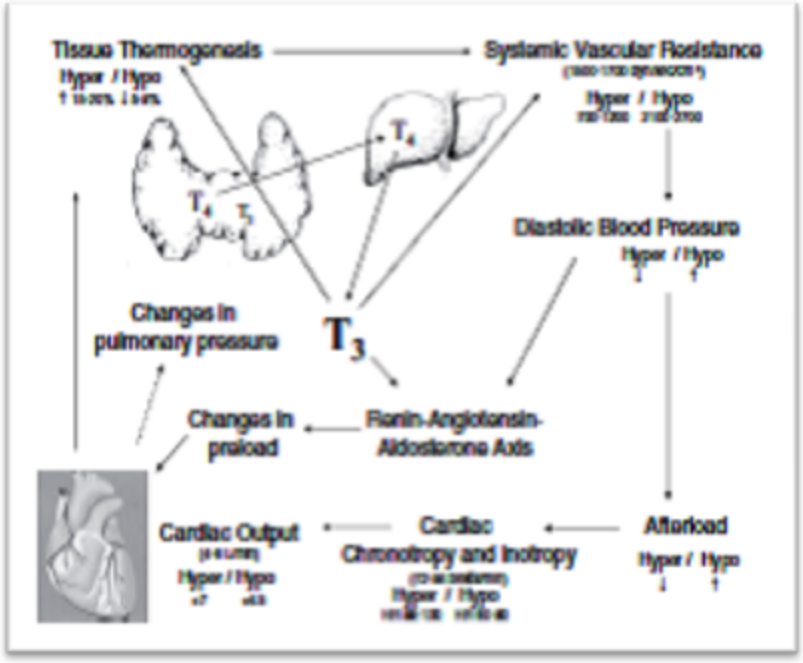
Thyroid hormone effects on the heart and peripheral vasculature include decreased superior venacaval resistance and increased resting heart rate, left ventricular contractility, and blood volume (Figure 9). Thyroid hormone causes decreased resistance in peripheral arterioles through a direct effect on VSM and decreased mean arterial pressure, which, when sensed in the kidneys, activates the renin-angiotensin-aldosterone system and increases renal sodium absorption. T3 also increases erythropoietin synthesis, which leads to an increase in red cell mass. These changes combine to promote an increase in blood volume and preload. In hyperthyroidism, these combined effects increase cardiac output 50% to 300% higher than in normal individuals. In hypothyroidism, the cardiovascular effects are diametrically opposite and cardiac output may decrease by 30% to 50%.

In the VSM cell, thyroid hormone mediated effects are the result of both genomic and nongenomic actions. Nongenomic actions target membrane ion channels and

endothelial nitric oxide synthase, which serves to decrease SVR. Relaxation of VSM leads to decreased arterial resistance and pressure, which thereby increases cardiac output. Increased endothelial nitric oxide production may result, in part, from the T3-mediated effects of TR on the protein kinase pathway either via non- genomic or genomic mechanisms. Nitric oxide synthesized in endothelial cells then acts in a paracrine manner on adjacent VSM cells to facilitate vascular relaxation. . Impaired endothelium dependent vasodilatation as a result of a reduction in nitric oxide availability has been demonstrated in subclinical hypothyroidism as well.

The juxtaglomerular apparatus of the kidneys is volume and pressure sensitive and in response to a decrease in mean arterial pressure, the renin-angiotensin-aldosterone system is activated and renin secretion is increased. The cascade of events that follow include increased levels of angiotensin I and II, angiotensin-converting enzyme (ACE) (characteristic of hyperthyroidism), and aldosterone. Thyroid hormone acts first to lower SVR through pathways discussed above, which causes mean arterial pressure to decrease. This is sensed by the juxtaglomerular apparatus, which leads to increased renin synthesis and secretion. hypothyroidism is often accompanied by a rise in diastolic blood pressure. Because cardiac output is low, the pulse pressure is narrowed.

FIG 9 : SHOWS EFFECTS OF THYROID HORMONE ON CARDIOVASCULAR HEMODYNAMICS



Thyroid Hormone Effects on Blood Pressure Regulation

Thyroid hormone increases basal metabolic rate in almost every tissue and organ system in the body, and the increased metabolic demands lead to changes in cardiac output, SVR, and blood pressure. In hypothyroidism, endothelial dysfunction and impaired VSM relaxation lead to increased SVR. These effects lead to diastolic hypertension in nearly 30% of patients, and thyroid hormone replacement therapy restores endothelial-derived vasorelaxation and blood pressure to normal in most.

Thyroid Hormone Effects on Lipid Metabolism

The reported mechanisms for the development of hypercholesterolemia in hypothyroidism include decreased fractional clearance of LDL by a reduced number of LDL receptors in the liver in addition to decreased receptor activity. The catabolism of cholesterol into bile is mediated by the enzyme cholesterol 7 α -hydroxylase. This liver-specific enzyme is negatively regulated by T3 and may contribute to the decreased catabolism and increased levels of serum cholesterol associated with hypothyroidism. The increased serum lipid levels in subclinical hypothyroidism as well as in overt disease are potentially associated with increased cardiovascular risk. Treatment with thyroid hormone replacement to restore euthyroidism reverses the risk ratio.

MATERIAL AND METHODS

A cross sectional study of serum lipid profile in subclinical hypothyroidism subjects was carried out from November 2011 to May 2013. We selected 60 subclinical hypothyroidism cases aged ≥ 30 years from among the patients referred to the clinical biochemistry department, BLDEU's Shri B M Patil Medical College, Hospital and Research Centre, Bijapur, Karnataka, and 30 healthy euthyroid controls from the general population according to the inclusion and exclusion criteria mentioned below. This study was approved by the Ethical and Research Committee of BLDEU's Shri B M Patil Medical College, Bijapur and all the subjects gave an informed consent before undergoing further investigations.

Inclusion criteria: subclinical hypothyroidism cases having TSH in the range of 4.50 to 14.99 mU/L and T3 and T4 values within normal limits. The euthyroid controls having normal TSH values.

Exclusion criteria: smokers, those with known hypothyroidism, previous radioactive iodine therapy, thyroidectomy, external radiation, consumption of drugs known to cause SCH, primary or secondary dyslipidemia, diabetes mellitus, renal and hepatic failure, or other systemic diseases were excluded from the study.

Venous blood samples were drawn at 8 AM following a 12 hours fast, in a plain bulb from the subjects, with all the aseptic precautions. Blood samples were centrifuged within 30 minutes at 3000 rpm for 5 min. and serum was separated. Serum samples were stored at 20°C until assayed

ESTIMATION OF THYROID STIMULATING HORMONE (TSH)

Method: Enzyme linked immuno sorbent assay (ELISA)[24,25].

Principle: Specific anti-TSH antibodies are coated on to microtitration wells. Test sera are applied. Then goat anti-TSH labeled with Horseradish peroxidise enzyme (conjugate) is added. If human TSH is present in the sample it will combine with the antibody on the well and the enzyme conjugate, resulting in the TSH molecule being sandwiched between the solid phase and the enzyme linked antibodies. After incubation the wells are washed to remove the unbound labelled antibodies. On addition of the substrate, a color will develop only in those wells in which the enzyme conjugate is present, indicating the presence of TSH. The enzyme reaction is stopped by the addition of dilute hydrochloric acid and the absorbance is measured at 450 nm.

Reagent preparation: all the reagents should be brought to room temperature and mixed gently prior to use. Do not induce foaming.

Standards: add 1 mL distilled water to each standard vial in order to reconstitute the lyophilised standards. Allow to stand for a minimum of 20 minutes before use. Rehydrated standards will be stable for up to 30 days when stored at 2° to 8° C. For longer storage store sealed at -20° C when not in use.

Wash buffer: dilute the concentrated wash buffer using 1 part wash buffer concentrate with 19 parts distilled water. For every 8 well breakable strip, prepare 25 mL of diluted wash buffer by adding 1.25 mL of concentrated wash buffer to 23.75 mL of distilled water. Prepare fresh diluted wash buffer prior to every assay run. Extra wash buffer is supplied to enable priming of automatic washing machines.

The washing procedure is critical to the outcome of this test. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Assay procedure.

1. Bring all the kit components and test serum to the room temperature (20° C to 25° C) prior to the start of the assay.
2. One set of standards should be run with each batch of test serum. Secure the desired number of coated wells in the holder. Record the position of the standards and the test serum on the EIA data recording sheet provided.
3. Unused strips should be resealed in the foil bag containing the desiccant, using the resealing zip-lock before being replaced at 2° C to 8° C.
4. Dispense 100 μL of standards and test serum in to the appropriate wells.
5. Dispense 100 μL of anti-TSH conjugate in to each well. Mix thoroughly for 30 seconds. It is very important to mix completely.
6. Incubate for 60 minutes at room temperature.
7. Hand washing: at the end of incubation period, discard the contents of the wells by flicking plate contents in to a biohazard container. Then strike the wells sharply against absorbent paper. Ensure adequate disinfectant is contained in the biohazard container.
8. Fill the wells with a minimum of 300μL of wash buffer per well. Flick plate contents in to a biohazard container. Then strike the wells sharply against absorbent paper. Wash the empty wells 5 times.
9. Strike the wells sharply on to absorbent paper or paper towel to remove all residual water droplets.

10. Dispense 100 μL substrate solution in to each well and mix gently for 5 seconds.
11. Incubate in the dark for 20 minutes at room temperature.
12. Stop the reaction by adding 100μL stop solution to each well.
13. Gently mix for 30 seconds to ensure that the blue colour changes completely to a yellow colour.
14. Read the optical density immediately (no later than 10 minutes) using a micropipette reader with a 450nm filter.

Calculation of the results: calculate the mean absorbance value for each set of standards and specimens. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration in μIU/mL on graph paper, with absorbance values on the Y axis and concentrations on the X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of TSH in μIU/mL from the standard curve. If levels of calibrators or users known samples do not give expected results, test results must be considered invalid.

Expected values and sensitivity: the graph produced by the calibrators should be hyperbolic in shape with the OD 450 of the calibrators proportional to their concentration. The OD of calibrator A should be less than 0.2 and the OD of calibrator F should be greater than 1.5 for the assay results to be valid. Normal values for adults between the ages of 21 and 54 years is 0.4 to 4.2 μIU/mL rising to 0.5 to 8.9 μIU/mL between the ages of 55 and 87.

ESTIMATION OF TOTAL TRIIODOTHYRONINE (T3)

Method: Enzyme linked immuno sorbent assay (ELISA)[26,27].

Principle: Goat anti-mouse IgG antibody is coated on to microtitration wells. Test sera are applied along with antibody reagent. T3 enzyme conjugate is added which competes with the serum T3 for available binding sites on the solid phase. After incubation, the wells are washed to remove any unbound T3 or T3 enzyme conjugate. On addition of the substrate (TMB), a color develops only in those wells in which enzyme is present indicating a lack of serum T3. The reaction is stopped by the addition of dilute hydrochloric acid and the absorbance is then measured at 450nm.

Reagent preparation: all the reagents should be brought to room temperature (20° C to 25° C) and mixed gently prior to use. Do not induce foaming.

Conjugate: Dilute the concentrated conjugate using 1 part concentrated conjugate with 10 parts conjugated diluents. Eg. Add 0.1 mL concentrated conjugate to 1.0 mL conjugated diluent. This should be done 20 minutes prior to initiation of the assay. Ensure that the diluted conjugate is at room temperature. Do not induce foaming. Use within 24 hours.

Wash buffer: Dilute the concentrated wash buffer using 1 part wash buffer concentrate with 19 parts distilled water. For every 8 well breakable strip, prepare 25 mL of diluted wash buffer by adding 1.25 mL of concentrated wash buffer to 23.75 mL of distilled water. Prepare fresh diluted wash buffer prior to every assay run. Extra wash buffer is supplied to enable priming of automatic washing machines.

The washing procedure is critical to the outcome of this test. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Assay procedure.

1. Bring all the kit components and test serum to the room temperature (20° C to 25° C) prior to the start of the assay.
2. One set of standards should be run with each batch of test serum. Secure the desired number of coated wells in the holder. Record the position of the standards and the test serum on the EIA data recording sheet provided.
3. Unused strips should be resealed in the foil bag containing the desiccant, using the resealing zip-lock before being replaced at 2° C to 8° C.
4. Dispense 50 µL of standards and test serum in to the appropriate wells.
5. Dispense 50 µL of antibody reagent in to each well. Mix thoroughly for 30 seconds. It is very important to mix completely.
6. Dispense 100 µL of working strength conjugate in to each well. Mix thoroughly for 30 seconds.
7. Incubate for 60 minutes at room temperature (20° C to 25° C).
8. Hand washing: at the end of incubation period, discard the contents of the wells by flicking plate contents in to a biohazard container. Then strike the wells sharply against absorbent paper. Ensure adequate disinfectant is contained in the biohazard container.
9. Fill the wells with a minimum of 300µL of wash buffer per well. Flick plate contents in to a biohazard container. Then strike the wells sharply against absorbent paper. Wash the empty wells 5 times.

10. Strike the wells sharply on to absorbent paper or paper towel to remove all residual water droplets.
11. Dispense 100 μL substrate solution in to each well and mix gently for 5 seconds.
12. Incubate in the dark for 20 minutes at room temperature (20° C to 25° C).
13. Stop the reaction by adding 100μL stop solution to each well.
14. Gently mix for 30 seconds to ensure that the blue colour changes completely to a yellow colour.
15. Read the optical density immediately (no later than 10 minutes) using a micropipette reader with a 450nm filter.

Calculation of the results: calculate the mean absorbance value for each set of standards and specimens. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration in ng/mL on graph paper, with absorbance values on the Y axis and concentrations on the X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of T3 in ng/mL from the standard curve.

If levels of calibrators or users known samples do not give expected results, test results must be considered invalid.

Expected values and sensitivity: the graph produced by the calibrators should be hyperbolic in shape with the OD 450 of the calibrators inversely proportional to their concentration. The OD of calibrator A should be greater than 1.5 and the OD of calibrator F should be less than 0.75 for the assay results to be valid. Normal range of T3 is 0.8 to 1.9 ng/ml

ESTIMATION OF TOTAL THYROXIN (T4)

Method: Enzyme linked immuno sorbent assay (ELISA)[28,29].

Principle: specific anti-T4 antibodies are coated on to microtitration wells. Test sera are applied. T4 with Horseradish peroxidase enzyme (conjugate) is added which competes with the serum T4 for available binding sites on the solid phase. After incubation, the wells are washed to remove any unbound T4 or T4 enzyme conjugate. On addition of the substrate, a colour develops only in those wells in which enzyme is present indicating a lack of serum T4. The reaction is stopped by the addition of dilute hydrochloric acid and the absorbance is then measured at 450nm.

Reagent preparation: All the reagents should be brought to room temperature (20° C to 25° C) and mixed gently prior to use. Do not induce foaming.

Conjugate: Dilute the concentrated conjugate using 1 part concentrated conjugate with 10 parts conjugated diluents. Eg. Add 0.1 mL concentrated conjugate to 1.0 mL conjugated diluent. This should be done 20 minutes prior to initiation of the assay. Ensure that the diluted conjugate is at room temperature. Do not induce foaming. Use within 24 hours.

Wash buffer: Dilute the concentrated wash buffer using 1 part wash buffer concentrate with 19 parts distilled water. For every 8 well breakable strip, prepare 25 mL of diluted wash buffer by adding 1.25 mL of concentrated wash buffer to 23.75 mL of distilled water. Prepare fresh diluted wash buffer prior to every assay run. Extra wash buffer is supplied to enable priming of automatic washing machines.

The washing procedure is critical to the outcome of this test. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

Assay procedure.

1. Bring all the kit components and test serum to the room temperature (20° C to 25° C) prior to the start of the assay.
2. One set of standards should be run with each batch of test serum. Secure the desired number of coated wells in the holder. Record the position of the standards and the test serum on the EIA data recording sheet provided.
3. Unused strips should be resealed in the foil bag containing the desiccant, using the resealing zip-lock before being replaced at 2° C to 8° C.
4. Dispense 25 µL of standards and test serum in to the appropriate wells.
5. Dispense 100 µL of working strength conjugate in to each well. Mix thoroughly for 30 seconds. It is very important to mix completely.
6. Incubate for 60 minutes at room temperature (20° C to 25° C).
7. Hand washing: at the end of incubation period, discard the contents of the wells by flicking plate contents in to a biohazard container. Then strike the wells sharply against absorbent paper. Ensure adequate disinfectant is contained in the biohazard container.
8. Fill the wells with a minimum of 300µL of wash buffer per well. Flick plate contents in to a biohazard container. Then strike the wells sharply against absorbent paper. Wash the empty wells 5 times.
9. Strike the wells sharply on to absorbent paper or paper towel to remove all residual water droplets.
10. Dispense 100 µL substrate solution into each well and mix gently for 5 seconds.
11. Incubate in the dark for 20 minutes at room temperature (20° C to 25° C).

12. Stop the reaction by adding 100μL stop solution to each well.
13. Gently mix for 30 seconds to ensure that the blue colour changes completely to a yellow colour.
14. Read the optical density immediately (no later than 10 minutes) using a micropipette reader with a 450nm filter.

Calculation of the results: calculate the mean absorbance value for each set of standards and specimens. Construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration in ng/mL on graph paper, with absorbance values on the Y axis and concentrations on the X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of T3 in ng/mL from the standard curve.

If levels of calibrators or users known samples do not give expected results, test results must be considered invalid.

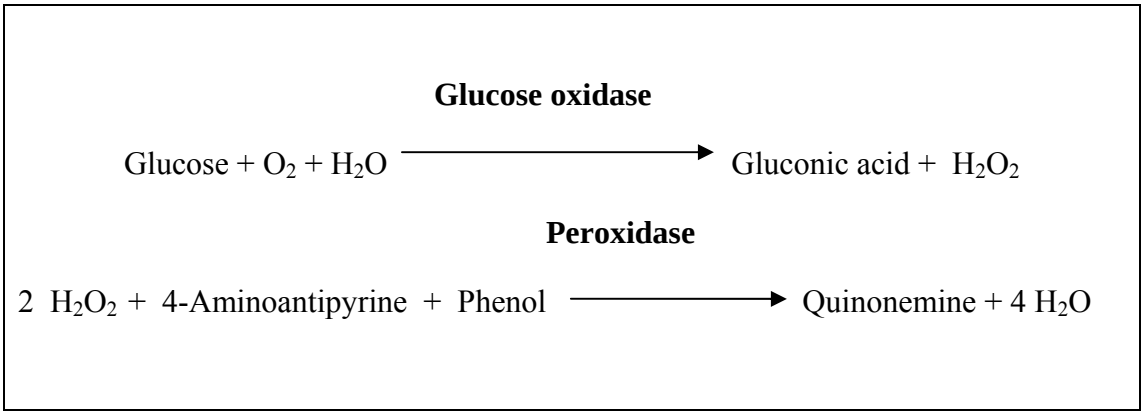
Expected values and sensitivity: the graph produced by the calibrators should be hyperbolic in shape with the OD 450 of the calibrators inversely proportional to their concentration. The OD of calibrator A should be greater than 1.5 and the OD of calibrator F should be less than 0.75 for the assay results to be valid. Normal range of T3 is 50 to 130 ng/mL.

ESTIMATION OF FASTING SERUM GLUCOSE

Method: Glucose oxidase (GOD-POD) method [30].

Principle:

The enzyme glucose oxidase catalyses the oxidation of glucose to gluconic acid and hydrogen peroxide (H_2O_2). The colorimetric indicator, quinoneimine is generated from 4-aminoantipyrine and phenol by H_2O_2 under the catalytic action of peroxidase. Intensity of color generated is directly proportional to glucose concentration.



Reagents:

1. Glucose Reagent : R1:

Active reagent	**Concentration**
Phosphate buffer pH 7.0	100 mmol / L
Phenol	10 mmol /L
R2 Active reagent	**Concentration**
4-Aminoantipyrine	270 mmol /L
Glucose oxidase	>10000U / L
Peroxidase	>600U / L

3. Glucose standard – 100 mg / dL.

Assay Procedure:

- Wavelength : 500(500-540) nm
- Cuvette : 1 cm light path
- Temperature : 37° C

Pipette into tubes marked	**Blank**	**Standard**	**Test**
Working reagent	1000 µl	1000 µl	1000 µl
Standard	-	10 µl	-
Test (serum)	-	-	10 µl

Mixed and incubated for 10 minutes at 37^0C. Measure the change in absorbance of standard and test against reagent blank

Calculations :

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \text{ X Concentration of Standard}$$

.....mg/dl

Linearity : The assay is linear up to 500 mg/dl

ESTIMATION OF SERUM TRIGLYCERIDES

Method: Enzymatic (GPO-POD method)[31].

Principle: Triglycerides are hydrolyzed by lipoprotein lipase to glycerol and fatty acids. Glycerol is first phosphorylated to glycerol-3-phosphate by glycerol kinase and then oxidized by glycerol phosphate oxidase forming hydrogen peroxide and dihydroxy acetone phosphate. This hydrogen peroxide in the presence of peroxidase causes oxidative coupling of 4- chlorophenol and 4- aminoantipyrine to form red colored quinoneimine dye which is measured at 505 nm. Intensity of colour generated is directly proportional to the concentration of triglycerides.

$$\text{Triglycerides} \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol + free fatty acids}$$

$$\text{Glycerol + ATP} \xrightarrow[Mg^{2+}]{\text{Glycerol kinase}} \text{Glycerol-3-phosphate+ADP}$$

$$\text{Glycerol-3-phosphate + } O_2 \xrightarrow{\text{GPO}} \text{Dihydroxy acetone phosphate + } H_2O_2$$

$$H_2O_2\text{+ 4 aminoantipyrine + 4chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine + HCl + } H_2O$$

Reagent 1: Triglycerides reagent composition

Active reagent	Concentration
ATP	3.15 mmol/L
Mg^{2+}	17 mmol/L
4 aminoantipyrine	0.9 mmol/L
p-Chlorophenol	5.3 mmol/L
Peroxidase	>450 U/L
Glycerol kinase	> 450 U/L
Potassium ferrocynate	10micromol/L
Glycerol-3 phosphate oxidase	>3500U/L
Lipoprotein lipase	> 1800 U/L
Buffer (pH 7.0)	50 mmol/L

Reagent 2: Triglyceride standard – 200 mg/dl

Assay Procedure:

- Wavelength : 505 nm
- Cuvette : 1 cm light path
- Temperature : 37^0C

Pipette into tubes marked	Blank	Standard	Test
Working reagent	1000 µl	1000 µl	1000 µl
Standard	-	10 µl	-
Test (serum)	-	-	10 µl

Mixed and incubated for 5 minutes at 37^0C. Measure the absorbance of sample and standard against reagent blank.

Calculations:

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \text{ X Concentration of Standard}$$
.....mg/dl

Linearity: The assay is linear upto 1000 mg/dl

ESTIMATION OF SERUM TOTAL CHOLESTEROL

Method: Enzymatic Cholesterol Oxidase - CHOD-PAP method[27].

Principle: Cholesterol esterase hydrolyzes cholesterol esters to free cholesterol and fatty acids. Cholesterol is oxidized by cholesterol oxidase forming hydrogen peroxide and cholest-4ene-3one. In presence of peroxidase, hydrogen peroxide formed brings about oxidative coupling of phenol and antipyrine to form red colored quinoneimine dye. Intensity of colour generated is directly proportional to total cholesterol concentration.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{Cholesterol esterase}} \text{Cholesterol} + \text{fatty acid}$$

$$\text{Cholesterol ester} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{Cholest-4ene-3one} + H_2O_2$$

$$2H_2O_2 + \text{4- aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Red Quinone} + 4H_2O$$

Reagents: Enzyme reagent: Phosphate buffer (pH 6.7) – 50 mmol/L

4- amino antipyrine – 0.5 mmol/L

Peroxidase - > 1000 IU/L

Cholesterol esterase - > 200 IU/L

Cholesterol Oxidase - > 180 IU/L

Sodium cholate – 0.50 mmol/L

Phenol – 24 m mol/L

Standard: Cholesterol 200 mg/dl

Assay Procedure:

- Wavelength : 505 nm(492-550nm)
- Cuvette : 1 cm light path
- Temperature : 37^0C

	Blank	Standard	Sample
Enzyme reagent	1000 µl	1000 µl	1000 µl
Standard	--	10 µl	--
Sample (serum)	--	--	10 µl

Mixed and incubated for 5 minutes measure the absorbance of sample and standard against the reagent blank.

Calculations:

$$\text{Concentration of total cholesterol} = \frac{\text{Absorbance (sample)}}{\text{Absorbance (standard)}} \text{ x } 200$$

= …….mg/dl

Linearity: The assay is linear up to 600mg/dL

ESTIMATION OF SERUM HDL CHOLESTEROL

Method: Enzymatic CHOD-PAP method[31].

Principle: Chylomicrons, VLDL and LDL are precipitated by adding phosphotungstic acid and magnesium ions to sample. Centrifugation leaves only HDL in the supernatant. The cholesterol content in it is determined enzymatically.

Reagents: Precipitating reagent

Phosphotungstic acid	2.4 mmol/L
Magnesium chloride	40 mmol/L

HDL cholesterol Standard – 25 mg/dl

Procedure:

Precipitation:

Pipette	**Volume**
Test(serum)	250 µl
Precipitating reagent	500 µl

Mixed well and allowed the reaction mixture to stand for 10 minutes at room temperature, centrifuge at 4000 rpm (1800xg) for 10 minutes to obtain a clear supernatant. The supernatant was used to determine the concentration of HDL cholesterol in the sample.

Assay Procedure:

Wavelength : 505 nm

Optical path : 1cm

Temperature : 37^0c

Pipette in to tubes marked	Blank	Standard	Test
Cholesterol working reagent	1000 µl	1000 µl	1000 µl
Distilled water	50 µl	--	--
HDL standard	--	50 µl	--
Supernatant	--	--	50 µl

Mixed well, incubated for 10 minutes at 37^0C. Read the absorbance of the standard and each test at 505 nm against reagent blank.

Calculation:

$$\text{HDL Cholesterol} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \text{ X Concentration of Standard X Dilution factor}$$

$$= \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \text{ x 25 x 3}$$

$$= \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \text{ x 75}$$

= ---------- mg/dl

Linearity: The assay is linear up to 125 mg/dl of HDL.

Normal Values:

- Males : $\geq$ 40 mg / dL
- Females: $\geq$ 50 mg / dL

ESTIMATION OF SERUM LDL CHOLESTEROL

Method: Enzymatic CHOD-PAP method[32].

Principle: HDL, VLDL and Chylomicrons are specifically hydrolysed by detergent-1 so that LDL particles remain intact. Addition of second detergent with coupler NN bis (4 sulfobutyl)-m-toluidine release cholesterol in LDL particle and is subjected to enzymatic action forming a red quinoneimine dye whose absorbance is measured at 552 nm. The colour intensity of dye is directly proportional to LDL concentration.

I step:

HDL, VLDL, Chylomicrons + Detergent1 ⟶ released cholesterol

Cholesterol esterase

Cholesterol ⟶ oxidized cholesterol + H_2O_2

Cholesterol oxidase

Peroxidase

H_2O_2 + 4 AAP ⟶ colourless product

II STEP:

LDL + Detergent-2 → released cholesterol

Cholesterol esterase

Cholesterol ⟶ oxidized cholesterol + H_2O_2

Cholesterol oxidase

Peroxidase

H_2O_2 + 4 AAP + DSBmT ⟶ Quinoneimine dye

Expected values for LDL cholesterol:

Recommendation of National Cholesterol Education Program (NCEP)

Risk Classification: LDL cholesterol

- Desirable : < 130 mg/dl (< 3.36 mmol/L)
- Border line : 130-160 mg/dl (3.36 – 4.13 mmol/L)
- High risk : > 160 mg/dl (>4.13 mmol/L)

STATISTICAL ANALYSIS

Data is analyzed with

- Diagrammatic representation.
- Mean $\pm$ SD
- Comparison of the groups is done by unpaired t test and Man Whiteny U test
- To find association, Spearman correlation test is done.
- For all the tests, p-value of 0.05 or less is considered as statistically significant.

RESULTS

The present study includes 60 subclinical hypothyroidism patients and 30 euthyroid controls.

Table 1: Age Distribution Of Study Group

Age in years	Subclinical hypothyroidism n = 60	Percentage	Euthyroid controls n = 30	Percentage
35-40	14	23%	1	3.3%
40-45	10	16%	4	13.3%
45-50	15	25%	11	36.6%
50-55	12	20%	7	23%
55-60	9	15%	7	23%

Table 1 shows age distribution of cases and controls studied. The study included 60 subclinical hypothyroidism cases with a mean age of 46.1 $\pm$ 7.2 years, and 30 healthy controls with a mean age of 49.5 $\pm$ 5.9 years. Subclinical hypothyroidism and euthyroid control subjects were well matched with respect to age . The table shows that subclinical hypothyroidism is more common in the age group of 45-50 years.

Chart 1: Shows mean age distribution of study group

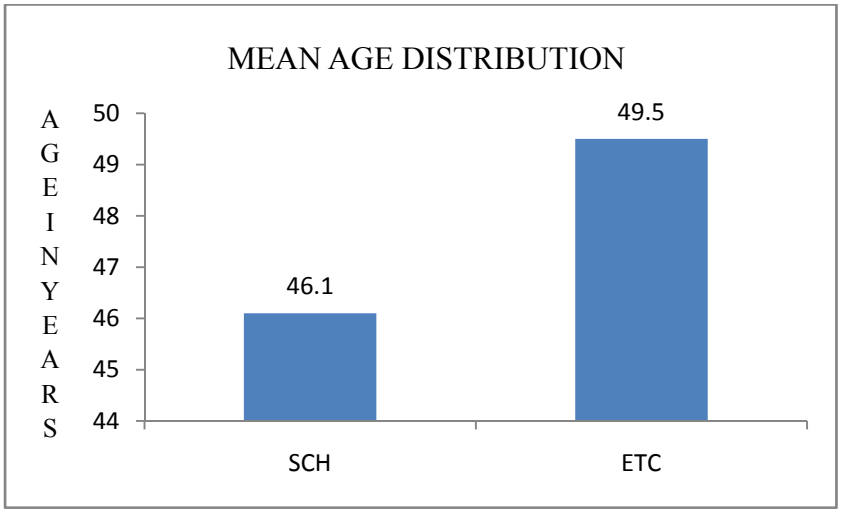
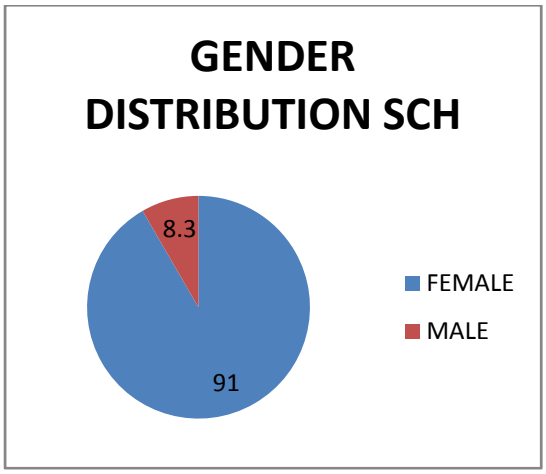


Table 2: Gender distribution of study group

Gender	Subclinical hypothyroidism	Euthyroid controls
FEMALE	55(91%)	26 (86.6%)
MALE	5 (8.3%)	4 (13.3%)

Table 2 shows the gender distribution of the study group . In Subclinical Hypothyroid cases out of 60 cases 55(91%) were female and 5(8.3%) were male . In Euthyroid controls out of 30 controls 26 (86.6%) were females and 4 (13.3%) were males. This shows that gender distribution is well matched between the two groups and indicates that Subclinical Hypothyroidism is more common in female population.

Chart 2: Shows gender distribution of study group



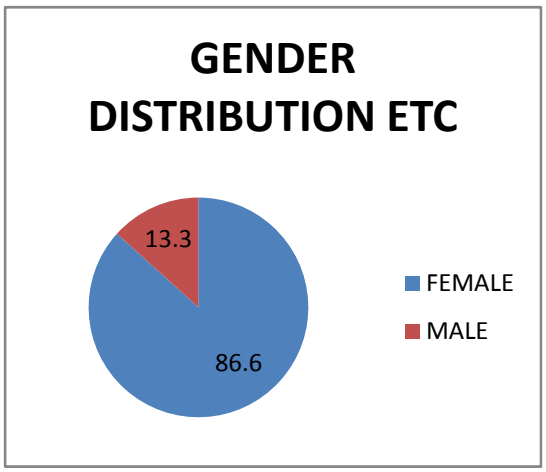


TABLE – 3: Comparison of parameters between subclinical hypothyoidism subjects and euthyroid controls

VARIABLE	SCH Patients (Mean±SD)	Euthyroid controls (Mean±SD)	P value	Statistical significance
Age (yrs)	46.1± 7.2	49.5 ± 5.9	-	-
TSH (μIU/ml)	7.68 ± 2.46	2.64 ± 1.09	P<0.0001	HS
T3 (nmol/l)	1.36 ± 0.41	1.83 ± 0.97	P<0.0035	HS
T4 (nmol/l)	85.12 ± 16.72	89.27 ± 22.78	P<0.4060	NS
FBG (mg/dL)	80.30 ± 7.13	78.73 ± 6.21	P=0.3085	NS
TC (mg/dL)	245.72 ± 38.36	183.66 ± 39.13	P<0.0001	HS
TG (mg/dL)	165.25 ± 18.74	104.37 ± 31.58	P<0.0001	HS
LDL-C (mg/dL)	185.63 ± 37.94	126.24 ± 36.39	P<0.0001	HS
HDL-C (mg/dL)	27.03 ± 4.07	36.23 ± 6.63	P<0.0001	HS
SBP (mm Hg)	127.36 ± 5.65	122.73 ± 4.35	P=0.0002	HS
DBP (mm Hg)	91.43 ± 3.08	80.86 ± 4.16	P<0.0001	HS

P value ≤ 0.05 is considered as statistically significant

TSH = Thyroid stimulating hormone; T3 = Tri-iodothyronine; T4 = Tetra-iodothyronine; FBG = Fasting blood glucose; TC = Total cholesterol; TG = Triglycerides; LDL-C = Low density lipoprotein Cholesterol; HDL-C = High density lipoprotein Cholesterol; SBP = Systolic blood pressure; DBP = Diastolic blood pressure; NS = Statistically Not significant; HS = Highly significant.

Table 3 shows comparison of parameters among the study groups. Serum mean levels of TSH (7.68 ± 2.46), T3 (1.36 ± 0.41), Total cholesterol (245.72 ± 38.36), Triglycerides (165.25 ± 18.74), LDL-C (185.63 ± 37.94), HDL-C (27.03 ± 4.07),Systolic blood pressure

(127.36 ± 5.65),and diastolic blood pressure (91.43 ± 3.08) were significantly higher in SCH patients than in controls (2.64 ± 1.09 , 1.83 ± 0.97, 183.66 ± 39.13, 104.37 ± 31.58, 126.24 ± 36.39, 36.23 ± 6.63, 122.73 ± 4.35, 80.86 ± 4.16, respectively) and were statistically significant ($p < 0.05$). Serum mean levels of T4 (85.12 ± 16.72), Fasting Blood Glucose (80.30 ± 7.13) were not significantly different from the values in controls (89.27 ± 22.78, 78.73 ± 6.21 respectively).

CHART 3 shows the mean thyroid profile in the study group. CHART 3A shows that serum TSH levels were significantly higher in SCH patients than controls ($P < 0.0001$), CHART 3B shows that serum T3 and T4 levels, although within normal range , T3 was statistically significant ($p < 0.0035$),whereas serum T4 levels were not statistically significant ($p < 0.40$).

CHART 3A: SHOWS MEAN TSH LEVELS IN STUDY GROUP

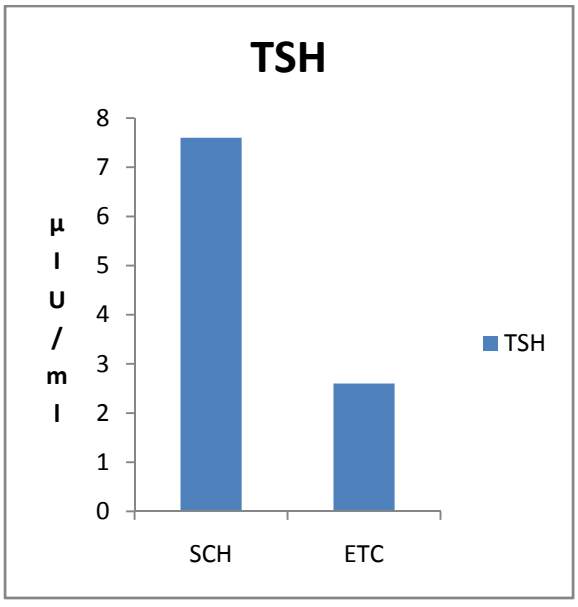


CHART 3B : SHOWS MEAN T3 AND T4 LEVELS IN STUDY GROUP

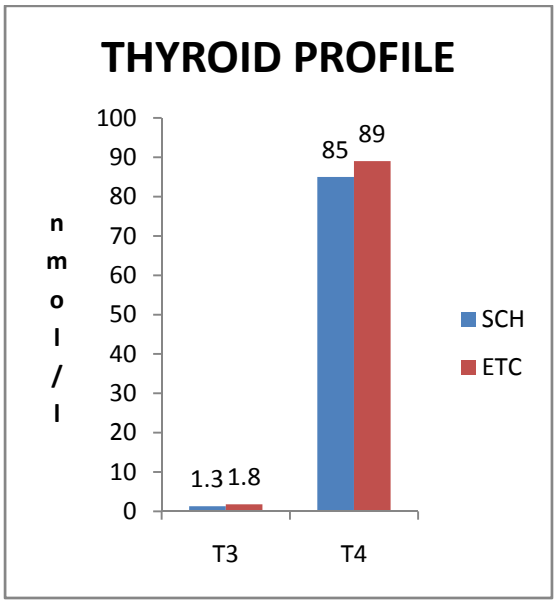


Chart 4: Shows mean lipid profile in study group

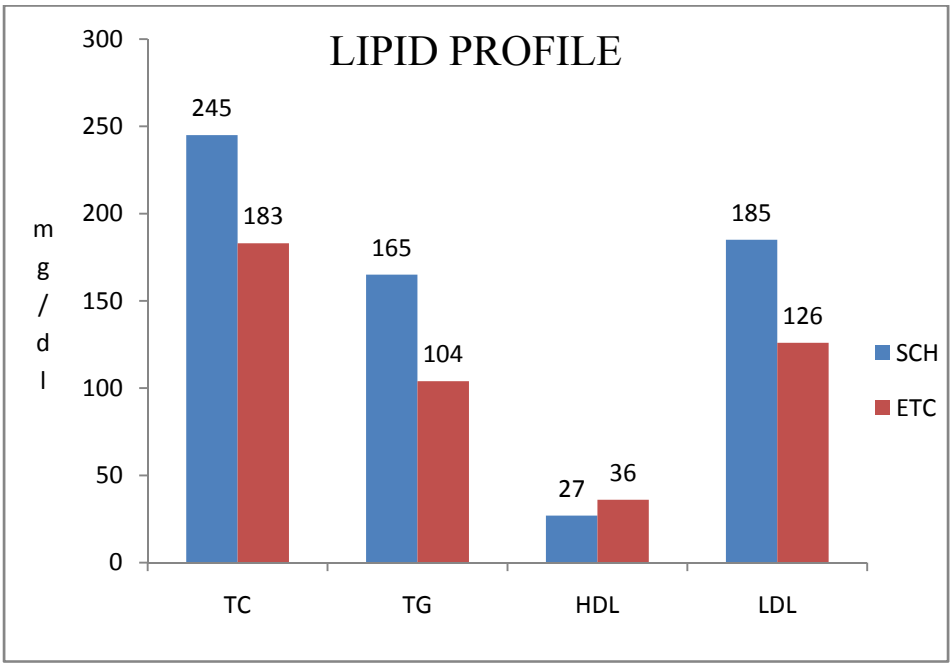


CHART 4 shows mean lipid profile in study group . Subclinical Hypothyroid cases show significantly higher levels of Total Cholesterol ($p < 0.0001$), Triglycerides ($p < 0.0001$) , High density lipoprotein cholesterol levels ($p < 0.0001$), and Low density lipoprotein cholesterol levels ($p < 0.0001$) .

Chart 5: Shows mean blood pressure in study group

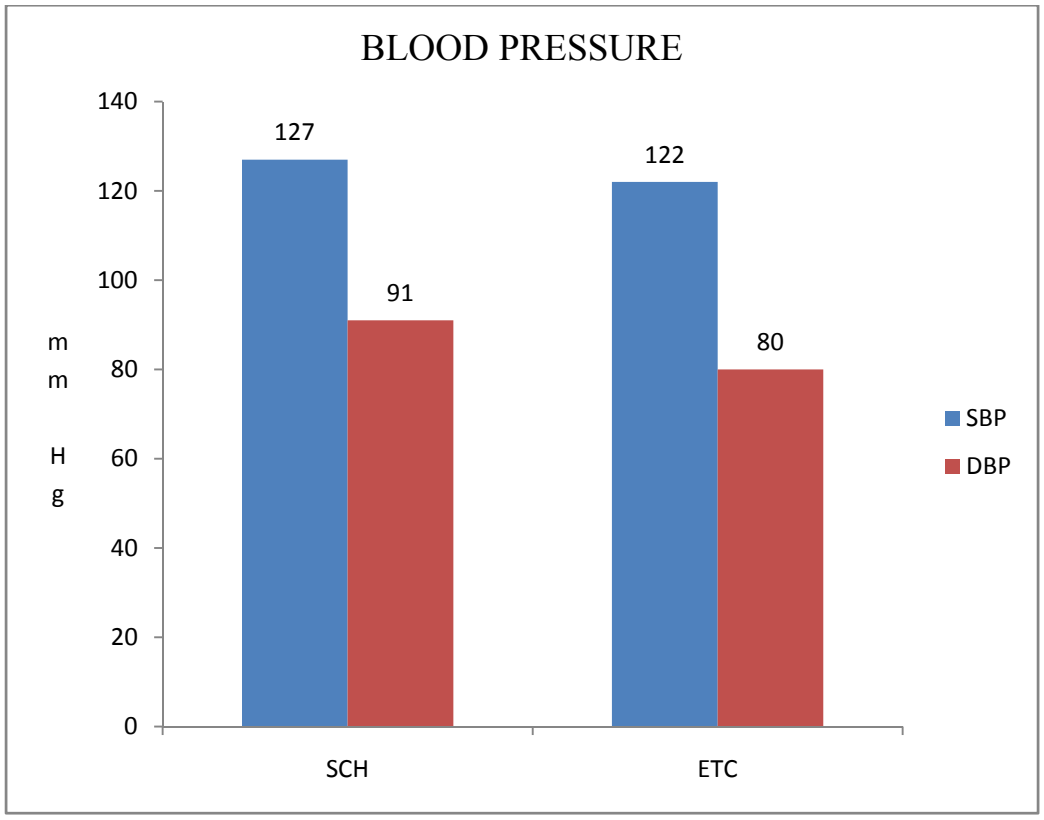


Chart 5 Shows mean Blood pressure in study group. Mean systolic blood pressure and diastolic blood pressure in Subclinical Hypothyroid cases was significantly higher ($p < 0.0002$, $p < 0.0001$ respectively) as compared to controls.

Table – 4: Risk factors for cardiovascular disease in SCH patients

Variable	SCH Patients (%)	Euthyroid controls (%)
FBS (≥ 110 mg/dL)	0	0
TC (> 200 mg/dL)	83.3	30
TG (> 150 mg/dL)	73.3	10
LDL (> 130 mg/dL)	95	33.3
HDL (< 30 mg/dL)	63.3	13.3
SBP (≥ 140 mm Hg)	6.6	0
DBP (≥ 90 mm Hg)	76.6	0

Table 4 shows the percentage of cases with higher blood pressure and lipid profile parameters in the study group. The percentage of subjects having hypertension (>140/90 mm Hg), elevated TC (>200 mg/dL), LDL (130> mg/dL), TG (150> mg/dL), and decreased HDL (<30 mg/dL) was higher in SCH patients than in controls.

Table 5 : Spearmans correlation between TSH and other parameters

VARIABLE	r VALUE
TSH and TC	0.157
TSH and TG	0.485
TSH and HDL	-0.020
TSH and LDL	0.115
TSH and FBS	-0.084
TSH and SBP	0.156
TSH and DBP	-0.168

(r = Spearman correlation coefficient)

Table 5 shows the correlation between TSH and other parameters. A significant positive correlation was observed between TSH and Triglycerides ($r = 0.485, p < 0.0001$). Serum total cholesterol($r = 0.157$), LDL-C($r = 0.115$) and systolic blood pressure($r = 0.156$) showed moderately weak positive correlation with TSH levels. Serum HDL-C($r = -0.020$), FBS ($r = -0.084$), and diastolic blood pressure($r = -0.168$) showed weak negative correlation with TSH.

Chart 6 : Relationship between serum TSH and serum TC levels in the study subjects

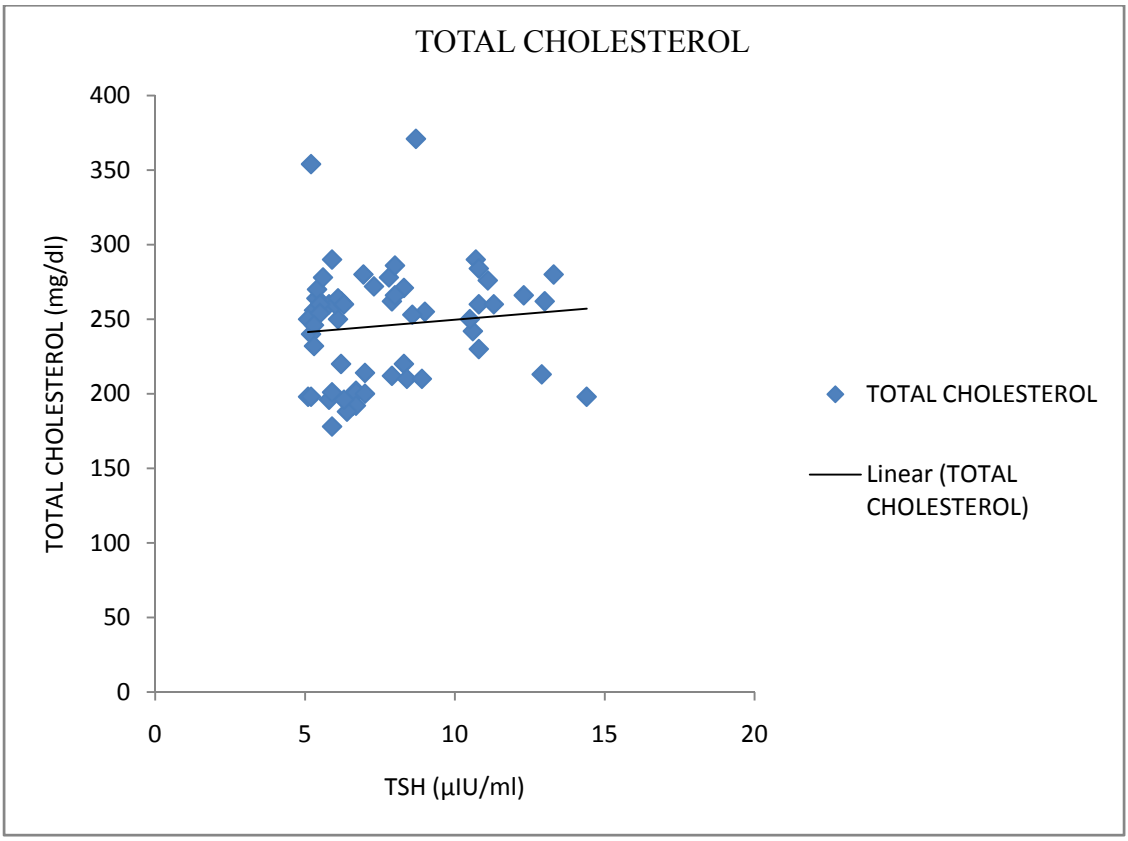


Chart 6 shows that the correlation between TSH and Total Cholesterol is moderately weak positive

CHART 7 : Relationship between serum TSH and serum triglycerides levels in the study subjects.

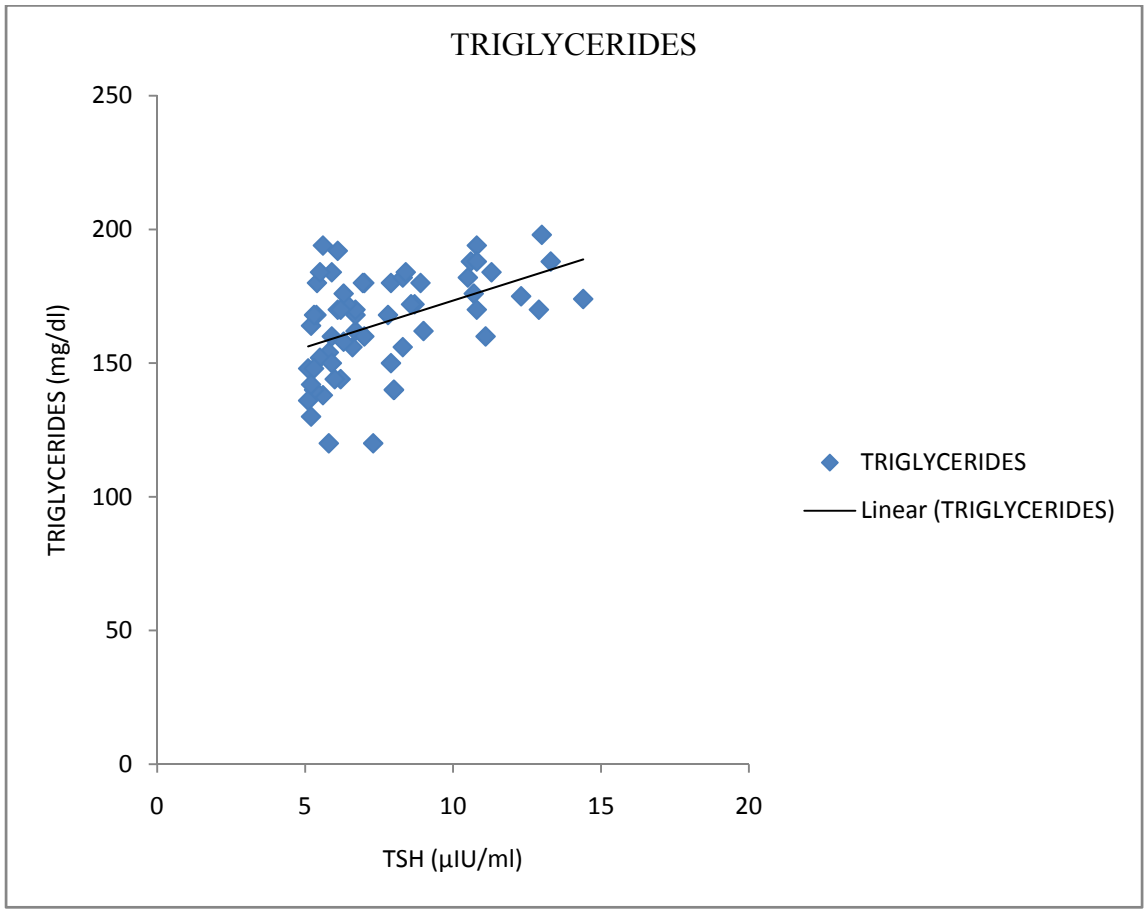


Chart 7 shows the correlation between TSH and Triglycerides is moderately weak positive

CHART 8: Relationship between serum TSH and serum HDL-C levels in the study subjects.

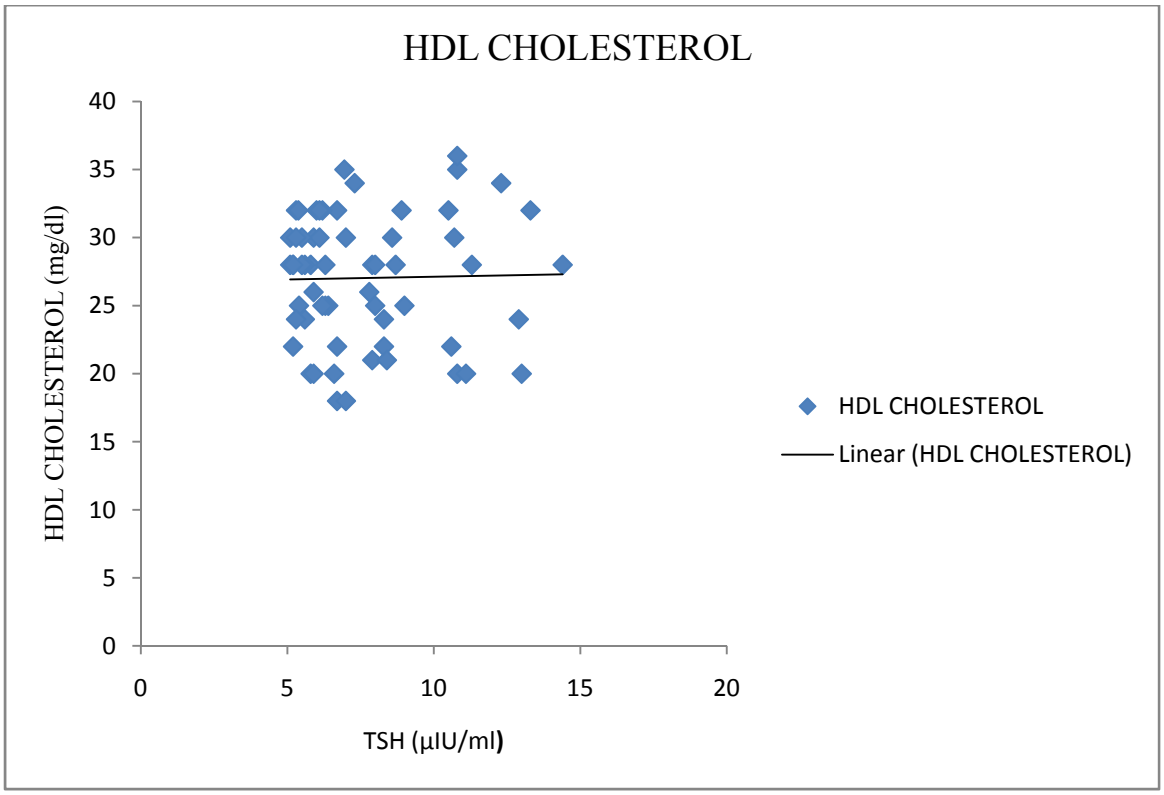


Chart 8 shows the correlation between TSH and HDL Cholesterol is weak negative

Chart 9: Relationship between serum TSH and serum LDL-C levels in the study subjects.

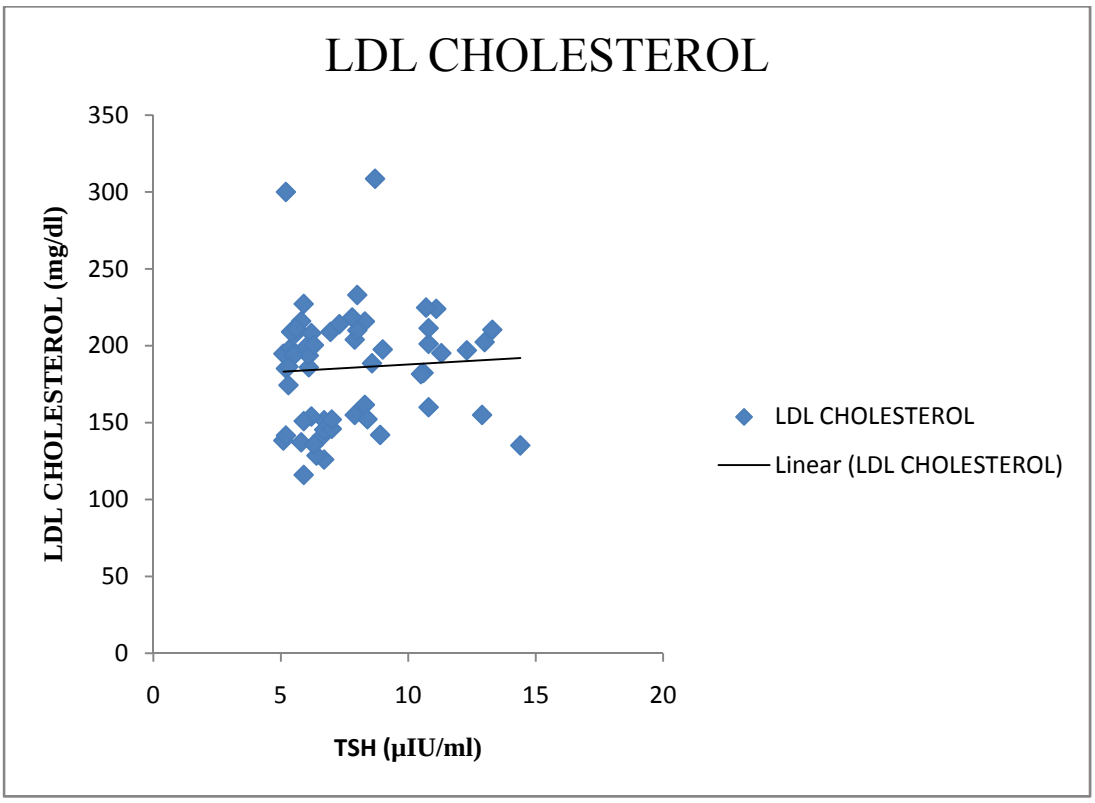


Chart 9 shows the correlation between TSH and LDL Cholesterol is moderately weak positive

Chart 10 : Relationship between serum TSH and fasting blood glucose levels in the study subjects .

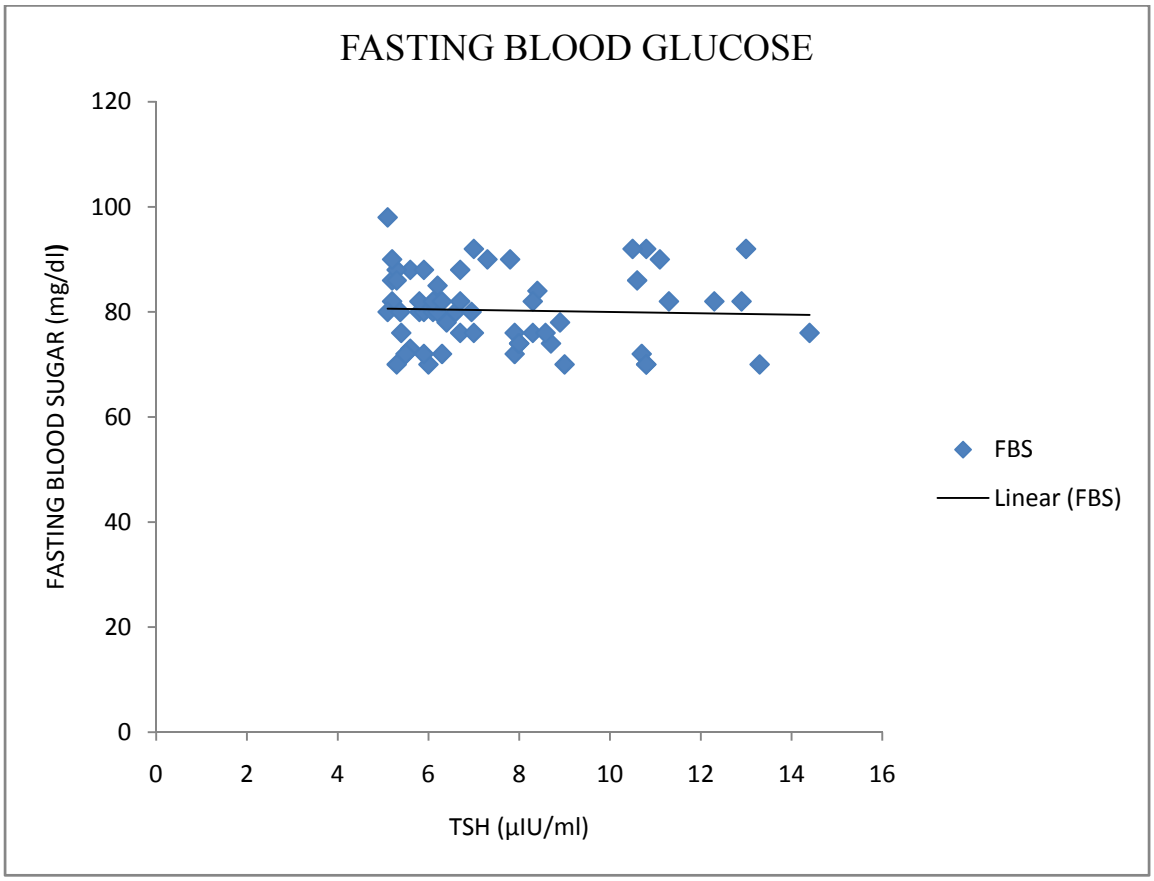


Chart 10 shows the correlation between TSH and Fasting Blood Glucose is moderately weak negative

Chart 11 : **Relationship between serum TSH and systolic blood pressure in the study subjects.**

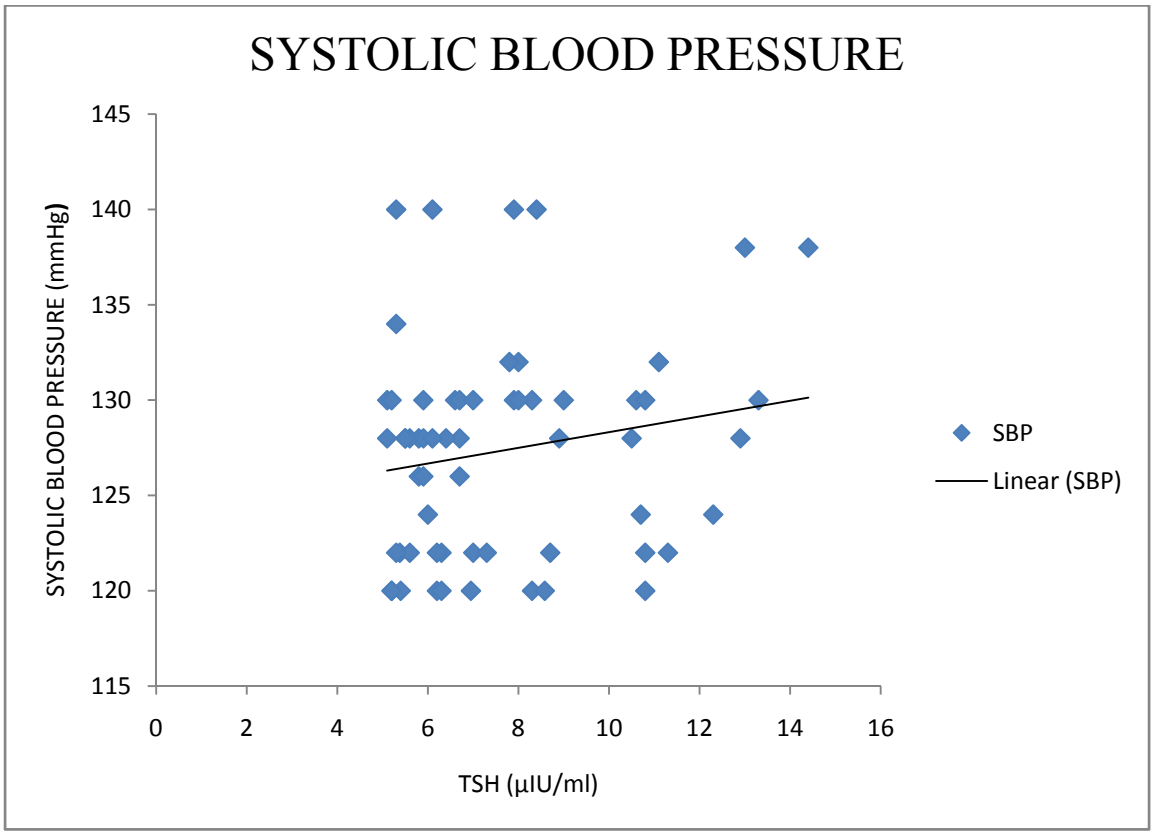


Chart 11 shows the correlation between TSH and Systolic blood pressure is moderately weak positive

Chart 12: Relationship between serum TSH and diastolic blood pressure in the study subjects.

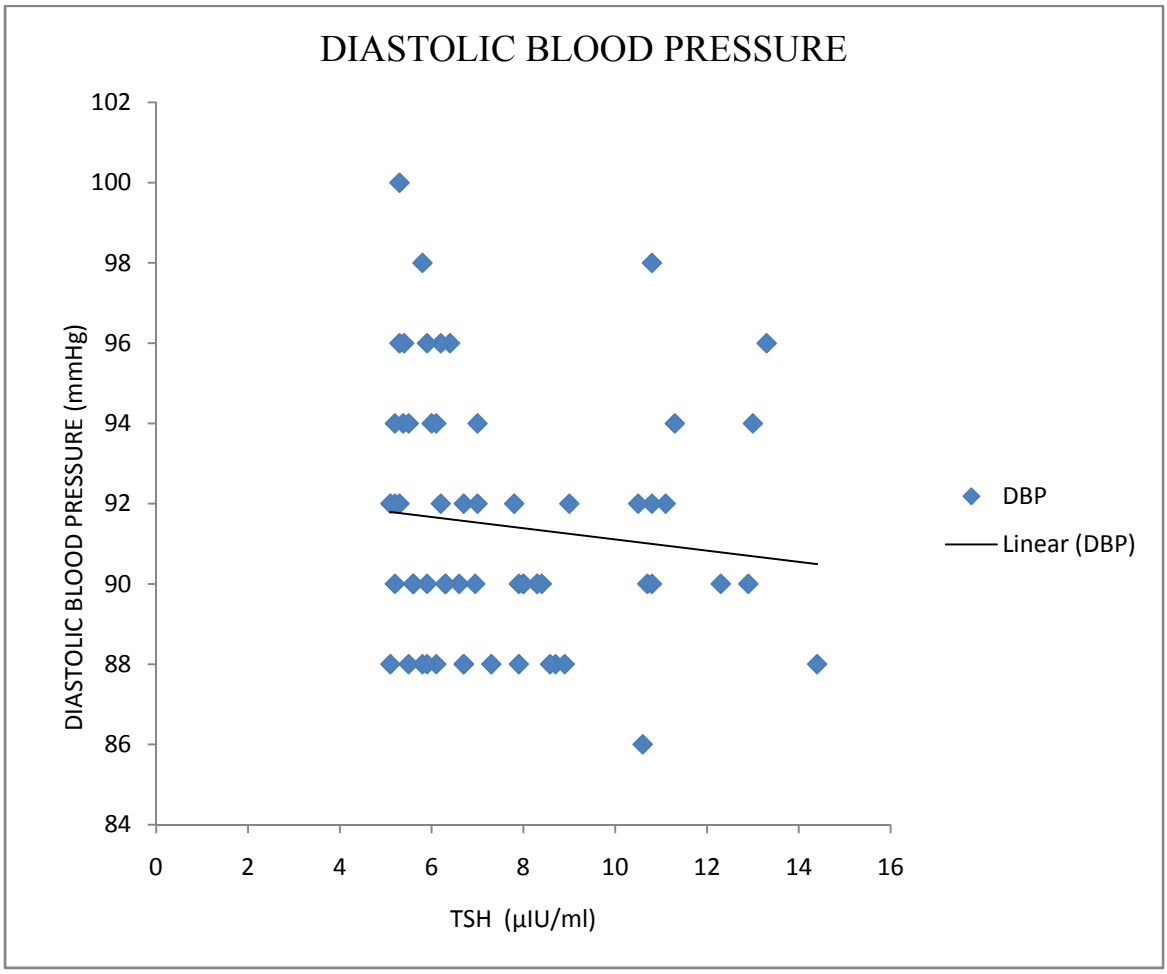


Chart 12 shows the correlation between TSH and Diastolic blood pressure is moderately weak negative

DISCUSSION

Subclinical Hypothyroidism (SCH) is more common than overt hypothyroidism. Although the view that overt hypothyroidism causes secondary hyperlipidemia and promotes atherosclerosis has been generally accepted[48], studies examining the relationships between hyperlipidemia, atherosclerosis, and SCH have yielded less convincing results. In recent times subclinical hypothyroidism is being diagnosed more frequently than overt hypothyroidism[12].Overt hypothyroidism is associated with abnormalities of lipid metabolism, which may predispose to the development of atherosclerotic coronary artery disease (CAD) [14].

Despite that subclinical hypothyroidism being more common, its clinical significance is still debatable. Still there is controversy pertaining to routine screening of SCH so as to prevent it from progressing to overt hypothyroidism[4]. Subclinical hypothyroidism has been associated with increased risk for atherosclerosis. Data on coronary heart disease (CHD) in subjects with subclinical hypothyroidism are conflicting[8]. The possible effects of subtle alterations of thyroid function as in SCH on lipid profile and atherogenesis remain unclear[33]. There is growing evidence, that SCH is an indicator of increased risk for atherosclerosis and myocardial infarction in elderly women[14].

The present study revealed that subclinical hypothyroid cases showed the statistical significance for serum TSH, Total Cholesterol, Triglycerides, HDL-C , LDL-C , Systolic Blood Pressure, Diastolic Blood Pressure where as no significance was found for T4, and fasting blood glucose as compared to the euthyroid controls .

Results of serum lipid concentrations in SCH revealed conflicting data. Present study demonstrated that the mean levels of Total Cholesterol, Triglycerides, LDL-C, were higher where as mean values for HDL-C was lower in subclinical hypothyroid cases as compared to euthyroid controls. Zoe Efstathiadou et al[14] studied lipid profile in subclinical hypothyroidism and concluded that serum total cholesterol and LDL-C were significantly increased in SCH as compared to controls. Our study also showed the similar results. Our study is in accordance with study done by Rafael Luboshitzky et al[18] , who demonstrated that the percentage of subjects with increased total cholesterol ,triglycerides , LDL-C were more in SCH as compared to euthyroid controls .Study done by Nadia Caraccio[15] showed the similar results as our study indicating increased levels of Total cholesterol and LDL-C in subclinical hypothyroidism .Total cholesterol and HDL-C were elevated in several reports, but were not different from those in the controls in most studies[34, 13]. Lower serum HDL-C levels were reported in few studies and were not different from the euthyroid controls in most other studies[34, 35]. In a substantial number of studies, TC and/or LDL-C seem to be elevated in SCH compared with controls. However, there are studies that do not confirm this observation[14]. Since LDL-C is atherogenic while HDL-C is protective, elevated TC/HDL and LDL/HDL ratios have been used as index of increased risk for atherosclerosis. As per our study mean TC/HDL ratio was 9.0 and LDL/HDL ratio was 6.8 which are above the normal range (normal TC/HDL is 5.0 and LDL/HDL ratio is 3.5) indicating increased risk for atherosclerosis . In our study we found that the percentages of patients with atherogenic lipid profiles were higher in subclinical hypothyroid cases than in euthyroid controls.

There is a growing body of evidence indicating that elevated triglyceride levels are an independent risk factor for atherosclerosis. Stephen Rahula et al[20] showed that triglyceride levels were significantly increased in subclinical hypothyroid cases. Our study is in accordance with study done by stephen Rahula indicating that Triglyceride levels are increased in subclinical hypothyroidsm. Hypertriglyceridaemic patients often develop a lipoprotein profile characterized by elevated triglycerides and LDL-C and low HDL-C. It is estimated that the aggregated risk associated with triglycerides greater than 2.28nmol/L and a TC/ HDL-C ratio greater than 5.0 contributes 25% of the cardiovascular events[36, 16].

Several studies, have reported variable and inconsistent increase in total cholesterol, LDL-C, higher and inconsistent changes in serum HDL-C. Two large population based studies were done in this aspect, Whickham survey and NHANES III. SCH was not associated with hyperlipidemia, in Whickham survey[12], where as NHANES III[3] reported , higher levels of mean Total cholesterol in SCH subjects as compared to euthyroid but no difference was reported in LDL-C or HDL-C. Bindels et al.[37] estimated that an increase of 1 mIU/L in serum TSH was associated with a rise in serum cholesterol of 0.09 mmol/L (3.5 mg/dl) in women and 0.16 mmol/L (6.2 mg/dl) in men. Present study showed a weak positive correlation between TSH and Total Cholesterol, Triglycerides and LDL-C and a weak negative correlation between TSH and HDL-C indicating atherogenic lipid profile as a risk factor of cardiovascular diseases.

The association between hypertension and subclinical hypothyroidism is a matter of debate. Present study demonstrated that patients with SCH have significantly higher systolic and diastolic blood pressure than the control group. We found that approximately

6.6 % of SCH patients had elevated systolic blood pressure and 76.6% had elevated diastolic blood pressure compared with 0% in the euthyroid, control group. Our study is similar to the study done by Rafael Luboshitzky[16] who demonstrated that percentage of subjects with elevated systolic and diastolic blood pressure were more in subclinical hypothyroidism as compared to euthyroid controls. Several studies have reported impaired left ventricular diastolic and systolic myocardial functions in subclinical hypothyroidism leading to elevated diastolic and systolic blood pressure[16, 18, 39]. Exposure of aortic endothelial and vascular smooth muscle cells to triiodothyronine (T 3) resulted in cellular relaxation. Two binding sites specific for T 3 were identified. When cells were exposed to T 3, no effect on phosphorylation or nitric oxide production were observed, suggesting that T 3 acted directly on the vascular smooth muscle cells to cause vascular relaxation[16].

So far, the data addressing the relationship between thyroid function, insulin resistance and fasting blood glucose in subclinical hypothyroidism are inconsistent. Present study revealed that the mean levels of fasting blood glucose was not significant in subclinical hypothyroid cases as compared to euthyroid controls. Our study is in accordance with the study done by Stephen Rahula [20] who demonstrated that TSH was not related to fasting blood glucose. Some studies described decreased insulin sensitivity in hypothyroidism[40, 41], while others did not[42, 43], and another study even reported an increased insulin sensitivity[44].

Thus there is increasing evidence that SCH is associated with dyslipidemia and hypertension which can be a potential risk factor for the development of CVD in the near future. The effectiveness of L-T4 replacement therapy in both reducing the levels of

Total Cholesterol, Triglycerides, LDL cholesterol levels and improving the condition of SCH patients is still controversial. Tzotzas et al.[45] reported that none of the commonly measured lipoproteins differed between SCH patients and controls, nor did the lipoprotein profile change significantly in SCH patients upon achieving euthyroidism. In contrast, Caron et al[46] reported lower HDL-C levels in SCH patients than in a control group and demonstrated a significant increase in ApoA and HDL-C levels after L-T4 therapy, with normalization of the TC/HDL-C ratio. Arem and Patsch[47], on the other hand, reported a reduction in LDL-C, ApoB, and the TC/HDL ratio after L-T4 replacement in a group of SCH patients with a mean TSH level of 16.6 mIU/liter.

Thus it is of prime importance to evaluate subclinical hypothyroidism, dyslipidemia and hypertension associated with it that can lead to cardiovascular disease. The high rate of conversion of subclinical hypothyroidism to overt hypothyroidism makes it necessary to treat asymptomatic subclinical hypothyroidism. Starting the therapy may help in different ways as First, progression to overt hypothyroidism, with its attendant morbidity, would be prevented by thyroxine therapy. Second, thyroxine therapy may improve the serum lipid profile and thereby potentially decrease the risk of death from cardiovascular causes. Finally, treatment may reverse the symptoms of mild hypothyroidism, including psychiatric and cognitive abnormalities**.**

The findings of this study must be interpreted within the limitations of the study design. Our assumption that the subclinical hypothyroid group is homogeneous might ignore the possibility that a subgroup of these persons might be at greater risk for hyperlipidemia. For example, patients with TSH values between 10 and 15 mU/L have been found to be at greater risk of advancing to overt hypothyroidism during the next few

years. It is possible that this group or another subgroup (like TSH values between 5 and 10 mU/L) also could be at higher risk for hypercholesterolemia or other squeal related to overt hypothyroidism before the thyroid completely fails. Due to the cross sectional nature of the study it is difficult to interpret whether thyroid test abnormalities preceded elevations in triglyceride levels, it cannot be definitely stated that one leads to the other. Further evaluation of this relationship with longitudinal data and a large population size would be necessary to support the present findings.

SUMMARY AND CONCLUSION

Subclinical hypothyroidism or mild thyroid failure is a common problem. Subclinical hypothyroidism is a laboratory diagnosis with few or no definitive clinical signs or symptoms of thyroid dysfunction. The clinical importance of SCH and therapy for its management remain subjects of debate. Subclinical hypothyroidism has been associated with higher levels of some cardiovascular risk factors. Despite some conflicting results, many studies found that subjects with subclinical hypothyroidism have higher total cholesterol and low density lipoprotein cholesterol levels than euthyroid subjects. Data on coronary heart disease (CHD) in subjects with subclinical hypothyroidism are conflicting. The decision about whether to screen patients for this disorder is clouded by inconsistent evidence of association of dyslipidemia and other risk factors of cardiovascular disease with SCH and also any benefit from early treatment. There is, in fact, doubt as to whether SCH should be treated because the evidence in terms of dyslipidemia, hypertension or glucose intolerance provided by different authors is controversial.

The objective of the present study was to quantitatively detect the levels of known risk factors of cardiovascular disease such as lipid profile, fasting blood glucose and range of blood pressure, in subclinical hypothyroid and euthyroid subjects and to detect the association between subclinical hypothyroidism and risk factors of cardio vascular diseases.

The study revealed significantly increased mean serum levels of TSH, Total Cholesterol, Triglycerides, LDL-C, and systolic blood pressure and diastolic blood

pressure in subclinical hypothyroid cases as compared to euthyroid controls. The percentage of subjects with increased Total Cholesterol, Triglycerides, LDL-C, and systolic blood pressure and diastolic blood pressure in subclinical hypothyroid cases were more compared to euthyroid controls. Weak positive correlation was seen between TSH and Total cholesterol, triglycerides, LDL-C and systolic blood pressure where as weak negative correlation was seen between TSH and HDL-C.

In conclusion hypertension and dyslipidemic state is seen in subjects with subclinical hypothyroidism leading to increased risk for cardiovascular disease and pointing towards the association between subclinical hypothyroidism and risk factors of cardiovascular disease. This indicates the importance of screening the patients for subclinical hypothyroidism and weigh in favor of treating the patients with subclinical hypothyroidism.

CONCLUSION:

Present study reveals that significantly higher levels of serum TSH , Total Cholesterol , Triglycerides , LDL-C , systolic blood pressure , and diastolic blood pressure and lower levels of HDL-C are seen in subclinical hypothyroidism as compared to euthyroid controls indicating hypertensive and dyslipidemic state associated with SCH leading to increased risk for cardiovascular disease. Present study showed weak positive correlation between TSH and Total Cholesterol, Triglyceride, LDL-C, and systolic blood pressure where as weak negative correlation between TSH and HDL-C pointing towards the association between subclinical hypothyroidism and risk factors of cardiovascular disease. This indicates the importance of screening the patients for subclinical

hypothyroidism and weigh in favor of treating the patients with subclinical hypothyroidism and reducing further development of cardiovascular disorders risks in future.

REFERENCES

1. Surks MI, Ortiz E, Daniels GH, Sawin CT, Col NF, Cobin RH, et al. Subclinical Thyroid Disease Scientific Review and Guidelines for Diagnosis and Management. JAMA, January 14, 2004—Vol 291, No. 2, 228-238.
2. Fatourechi V. Subclinical Hypothyroidism: An Update for Primary Care Physicians. Mayo Clin Proc. 2009;84 (1):65-71.
3. Hollowell JG, Staehling NW, Flanders WD, et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab. 2002;87 (2):489-499.
4. Cooper DS. Subclinical hypothyroidism. N Engl J Med. 2001;345(4): 260-265.
5. Surks MI, Hollowell JG. Age-specific distribution of serum thyrotropin and antithyroid antibodies in the US population: implications for the prevalence of subclinical hypothyroidism. J Clin Endocrinol Metab. 2007 Dec;92(12):4575-4582.
6. Gharib H, Tuttle RM, Baskin HJ, Fish LH, Singer PA, McDermott MT. Subclinical thyroid dysfunction: a joint statement on management from the American Association of Clinical Endocrinologists, the American Thyroid Association, and the Endocrine Society. Thyroid. 2005;15(1):24-28.
7. Chu JW, Crapo LM. The treatment of subclinical hypothyroidism is seldom necessary. J Clin Endocrinol Metab. 2001;86(10):4591-4599.

8. Rodondi N, Newman AB, Vittinghoff E, Rekeneire ND, Satterfield S, Harris TB, et al. Subclinical Hypothyroidism and the Risk of Heart Failure, Other Cardiovascular Events, and Death. Arch Intern Med. 2005;165:2460-2466.
9. Hueston WJ and Pearson WS. Subclinical Hypothyroidism and the Risk of Hypercholesterolemia. Ann Fam Med 2004;2:351-355.
10. Cappola AR, Fried L P, Arnold AM, Danese MD, Kuller LH, Burke GL, et al. Thyroid Status, Cardiovascular Risk, and Mortality in Older Adults. JAMA. 2006;295:1033-1041
11. Helfand M, Redfern CC. Clinical guideline, part 2. Screening for thyroid disease: an update. American College of Physicians. Ann Intern Med. 1998;129:144-58.
12. Tunbridge WM, Evered DC, Hall R, Appleton D, Brewis M, Clark F, et al. The spectrum of thyroid disease in a community: the Wickham survey. Clin Endocrinol (Oxf). 1977;7:481-93.
13. Hak A E, Pols HAP, Visser TJ, Drexhage HA, Hofman A, and Witteman JCM. Subclinical Hypothyroidism Is an Independent Risk Factor for Atherosclerosis and Myocardial Infarction in Elderly Women: The Rotterdam Study. Ann Intern Med. 2000;132:270-278.
14. Efstathiadou Z, Bitsis S, Milionis HJ, Kukuvitis A, Bairaktari ET, Elisaf MS and Tsatsoulis A. Lipid profile in subclinical hypothyroidism: is L-thyroxine substitution beneficial? European Journal of Endocrinology 2001;145;705–710.
15. Caraccio N, Ferrannini E and Monzani F. Lipoprotein Profile in Subclinical Hypothyroidism: Response to Levothyroxine Replacement, a Randomized Placebo-Controlled Study. J Clin Endocrinol Metab 2002;87:1533–1538.

16. Luboshitzky R, Aviv A, Herer P, and Lavie L. Risk Factors for Cardiovascular Disease in Women with Subclinical Hypothyroidism. Thyroid. 2002;12:5;421-25.
17. Imaizumi M, Akahoshi M, Ichimaru S, Nakashima E, Hida A, Midori Soda M, et al. Risk for Ischemic Heart Disease and All-Cause Mortality in Subclinical Hypothyroidism. The Journal of Clinical Endocrinology & Metabolism.2004;89(7):3365–70.
18. Luboshitzky R & Herer P. Cardiovascular risk factors in middle-aged women with subclinical hypothyroidism. Neuroendocrinol Lett 2004; 25(4):262–266.
19. Anne R. Cappola, MD, ScM Linda P. Fried, MD, MPH Alice M. Arnold, PhD et al Thyroid Status, Cardiovascular Risk, and Mortality in Older Adults JAMA, March 1, 2006;Vol 295, No. 9: 1033-1041.
20. Ruhla S, Weickert MO, Arafat AM, Osterhoff M, Isken F, Spranger J, et al. A high normal TSH is associated with the metabolic syndrome. Clinical Endocrinology.2010;72:696–701.
21. Sharma R, Sharma TK, Kaushik GG, Sharma S, Vardey SK, Sinha M. Subclinical hypothyroidism and its association with cardiovascular risk factors. Clin Lab 2011;57(9-10):719-24.
22. David Nanchen, Jacobijn Gussekloo, Rudi G. J. Westendorp et al David Nanchen, Jacobijn Gussekloo, Rudi G. J. Westendorp, Journal of Clinical Endocrinol Metabolism, March 2012;97(3):852–861
23. Gencer B, Collet TH, Virgini V, Auer R, Rodondi N. Subclinical thyroid dysfunction and cardiovascular outcomes among prospective cohort studies. Endocr Metab Immune Disord Drug Target. 2013 Jan 15.

24. Soos M, Siddle K. Immunol methods. 1982;51;57-68.

25. Wada HG, Danisch RJ, Baxter SR. Clin Chem. 1982;28:1862-66

26. Walker, W H O, Introduction: an approach to immunoassay. Clin Chem. 1977;23:384.

27. Kerkegaard C, Friis T, Siersback-Nielsen K, Acta Endocrinol. 1974;77;71.

28. Wistom GB. Enzyme immunoassay. Clin Chem.1976; 22:1243.

29. Schuurs AH, Van Weeman BK. Review. Enzyme-immuneassay. Clin. Chem. Acta.1977 81;1.

30. Burtis CA, Ashwood ER. Teitz Textbook of Clinical Chemistry, 2nd ed. W.B.Saunders company; 1994: 961-2.

31. Nader R, Warnick GR. Lipids, lipoproteins, apolipoproteins and other cardiovascular risk factors. In : Burtis CA, Ashwood ER and Bruns DA, eds. Tietz text book of clinical chemistry and molecular diagnostics, 4th edn. New Delhi : Elsevier Co., 2006; 916-52.

32. Rifai N, Iannotti E, DeAngelis K, Law T. Analytical and clinical performance of a homogeneous enzymatic LDL – cholesterol assay compared with the ultracentrifugation-dextran sulfate-Mg^{2+} method. Clinical Chemistry. 1998; 44(6): 1242-50.

33. Kahaly GJ. Cardiovascular and atherogenic aspects of subclinical hypothyroidism. Thyroid 2000 ;10: 665–679.

34. Kung AWC., Pang RWC., Janus ED.: Elevated serum lipoprotein (a) in subclinical hypothyroidism. Clinical Endocrinology;1995; 43: 445–449.

35. Althaus BU., Staub JJ., Ryff-de Leche A., Oberhansli A., Stahelin HB.: LDL/HDL-changes in subclinical hypothyroidism: Possible risk factors for coronary artery disease. Clin Endocrinol;1988; 28: 157–163.
36. Velkoska Nakova V. Krstevska B, Bosevski M, Dimitrovski Ch.,1 Serafimoski V Dyslipidaemia and hypertension in patients with subclinical hypothyroidism Sec. Biol. Med. Sci., MASA;2009:93–102.
37. Bindels AJ, Westendorp RG, Frolich M, Seidell JC, Blokstra A, Smelt AH. The prevalence of subclinical hypothyroidism at different total plasma cholesterol levels in middle aged men and women:a need for case finding? ClinEndocrinol.1999;50:217–20.
38. Georgia Michalopoulou, Maria Alevizaki, Gregory Piperingos, Demetrios Mitsibounas, Emily Mantzos, Panayotis Adamopoulos and Demetrios A Koutras, High serum cholesterol levels in persons with 'high-normal' TSH levels: should one extend the definition of subclinical hypothyroidism? European Journal of Endocrinology :1998:138 141–145
39. Forfar JC, Wathen CG, Todd WT, Bell GM, Hannan WJ, Muir AL, Toft AD Left ventricular performance in subclinical hypothyroidism.Q J Med 1985;224:857–865
40. Rochon, C., Tauveron, I., Dejax, C. et al Response of glucose disposal to hyperinsulinaemia in human hypothyroidism and hyperthyroidism. Clinical Science, 2003;104:7–15.
41. Stanicka, S., Vondra, K., Pelikanova, T. et al. Insulin sensitivity and counter-regulatory hormones in hypothyroidism and during thyroid hormone replacement therapy. Clinical Chemistry and Laboratory Medicine, 2005;43:715–720.

42. Harris, P.E., Walker, M., Clark, F. et al. Forearm muscle metabolism in primary hypothyroidism. European Journal of Clinical Investigation,1993;23:585–588.
43. Owecki, M., Nikisch, E. & Sowinski, J. Hypothyroidism has no impact on insulin sensitivity assessed with HOMA-IR in totally thyroidectomized patients. Acta clinica Belgica,2006; 61: 69–73.
44. Jackson, I.M., Prentice, C.R. & McKiddie, M.T. The effect of hypothyroidism on glucose tolerance and insulin metabolism. Journal of Endocrinology,1970;47:257–258
45. Tzotzas T, Krassas GE, Konstantinidis T, Bougoulia M Changes in lipoprotein(a) levels in overt and subclinical hypothyroidism before and during treatment. Thyroid 2000;10:803–808
46. Caron P, Calazel C, Parra HJ, Hoff M, Louvet JP Decreased HDL cholesterol in subclinical hypothyroidism: the effect of levothyroxine therapy.Clin Endocrinol (Oxf) 1990 ;33:519–523
47. Arem R, Patsch W Lipoprotein and apolipoprotein levels in subclinical hypothyroidism. Effect of levothyroxine therapy. Arch Intern Med 1990 ;150:2097– 2100
48. Becker C Hypothyroidism and atherosclerotic heart disease: pathogenesis, medical management, and the role of coronary artery bypass surgery. Endocr Rev,1985; 6:432–440
49. Laurence M. Demers. Thyroid Disorders. In:Carl A Burtis, Edward R. Ashwood, David E. Bruns editors.Tietz Fundamentals of Clinical Chemistry.6th edition,Saunders.2012:766-779

ANNEXURE 1

Ethical Committee
BIJAPUR-586103
OUT WARD
No 27/2011
Date 21-10-11
BLDEA's Shri B. M. PATIL Medical College

B.L.D.E.UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 20-10-2011 *at* 10-30 am *to scrutinize the Synopsis/Research projects of postgraduate/undergraduate student/Faculty members of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis/Research project has been accorded Ethical Clearance.*

Title "Study of association between Subclinical hypothyroidism and risk factors of cardiovascular diseases"

Name of P.G./U.G. student/Faculty member Dr. Smita. Kottagi
Dept of Biochemistry

Name of Guide/Co-investigator Dr. Dileep Rathi, Prof & HOD.
Biochemistry

DR.M.S.BIRADAR,
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Chairman
Ethical Committee
BLDEA'S Shri. B.M. Patil
Madical College
Bijapur-586103

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

ANNEXURE 2

PROFORMA

Name: **I.P. / O.P. No.:**

Age: **Date of admission:**

Sex: **Date of examination:**

Occupation: **Case / Control no.:**

Address:

Chief complaints and history of presenting illness:

Past history:

H/O similar complaints in the past – Yes / No

H/O drug intake / diabetes mellitus / coronary artery disease / renal disease / neoplasia, in the past.

Family history:

H/O similar complaints in the family members – Yes / No

Personal history:

1. Diet –Veg / Mixed
2. Appetite – Normal / Decreased
3. Weight – Normal / Decreased

4. Sleep – Normal / Decreased
5. Bowel and bladder habits – Regular / Disturbed
6. Alcohol consumption – Yes / No; if yes, then

   Duration - Amount - Frequency -

7. Smoking – Yes / No; if yes, then

   Duration - Frequency –

General physical examination:

- Nourishment – Well / Moderate / Poor
- Built – Well / Moderate / Poor
- Consciousness – Conscious / Drowsy / Comatose
- Pallor / Icterus / Clubbing / Lymphadenopathy / Oedema / Parotid enlargement / Gynaecomastia / Palmar erythema.
- Pulse rate –
- Blood pressure –
- Respiratory rate –

Systemic examination:

1. Per abdomen:
2. Respiratory system:
3. Cardiovascular system:
4. Central nervous system:

Clinical diagnosis:

Study profile:

1. Serum TSH, T3, T4
2. Serum fasting glucose
3. Serum total cholesterol
4. Serum triglycerides
5. Serum HDL-C
6. Serum LDL-C

ANNEXURE 3

SAMPLE INFORMED CONSENT FROM BLDEU'S SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTER, BIJAPUR-586103

TITLE OF THE PROJECT	-	TO STUDY THE ASSOCIATION BETWEEN SUBCLINICAL HYPOTHYROIDISM AND RISK FACTORS OF CARDIOVASCULAR DISEASES
PRINCIPAL INVESTIGATOR	-	DR. SMITA. KOTTAGI.
GUIDE	-	DR. DILEEP. RATHI. MD PROFESSOR & HOD OF BIOCHEMISTRY
PURPOSE OF RESEARCH:	-	TO OBTAIN BETTER KNOWLEDGE ABOUT THE ASSOCIATION BETWEEN SUBCLINICAL HYPOTHYROIDISM AND RISK FACTORS OF CARDIOVASCULAR DISEASES
PROCEDURE:	-	ANALYSIS OF THE LAB REPORTS OF PATIENTS WITH SUBCLINICAL HYPOTHYROIDISM
RISK AND DISCOMFORTS:	-	NIL
BENEFITS:	-	BETTER UNDERSTANDING AND KNOWLEDGE ABOUT THE ASSOCIATION BETWEEN SUBCLINICAL HYPOTHYROIDISM AND RISK FACTORS OF CARDIOVASCULAR DISEASES
ALTERNATIVES:	-	NIL

I understand that my participation is voluntary and I may refuse to participate or withdraw from the study at any time.

Signature of the patient

ANNEXURE 4

MASTER CHART - CASE

Sl.No	Name of Patient	Age / Sex	IP No/OP NO	TSH	T3	T4	TOTAL CHOLESTEROL	TRIGLYCERIDES	HDL CHOLESTEROL	LDL CHOLESTEROL	FBS	SBP	DBP
1	KUSUMABAI	35/F	19866	5.8	1.4	95	196	154	28	137.2	80	128	88
2	BHARATI	40/F	226712	7.9	0.6	75	212	180	21	155	72	130	90
3	VANDANA	36/F	250558	5.1	1.6	80	198	148	30	138.4	98	128	92
4	NINGORAJ	35/M	256060	6.6	0.9	97	192	156	20	140.8	80	130	90
5	KAJABI	35/F	22076	8.3	1.1	104	220	182	22	161.6	76	120	90
6	SHARADA	38/F	272048	6.7	0.9	70	202	162	18	151.6	88	126	88
7	ROSALIN	47/F	262664	13	1	98	262	198	20	202.4	92	138	94
8	SHOBA	53/F	281882	8.4	0.8	96	210	184	21	152.2	84	140	90
9	AKKAMAHADEVI	53/F	282301	6.7	0.9	99	201	168	22	145.4	82	130	92
10	SARIKA	54/F	22219	6.7	1.1	72	192	170	32	126	76	128	88
11	JAYASHREE	35/F	278195	7	1.2	99	200	180	18	146	92	130	94
12	BORAMMA	48/F	23594	6.4	0.8	75	188	172	25	128.6	78	128	96
13	DEVAKAMMA	40/F	295951	10.6	1	106	242	188	22	182.4	86	130	86
14	SHEELA	41/F	298951	10.8	1.1	109	260	194	20	201.2	92	120	98
15	MANAKABAI	50/F	28471	5.9	2.2	119	178	160	30	116	80	128	90
16	KASTURI	40/F	27488	6.3	0.4	110	196	176	25	135.8	72	122	90
17	ANITA	38/F	362600	7.9	1.6	71	262	150	28	204	76	140	88
18	SHARADA	48/F	361895	8.3	1.6	75.6	271	156	24	215.8	82	130	90
19	TANGEMMA	56/F	29519	11.1	1.6	89.1	276	160	20	224	90	132	92

20	KASTURI	52/F	29829	5.6	2	134	260	138	24	208.4	73	128	90
21	GOURAKKA	58/F	19427	6.2	1.8	69.2	262	144	25	208.2	85	122	96
22	ROOPA K	36/F	352436	5.2	1.2	72	240	164	22	185.2	82	120	90
23	KASHIBAI	55/F	363156	5.3	1.3	63.7	256	140	32	196	70	134	92
24	MOHAN	35/M	2374	8	1.5	105	266	140	28	210	74	132	90
25	SHAR	39/F	1064	5.1	1.7	92	250	136	28	194.8	80	130	88
26	BASAVRAJ	48/M	17359	5.8	1.6	86.8	260	120	20	216	82	126	98
27	SHAYAWWA	60/F	2193	9	0.76	82.3	255	162	25	197.6	70	130	92
28	GIRIJAKKA	50/F	194217	6.1	1.8	69.2	250	170	30	186	80	128	88
29	SHILPA	53/F	42926	12.3	1.6	50.6	266	175	34	197	82	124	90
30	LALITA	46/F	47171	5.2	1	88.5	354	130	28	300	86	130	92
31	ARCHANA	42/F	3456	6	1.2	90	260	144	32	199.2	70	124	94
32	NEELAMMA	55/F	2736	7	1.6	110	214	160	30	152	76	122	92
33	RAVI BIDRI	38/M	57600	8.7	1.5	78	371	172	28	308.6	74	122	88
34	NEELU	35/F		6.95	1.6	118	280	180	35	209	80	120	90
35	JAGADEVI	49/F	4888	11.3	1.5	68.2	260	184	28	195.2	82	122	94
36	RENUKA	42/F	23500	8.58	2	73.6	253	172	30	188.6	76	120	88
37	RENUKA	51/F	64308	5.38	1.8	88.6	264	168	32	198.4	80	122	94
38	SUNIL	45/M	72228	5.9	1.6	73	290	184	26	227.2	72	126	88
39	YASMEEN	43/F	6315	5.4	1.9	82.3	270	180	25	209	76	120	96
40	SUREKHA	46/F	75418	5.6	1.9	109	278	194	28	211.2	88	122	90
41	RUKMINI	54/F	6987	10.7	0.4	60	290	176	30	224.8	72	124	90
42	SAMEENA	50	85885	6.2	1.7	76.4	220	170	32	154	80	120	92
43	SUMANGALA	52/F	88446	5.5	1.5	94.1	260	184	28	195.2	72	128	94
44	CHANDRAMMA	58/F	7776	7.3	1.8	84.7	272	120	34	214	90	122	88
45	SUNITA	48/F	89568	10.8	0.7	74.2	284	188	35	211.4	70	130	92
46	RAMABAI	54/F	91486	6.1	1.2	68.9	264	192	32	193.6	82	140	94

47	RAJESHWARI	46/F	98043	14.4	1.7	88.4	198	174	28	135.2	76	138	88
48	JYOTI	41/F	84514	5.3	1.9	91.1	232	168	24	174.4	88	122	96
49	VIJAYALAXMI	45/F	101853	10.5	1.4	66.5	250	182	32	181.6	92	128	92
50	WALI	48/F	108692	5.5	1.6	79.7	254	152	30	193.6	72	128	88
51	SHIVANAGANGA	55/F	109127	6.3	1	79.7	260	158	28	200.4	82	120	90
52	SWATI	38/F	111893	13.3	1.6	60.8	280	188	32	210.4	70	130	96
53	REKHA	49/F	117069	7.8	1.6	89.4	278	168	26	218.4	90	132	92
54	BIJANABAI	46/F	10508	5.3	1.7	82.9	246	148	30	186.4	86	140	100
55	ROSHINI	48/F	124269	8	1.2	92.1	286	140	25	233	74	130	90
56	NEELA	40/F	12351	12.9	1	72.7	213	170	24	155	82	128	90
57	NEELAMMA P	38/F	12633	5.2	1.3	94	198	142	28	141.6	90	120	94
58	KAMALA	44/F	146243	8.9	1.3	62	210	180	32	142	78	128	88
59	SHANKARAMMA	58/F	127534	10.8	1.2	68	230	170	36	160	70	122	90
60	SUJATA	56/F	13475	5.9	1.7	77.9	201	150	20	151	88	130	96

MASTER CHART – CONTROL

Sl. No.	Name of Patient	Age / Sex	IP No/OP NO	TSH	T3	T4	TOTAL CHOLESTEROL	TRIGLYCERIDES	HDL CHOLESTEROL	LDL CHOLESTEROL	FBS	SBP	DBP
1	GURUBASAPPA	46/M	338334	3.5	1.1	80.5	160	100	30	110	73	122	86
2	JAYASHREE	48/F	1938	2.5	1.7	104.6	201	122	42	134.6	80	126	82
3	RAMAWWA	45/F	367	3.5	0.83	100.9	155	107	38	95.6	75	124	80
4	SUMANGALA	48/F	4125	2.3	2.1	100.2	159	128	25	108.4	86	120	70
5	SHOBANA	36/F	5120	3	2.1	96	215	133	40	148.4	90	128	80
6	VANISHREE	42/F	8117	1.4	1.3	79.7	152.6	74	36	101.8	80	126	84
7	NALLINI	50/F	11645	4	1.3	111	158.3	110	30	106.3	70	120	82
8	JYOGYAL	58/F	11901	0.9	1.4	103	191	104	34	136.2	88	120	86
9	RENUKA	56/F	5982	1.7	1.5	83.2	182	80	45	121	75	128	80
10	PRABHAVATI	40/F	16279	2.8	1.8	68.3	155	99	36	99.2	73	118	82
11	SANJAY	42/M	16334	2.6	1.7	75.8	220	165	30	157	80	120	78
12	SHILPA	52/F	21223	2.2	1.4	58.2	200	109	28	150.2	82	128	84
13	ASHWINI	48/F	20919	1	2.1	86.6	162	145	35	98	84	130	80
14	CHANNAMMA	38/F	21264	1.1	1.5	76.5	210	161	32	145.8	70	126	82
15	AKKUBAI	57/F	17561	3.8	1.2	65.6	182	75	24	121.6	70	130	84
16	SUMITRA	46/F	1329	1.8	1.3	93.4	185	150	33	122	73	114	82
17	GULASUBAI	40/F	1342	4.1	1.8	99.4	254	117	28	202.6	82	122	78
18	AMBIKA	55/F	26807	2.9	1.9	82.4	154	60	40	102	73	118	82
19	LALITA	54/F	30423	0.9	6.4	101.6	168	64	38	117.2	76	120	78
20	NIKHA	48/F	28306	2	2.8	68.3	183	96	36	127.8	82	114	80
21	JYOTI	58/F	27791	3.5	2.3	77.3	150	58	31	107.4	78	120	86

22	RAVIKUMAR	46/M	29200	3.7	1.6	75.2	285	168	36	215.4	75	126	80
23	SAVITRIBAI	48/F	1217	3.8	1.1	184.2	90	102	42	39.6	85	124	88
24	RENUKA	49/F	30394	1	2.7	106	241	62.3	52	176.54	72	122	82
25	BIDRI	47/M	57617	4.1	1.7	93	253	84	41	195.2	72	118	72
26	GOURABAI	52/F	4445	4.3	1	62	180	90	35	127	76	120	78
27	BANGARAWWA	60/F	51660	3.9	1.8	80	164	80	42	106	84	122	84
28	SAVITRI	53/F	56099	2.7	2	89	158	88	44	96.4	80	126	80
29	SARASWATI	53/F	31061	2.5	2	95	162	80	48	98	90	122	72
30	SHASHIKALA	52/F	61136	1.89	1.7	81.2	180	120	36	120	88	128	84