"PSEUDOCHOLINESTERASE AS A DIAGNOSTIC AND PROGNOSTIC INDICATOR IN HYPOTHYROIDISM"

By

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Dissertation Submitted to the

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH, Karnataka

In partial fulfillment of the requirements for the degree of

DOCTOR OF MEDICINE

IN

GENERAL MEDICINE

Under the Guidance of

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KOLAR

2012

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ACKNOWLEDGEMENT

I take this opportunity to extend my sincere thanks and indebtedness to all those persons and dignitaries who helped me to complete this work.

It gives pleasure to express my sense of gratitude to my professor and head of department of medicine, Dr. B.N. Raghavendra Prasad, M.D., for his guidance, encouragement and constant source of inspiration during my postgraduate course. I am grateful to inspiration and constructive comments during the course of my study and preparation of this dissertation.

I am grateful to Dr. V. Lakshmaiah, M.D., Professor of medicine, who has been a good teacher and a constant source of inspiration all the time during my postgraduate course. My sincere thanks and gratitude to Professors Dr. K. Prabhakar, M.D., Dr.Venkatarathnamma P.N, M.D., Dr. Srinivas Rao, M.D. and Dr Raveesha. A, M.D., who have been constant source of inspiration all the time during my postgraduate course. I express my sincere thanks to Dr. Mukesh, M.D., Dr. Srinivas, M.D., Dr.Jayarama, M.D., Dr.Vidyasagar, M.D. and Dr. S.Kumar, M.D., for all the help they have done.

I thank Mr. K.P Suresh, Bio-statistician, for his valuable guidance. I must also give my sincere thanks to my parents for their moral support, constant encouragement and sincere advices throughout my career.

I am grateful to all my patients who were a part of this study. Above all I thank the almighty for the successful completion of this work.

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ABSTRACT

Background: Among adult people in India, the prevalence of hypothyroidism has been recently studied. In this population-based study done in Cochin on 971 adult subjects, the prevalence of hypothyroidism was 3.9%.

The prevalence of subclinical hypothyroidism was also high in this study, the value being 9.4%. In women, the prevalence was higher, at 11.4%, when compared with men, in whom the prevalence was 6.2%. The prevalence of subclinical hypothyroidism increased with age.

Studies from Mumbai have suggested that hypothyroidism can also occur in childhood. In a clinic-based study from Mumbai, out of 800 children with thyroid disease, 79% had hypothyroidism.

There is often a delay in the diagnosis of hypothyroidism in the country. This delay is attributable to the lack of awareness about the illness, as well as the lack of facilities available or screening program in place for comprehensive screening of this illness.

There are no well-coordinated studies on pseudocholinesterase activity in hypothyroidism available from the Indian subcontinent. Hence this study is undertaken to compare the diagnostic and prognostic efficiency of these methods in diagnosing hypothyroidism and to show that hypothyroidism is an important cause for decrease in pseudocholinesterase. So Pseudocholinesterase levels should be tested before a patient with hypothyroidism is subjected for anesthesia especially succinylcholine.

Objectives:

1. To assess the serum Pseudocholinesterase activity in hypothyroidism

2. To assess the correlation between Serum Pseudocholinesterase and Thyroid function test.

3. To assess Pseudocholinesterase as a diagnostic and prognostic indicator in hypothyroidism.

Methodology: This study was conducted in the R.L. Jalappa Hospital and research centre during the years 2011-2012. The study was a comparative case-control study done on 70 hypothyroid patients attending outpatient clinic and those admitted in the medical wards. The study group consisted of patients above the age of 18 years.

All freshly detected and old cases of hypothyroidism, irrespective of duration of hypothyroidism and type of treatment receiving were taken for the study. They were made into two groups. A separate control group of 54 euthyroid patients without any previous thyroid diseases were taken with age and sex matched. The exclusion criteria were all cases of organophosphorus poisoning, severe anemia, cardiac failure, uraemia, cirrhosis of liver, vysya community, third trimester of pregnancy, previously diagnosed cases of malignancies including head, neck, lung, cervix and colon.

Results: Among the 70 patients included in the study, 37 newly detected hypothyroid patients had a 50.48% decrease in mean activity for pseudocholinesterase level than that found in random normal group. When all hypothyroid patients (both newly detected and on treatment groups) were taken into consideration, a 31.21% decrease in mean activity for pseudocholinesterase level was found as compared with random normal group. The Mean Pseudocholinesterase (U/L) is significantly less in newly detected hypothyroidism cases with a mean activity of 3344.18±1304.56 U/L ($P < 0.001^{**}$). Pseudocholinesterase is found to be a diagnostic marker with an area under ROC curve of 0.837, indicating that it is a good marker with moderate sensitivity (71.43%) and high specificity (94.44%). Pseudocholinesterase is significantly less in cases not on treatment with $F=78.264$, $P<0.001^{**}$, however the cases on treatment are more close to controls with $P=0.100$ indicating this as a good prognostic factor. In addition there is convincing evidence from our study showing that there is a significant negative correlation (r value -0.436; p value 0.011^{*}) between pseudocholinesterase and TSH in hypothyroid patients on treatment.

Conclusion: In this comparative case control study, the correlation between pseudocholinesterase and TSH activity in hypothyroidism was examined. There is convincing evidence from the present study showing that there is a negative correlation between pseudocholinesterase and TSH in hypothyroid patients who are on treatment. There is a mean decrease in pseudocholinesterase level among hypothyroid patients with a cut off level of less than 5290 U/L. The results from this study suggest that pseudocholinesterase can be used both as a diagnostic and prognostic indicator in hypothyroidism.

LIST OF ABBREVIATIONS USED

TSH	Thyroid stimulating Hormone
T4	Thyroxine
fT4	free Thyroxine
T3	Triiodothyronine
EC	Enzyme Commission
LDL	Low density lipoprotein
HDL	High density lipoprotein
SHBG	Sex hormone binding globulin
ADIOL	Androstenediol
ADIOLS	Androstenediol 3-sulfate
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone sulfate
E_2	Estradiol
PREGS	Pregnenolone-sulfate
Gn	Gonadotropin
GnRH	Gonadotropin Releasing hormone
PRL	Prolactin
TRH	Thyroid releasing hormone
TPO	Thyroid peroxidase
PET	Positron emission Tomography
T	Testosterone
S.D	Standard Deviation
D3	Type 3 iodothyronine deiodinase

RTH	Resistance to thyroid hormone
PAS	Periodic acid Schiff
AUC	Area under Curve
mRNA	Messenger Ribonucleic acid
GLUT	Glucose Transporter
ECG	Electrocardiogram
AST	Aspartate Aminotransaminase
ALT	Alanine Transaminase
ALP	Alkaline Phosphatase
N	Population size
M	Margin of error, measure of precision.
CI	Confidence Interval
N	Sample size
σ	Standard deviation
z	Critical value based on Normal distribution at 95% Confidence Interval
+	Suggestive significance (P value: $0.05<P<0.10$)
*	Moderately significant (Pvalue:$0.01<P\leq0.05$)
**	Strongly significant (P value : $P\leq0.01$)
SAS 9.2	Statistical Analysis System
SPSS 15.0	Statistical Package for the Social Sciences
STATA 10	STATA Data Analysis and Statistical Software
MedCalc 9.0.1	Medical calculator statistical software for biomedical research
Systat 12.0	System statistics

R environment ver.2.11.1	R is a language and environment for statistical computing and graphics
ANOVA	Analysis of variance
HRP	Horseradish peroxidase

TABLE OF CONTENTS

PAGE NO.

List of Tables

List of Figures

INTRODUCTION

Hypothyroidism

Hypothyroidism results from reduced effects of thyroid hormone on tissues. Hypothyroidism is more common in women, has total prevalence of 1% to 2%,[1] and increases with age (~10% adults >65 years). In the U.S. population, prevalence of biochemical hypothyroidism is 4.6%, but clinically evident hypothyroidism is present in 0.3%.[2]

As thyroid hormones are universal determinants of organ function, there may be a multiplicity of symptoms. Hypothyroidism emerges insidiously and is nonspecific. They include a general slowing down, mental depression, modest weight gain, cold intolerance, constipation, vague aches and pains, dryness of the skin, and brittleness of the scalp hair.[1] The clinical features of hypothyroidism are dependent on patient's age , presence of other disease, rate at which hypothyroidism develops . The clinical features may be atypical, and the diagnosis may be missed easily particularly in the elderly.[2] Once the disorder is fully established, the classic features of myxedema of the skin, periorbital edema, hoarseness, sinus bradycardia, decrease in body temperature, and delayed relaxation of the deep tendon reflex appear.

Laboratory investigation may reveal a mild anemia, increased creatine phosphokinase concentrations, and an abnormal lipid profile with increased total and low-density lipoprotein cholesterol and decreased high-density lipoprotein cholesterol concentrations.[1] First line tests for hypothyroidism are analyses of the concentrations of free thyroxine (T4) and TSH in serum.[2]

Hypothyroidism in Indian Prospective:

Among adult people in India, the prevalence of hypothyroidism has been recently studied. In this population-based study done in Cochin on 971 adult subjects, the prevalence of hypothyroidism was 3.9%.[3] Prevalence of subclinical hypothyroidism was also high in this study, the value being 9.4%. In women, the prevalence was higher, at 11.4%, when compared with men, in whom the prevalence was 6.2%. The prevalence of subclinical hypothyroidism increased with age. [3]

Studies from Mumbai have suggested that congenital hypothyroidism is also common in India. The disease occurring in 1 out of 2640 neonates, when compared with the worldwide average value of 1 in 3800 subjects.[4] Hypothyroidism can also occur in childhood. In a clinic-based study from Mumbai, out of 800 children with thyroid disease, 79% had hypothyroidism. Common causes of hypothyroidism in these children were thyroid dysgenesis, dyshormonogenesis and thyroiditis.[4]

There are no studies relating to pseudocholinesterase activity in hypothyroidism in the Indian subcontinent. There is a delay in the diagnosis of hypothyroidism in the country. This delay is attributable to the lack of awareness about the illness, as well as lack of facilities available or screening program for comprehensive screening and testing of this illness.

OBJECTIVES

1. To assess the serum Pseudocholinesterase activity in hypothyroidism.

2. To assess the correlation between Serum Pseudocholinesterase and Thyroid function test.
3. To assess Pseudocholinesterase as a diagnostic and prognostic indicator in hypothyroidism.

REVIEW OF LITERATURE

HISTORICAL REVIEW

Historical Background And Terminology

In 1914, Dale discovered that the action of acetylcholine on the heart of the frog was short-lived, and he suggested that an enzyme was present in the blood which catalyzed the hydrolysis of choline esters. [5]

In 1932, Stedman et al. showed that liver esterases from the pig or cat were unable to catalyze the hydrolysis of acetylcholine, and a search was made for the enzyme responsible for the hydrolysis of this choline ester. They went on to describe and purify, for the first time, an enzyme present in horse serum which did catalyze the hydrolysis of acetylcholine. They called this enzyme "choline-esterase" and this name with or without the hyphen has persisted.[6]

During the following years, the picture became confused since acetylcholine-hydrolyzing enzymes obtained from various organs were found to have optimal activity at different concentrations of substrate. An explanation for this was put forward by Alles and Hawes. They demonstrated the existence of two choline-esterases: The first, whose activity was greatest at low concentrations and was inhibited by excess substrate, was present in human erythrocytes; and the second, whose activity increased throughout the range of substrate concentrations studied, was present in human serum. [7]

In later reports of work using purified enzymes, it was suggested that the erythrocyte enzyme was specific for choline esters and should be called "true cholinesterase", while the serum enzyme, which could also hydrolyze noncholine esters, should be called "Pseudocholinesterase". In fact, both enzymes are to some degree nonspecific, and these names are not recommended by the Commission on Biochemical Nomenclature of the International Union of Pure and Applied Chemistry and the International Union of

Biochemistry. Instead, the trivial names "acetylcholinesterase" and "cholinesterase" should be used for the erythrocyte and serum enzymes (EC 3.1.1. 7 and EC 3.1.1.8, respectively). [8]

CLINICAL BACKGROUND

The cholinesterases are generally accepted as being synthesized in the liver and the assay of cholinesterase first became of interest to the clinician and to the clinical chemist as a test of liver function. Low serum cholinesterase activities are found in acute hepatitis, acute cirrhosis and in liver metastasis. That is, in those conditions where the hepatic synthesis of the protein is impaired. The synthesis of several other proteins is also reduced in such conditions, so that cholinesterase assay has been largely superseded as a test of liver function by measurements related to such proteins as albumin and prothrombin. Nevertheless, cholinesterase still has a place in the assessment of hepatic and other diseases.

Pseudocholinesterase is synthesized in liver and its serum activity is influenced by liver disease. Pseudocholinesterase level in serum is a useful test of liver function.[9]

Sex-hormone binding globulin, ferritin or LDL cholesterol have been used as endpoints in clinical studies of the responsivity of the liver to thyroid hormone in patients with thyroid hormone resistance.[10]

Only liver and gonads display the marked sex difference in enzyme content characteristic of the serum. The gonads are not necessary to the synthesis since castrates respond to administration of estrogen by an elevation of liver and serum cholinesterases. In both sexes, a relatively constant enzyme threshold in the liver, above which level the esterase is liberated into the serum, constitutes a simpler explanation of the known facts than does a differential concentrating mechanism from serum to liver. The liver produces serum albumins [11], and serum cholinesterase has been associated with the albumin fraction of serum proteins.[12]

In keeping with the last statement are the facts that liver damage lowers serum albumins [13] and liver and serum cholinesterases [14]; liver diseases lower both serum albumins and cholinesterase in humans [15]; the "alarm reaction" lowers serum albumin concentrations [16] and serum choline esterase [17]; estrogens elevate serum albumin levels [18] and serum non-specific cholinesterase.[17] The slight sex difference in liver specific cholinesterase content (mecholyl hydrolysis) is not statistically significant ($P > 0.1$).

Two recent papers have demonstrated that the amount of non-specific cholinesterase in rat serum is controlled at least in part by sex hormones. Estrogen elevates the serum enzyme level and testosterone depresses it, while progesterone exerts no noticeable effect except indirectly through estrogen.[17,18, 19]

Immature male and female rats have a similar basal level of pseudocholinesterase activity in the liver and serum. Castration of male rats results in an increase of the enzyme activity to the basal level, whereas subsequent injection of testosterone-propionate brings about a fall to the level found in adult male rats. Three androgenic / anabolic steroids were compared for their effects on pseudocholinesterase activities in liver and serum of male castrates, testosterone-propionate, testosterone-phenyl-propionate and nor-testosterone-phenyl propionate. This was confirmed in experiments by direct protein determinations in liver and serum and by levator ani muscle/seminal vesicle ratios. At a dose of 1 mg daily for ten days, the former two substances had a similar considerable effect on pseudocholinesterase activity, whereas the effect of the nor-derivative was much smaller. It is concluded that the cholinesterase activity lowering effect of the compounds investigated is correlated with their androgenic rather than with their anabolic potencies.[20]

Hypothyroidism can lead to low levels of SHBG (sex hormone binding globulin) which in turn can lead to higher concentrations of free testosterone and increased testosterone throughout the body [21]**.** For both sexes, hyperthyroidism was associated with significant elevations of the mean total testosterone and sex hormone-binding globulin (SHBG) levels and significant depressions of the mean percentage and concentration of non-SHBG-bound testosterone and the mean percentage of free testosterone. For women, the mean free testosterone concentration was significantly lower during hyperthyroidism than during euthyroidism.[22]

Androstenediol (5-androsten-3beta, 17beta-diol, ADIOL) and androstenediol 3-sulfate (ADIOLS) are active metabolites of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS), respectively, and have estrogenic activity and immunoregulatory function. In hypothyroidism, serum levels of all these steroids were significantly decreased in both genders. Serum concentrations of all these steroids correlated with the serum concentration of the thyroid hormones in these patients. These findings indicate that serum concentrations of ADIOLS, ADIOL, DHEAS, DHEA and PREGS were decreased in hypothyroidism.[23]

Hypothyroid women have decreased rates of metabolic clearance of androstenedione and strone and exhibit an increase in peripheral aromatization.[24, 25] The 5α/β ratio of androgen metabolites is also decreased in hypothyroid women, and there is an increase in excretion of 2-oxygenated estrogens.[26] Plasma binding activity of SHBG is decreased, which results in decreased plasma concentrations of both total testosterone and E_2, but their unbound fractions are increased. Alterations in steroid metabolism disappear when a euthyroid state is restored.[27] Gn levels are usually normal.[28] However, blunted or delayed LH response to GnRH has been reported in some hypothyroid women. [29, 30] When there is a delayed LH response, serum PRL concentration may be increased, and this may be due to hypothalamic

TRH increasing both TSH and PRL secretion. Galactorrhea may also occur, but these disturbances disappear usually after T_4 administration. [31]

Transport of Testosterone in the Circulation

Since testosterone is relatively insoluble in water (and hence blood) it travels through the circulatory system bound to proteins. The major binding protein, Sex Hormone Binding Protein (SHBG) is responsible for carrying between 60 – 70% of the body's testosterone through the bloodstream. The testosterone that is bound to SHBG is bound so tightly that it is considered biologically inactive. Virtually all the remaining testosterone is bound loosely to a number of other binding proteins, the main one being albumin. Only about 2% of the body's testosterone circulates free, or unbound. However, as mentioned, the testosterone that is albumin bound is only attached very loosely to this transport protein and so is able to interact with the androgen receptor as if it were free. Hence it, like free testosterone, is considered to be biologically active. The free and albumin bound testosterone together are called the bioactive or bioavailable fraction.

Factors Influencing the Synthesis of SHBG and Albumin

SHBG itself is produced in the liver, and its production is under the control of a number of factors, including androgen and estrogen levels, insulin, and thyroid hormone. For example, androgens lower levels of SHBG, while estrogens raise SHBG. Thyroid hormone has been shown in numerous studies to elevate levels of SHBG. Albumin synthesis, like that of SHBG, is also under the control of thyroid hormone. In cases of hyperthyroidism, albumin levels have been observed to be lower than in controls.[32] Hyperthyroidism was characterized by SHBG and albumin levels of 187 nmol/l and 36.2 g/l respectively. SHBG and albumin in

controls were 50.9 nmol/l and 43.5 g/l. So we see that hyperthyroidism increases SHBG levels while it decreases albumin.[32]

Relationship between SHBG, albumin and total testosterone levels

Numerous studies have shown that total testosterone (free+SHBG bound+albumin bound) correlates with SHBG levels. As SHBG levels rise, testosterone is believed to partition out of the free and albumin bound phases into the SHBG phase under the law of mass action. Testosterone bound tightly to SHBG is less subject to the action of metabolic enzymes. Hence the increase in SHBG bound testosterone leads to a decrease in the metabolic clearance rate of testosterone, with a corresponding increase in total plasma testosterone .[32] So with decreased metabolic clearance of testosterone, we might anticipate that the elevated SHBG levels associated with natural and artificially induced hyperthyroidism would lead to an increase in total testosterone levels.

The body fails to compensate for the drop in bioavailable T, with either no increase or an insufficient increase in LH production. It turns out the latter scenario, an uncompensated drop in bioavailable T, is what is most commonly seen in studies that have measured bioavailable T under conditions of hyperthyroidism.

For example, in the study by Loric et al, hyperthyroid subjects had average non-SHBG-bound T levels of 0.097nmol/l. After 3 to 6 months of treatment with the antithyroid drug carbimazole non-SHBG-bound T levels had increased to 0.196nml/l. Free T increased from 2.59 pmol/l to 3.74 pmol/l. Control subjects in this study had non-bound and free T levels of 0.152 nmol/l and 1.05 pmol/l, respectively.[32]

Additional Abnormalities of the Hypothalmic Pituitary Gonadal Axis in Hyperthyroidism

In addition to the rather well documented decrease in bioavailable testosterone associated with hyperthyroidism discussed above, several additional abnormalities in hypothalamic-pituitary - gonadal function have been recorded in the literature in cases of hyperthyroidism. Free estradiol levels have been observed to be elevated out of proportion to the rise in SHBG. [33] The peripheral aromatization of androgens to estrogens has also been reported [34], as has increased levels of progesterone in both men and women with Grave's disease.[35] Elevated estradiol and depressed bioavailable testosterone have been cited as the cause of sexual dysfunction common in hyperthyroid individuals.

The elevated levels of estradiol and progesterone seen in hyperthyroidism undoubtedly contribute to the high incidence of gynecomastia in this disease. It has also been suggested that increased levels of androstenedione [36] and androstenediol [37] observed in cases of hyperthyroidism could also contribute to gynecomastia via their aromatization to estrogens.

Pseudocholinesterase activity in thyroid disease

In a study on Pseudocholinesterase activity in thyroid disease, 12 patients with myxoedema had a 30 % decrease in mean activity for pseudocholinesterase level than that found in random normals using either substrate (acetylcholine or benzoylcholine). Even when myxoedema is not associated with a subnormal esterase activity, a rise in activity does occur when the patient becomes euthyroid. Treatment restores the esterase level to the average mean activity of random normal as the patient becomes euthyroid. There is no evidence in the present investigation that any of the pseudocholinesterase variants modify these conclusions (Table 1).[38]

Table 1: Pseudocholinesterase activity in Thyroid disease[38].

Pathological Condition	**Number Tested**	**Substrate**			
		Acetylcholine		**Benzoylcholine**	
		Mean activity	SD	Mean activity	SD
Hypothyroid	12	75	25	73	35
Random Normals	94	107	19		
Random Normals				103	24
Hyperthyroid	48	124	24	124	30
Hyperthyroid now euthyroid	32	98	22	100	30

Decreased levels of Serum cholesterol and beta-lipoprotein**s** occurring in hyperthyroidism were found to be accompanied by an enhanced activity of pseudocholinesterase**,** while in patients with myxedema, the pathologically increased levels of serum cholesterol and beta-lipoproteins were associated with diminished serum pseudocholinesterase activity. The percentage of the pre-beta fraction was found to be increased in both hypothyroid and hyperthyroid patients, but mechanisms leading to this change are probably different in the two pathological conditions. The behavior of cholesterol and pseudocholinesterase activity and especially the ratio between these parameters might be used for the diagnosis of thyroid disease and for the control of therapeutic efficiency.[39]

Methodological Aspects

A good correlation exists between the activities using acetylcholine and benzoylcholine as substrates (Fig. 1). Pseudocholinesterase activity using acetylcholine as substrate was

determined micromanometrically (Callaway, Davies, and Rutland, 1951). The esterase activity using benzoylcholine as substrate was determined in M/15 phosphate buffer at pH 7-4 at 26 5°C. by the method of Kalow and Lindsay (1955).[38]

Dibucaine numbers were determined by the method of Kalow and Genest (1957) and fluoride numbers by the method of Harris and Whittaker (1961). The percentage inhibition of benzoyl cholinesterase by dibucaine is termed the dibucaine number and the fluoride number is similarly defined. All the readings on the spectrophotometer were measured directly and not automatically recorded.[38]

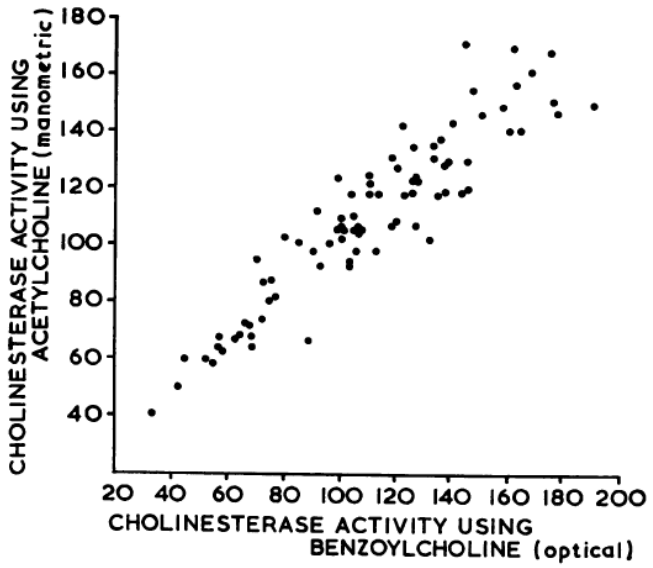

FIG. 1. *Scatter diagram for the two methods for estimating pseudocholinesterase activity.*

There is no evidence that any of the pseudocholinesterase variants will modify any conclusions. Even if either acetylcholine or benzoylcholine are measured there is no evidence that they will modify any conclusions (fig 1). [38]

6.1 SPECIMEN COLLECTION AND STORAGE

Much of the work involving cholinesterase variants has involved collecting serum specimens at one location and shipping them to another location for analysis. In some cases the specimens have been shipped in the frozen state but in others they have been shipped at ambient temperature, even though they were in transit from one continent to another. The effects of time and temperature of storage on the stability of cholinesterase variants have received comparatively little attention. There are conflicting reports concerning the stability of cholinesterase activity in blood. This subject has been included in the review of Witter , who stated that the enzyme in plasma is stable for several weeks at 0 to 5°C and for several months in the frozen state. The use of blood dried on filter paper was also discussed as there were reports that the enzyme is unstable when the plasma is stored for several days. However, other investigators have drawn opposite conclusions. Johnston and Huff reported that an average of 31 % of the cholinesterase activity was lost within 3-4 months when plasma was stored frozen under their conditions.

This stability of cholinesterase in stored plasma was systematically studied by Braid and Nix. They found that one reason for apparent loss of activity at room temperature is that the specimen slowly coagulates, and as it does, cholinesterase activity accumulates in the coagulum. Full activity could be restored by very vigorous mixing; 5-fold dilution of the plasma before storage prevented coagulation. Another way in which the activity was reduced was through bacterial contamination. Apparent increase in activity was produced through evaporation from inadequately stoppered specimens. It was also noted that the activity increased by about 10 % during the first 24 hours after the specimen was collected, but returned to the original value within 48 hours. These studies demonstrated that plasma cholinesterase is very stable. At - 20°C, specimens have retained more than 95 % of their original activity after 3 years of storage, and more than 85 % after 7 years. Eight freeze-thaw cycles over a period of 8 days resulted in less than 5 % deviation from the original value. At

5°C, 80 % of original activity remained after 4 months of storage. At 23°C, the enzyme appeared to be completely stable for up to about 80 days, and retained 85 % of its original activity at 240 days. Storage at 37°C resulted in a loss of about 1 % of the original activity per day. At 45 to 55°C, the loss of activity occurred in two phases-an initial rapid phase, and a slower second phase. The higher the temperature, the less activity remained at the time of switchover from phase 1 to phase 2. The rate of the phase 1 reaction increased with increasing temperature, whereas the rate of the phase 2 reaction decreased with increasing temperature.

In regard to the stability of the phenotype determined on fresh versus stored serum or plasma, there are numerous observations that the chemical tests lead to the same phenotype after specimen storage in the frozen state.

The recommended method of collecting, transporting, and storing the specimen for serum cholinesterase measurement and phenotyping is as follows: The site to be punctured should be washed free of possible cholinesterase inhibitors by cleansing with alcohol, which is allowed to evaporate before skin puncture. In those cases where only the plasma cholinesterase is to be studied, the blood is collected in the absence of anticoagulant and allowed to clot for 1 hour at room temperature. The tube is then centrifuged at 1000 g, and the serum separated from the clot. It is important to avoid hemolysis, especially if acetylcholine or acetyl thiocholine is used as substrate. If the analysis is not to be performed immediately, the serum may be stored for several days at 4 ° C. Otherwise it should be stored at - 20°C, or lower. If the serum is to be shipped, it is safest to freeze the separated serum, place it in a pre-cooled, insulated container along with solid carbon dioxide, and ship by an appropriate means of transportation such that it arrives at the laboratory while still frozen. This is recommended in order to avoid unwanted extreme increases in temperature during

transportation. If it is not possible to follow this latter recommendation, the specimens should be kept sterile. In this case, the transported specimens may still be analyzed for phenotyping purposes, but it should be recognized that a reduction of enzyme activity may have occurred during shipment.

PATHOPHYSIOLOGY OF Hypothyroidism

Reduced production of thyroid hormone is the central feature of the clinical state termed hypothyroidism.[39,40] Permanent loss or destruction of the thyroid, through processes such as autoimmune destruction or irradiation injury, is described as *primary hypothyroidism*. Hypothyroidism due to transient or progressive impairment of hormone biosynthesis is typically associated with compensatory thyroid enlargement. Central or secondary hypothyroidism, caused by insufficient stimulation of a normal gland, is the result of hypothalamic or pituitary disease or defects in the thyroid-stimulating hormone (TSH) molecule. Transient or temporary hypothyroidism can be observed as a phase of sub acute thyroiditis.

Primary hypothyroidism is the etiology in approximately 99% of cases of hypothyroidism. Reduced action of thyroid hormone at the tissue level in the face of normal or increased thyroid hormone production from the thyroid gland can also be associated with clinical hypothyroidism. Conditions associated with reduced thyroid hormone action are rare and include abnormalities of thyroid hormone metabolism and defects in nuclear signaling. Consumptive hypothyroidism, identified in an increasing number of clinical settings, is the result of accelerated inactivation of thyroid hormone by the type 3 iodothyronine deiodinase (D3). Defects of activation of the prohormone, thyroxine (T4), to the active form, triiodothyronine (T3), have also been identified. Polymorphisms in genes regulating thyroid hormone production and activation may influence thyroid hormone action in some tissues.[41] Resistance to thyroid hormone (RTH), the result of defects in the thyroid hormone nuclear receptor or nuclear cofactors, is associated with elevated circulating levels of thyroid hormone. Some tissues, depending on the level of expression of the mutant receptor and other forms of local compensation, have evidence of reduced thyroid hormone action. Estimates of the incidence of hypothyroidism vary depending on the population studied.[42,43]

In the United States, 0.3% of the population have overt hypothyroidism, defined as an elevated serum TSH concentration and reduced free thyroxine concentration (fT4), and 4.3% have what has been described as subclinical or mild hypothyroidism.[43] Although a number of clinical manifestations have been associated with this early or mild phase of hypothyroidism, the term *subclinical* is used here to describe this group, as in most clinical studies. Subclinical hypothyroidisms defined as an elevated serum TSH level with a normal serum fT4 concentration.[44]Subclinical hypothyroidism can progress to overt hypothyroidism,[45] and it can be associated with manifestations, that in some patients, can be improved with treatment.[44- 47] The incidence of hypothyroidism is higher among women, the elderly, and in some racial and ethnic groups.[43]

CLINICAL PRESENTATION

Hypothyroidism can affect all organ systems. These manifestations are largely independent of the underlying disorder but are a function of the degree of hormone deficiency. The following sections discuss the pathophysiology of each organ system at various levels of thyroid hormone deficiency, from mild to severe.

The term myxedema, formerly used as a synonym for hypothyroidism, refers to the appearance of the skin and subcutaneous tissues in the patient who is in a severely hypothyroid state. Hypothyroidism of this severity is rarely seen today, and the term should be reserved for description of the physical signs.

Skin and Appendages

Hypothyroidism causes an accumulation of hyaluronic acid that alters the composition of the ground substance in the dermis and other tissues.[48] This material is hygroscopic, producing the mucinous edema that is responsible for the thickened features and puffy appearance (myxedema) observed in patients with full-blown hypothyroidism. Myxedematous tissue is characteristically boggy and non pitting and is apparent around the eyes, on the dorsa of the hands and feet, and in the supraclavicular fossae. It causes enlargement of the tongue and thickening of the pharyngeal and laryngeal mucous membranes. A histologically similar deposit may occur in patients with Graves disease, usually over the pretibial area (infiltrative dermopathy or pretibial myxedema). In addition to having a puffy appearance, the skin is pale and cool as result of cutaneous vasoconstriction. Anemia may contribute to the pallor; hypercarotenemia gives the skin a yellow tint but does not cause scleral icterus. The secretions of the sweat glands and sebaceous glands are reduced, leading to dryness and coarseness of the skin, which in extreme cases may resemble that observed in patients with ichthyosis. Wounds of the skin tend to heal slowly. Easy bruising occurs because of an increase in capillary fragility. Head and body hair is dry and brittle, lacks luster, and tends to fall out. Hair may be lost from the temporal aspects of the eyebrows, although this feature is not specific for hypothyroidism. Growth of hair is retarded, so that haircuts and shaves are required less often.

The nails are brittle and grow slowly. Topical T3 has been shown to accelerate wound healing and stimulate hair growth in a euthyroid mouse model, demonstrating a role for thyroid hormone in these processes.[49] Histopathologic examination of the skin reveals hyperkeratosis with plugging of hair follicles and sweat glands. The dermis is edematous, and the connective tissue fibers are separated by an increased amount of metachromatically staining, periodic acid–Schiff (PAS)-positive mucinous material. This material consists of protein complexed with two mucopolysaccharides, hyaluronic acid and chondroitin sulfate B.

The hygroscopic glycosaminoglycans are mobilized early during treatment with thyroid hormone, leading to an increase in urinary excretion of nitrogen and hexosamine as well as tissue water.[48] Patients with hypothyroidism due to Hashimoto's thyroiditis may also have skin lesions with loss of pigmentation characteristic of the autoimmune skin condition called *vitiligo*. This is not a manifestation of reduced thyroid hormone action but reflects the common association of autoimmune endocrine disease and this skin condition, which is recognized as a component of autoimmune polyendocrine syndromes. [50]

Cardiovascular System

The cardiac output at rest is decreased because of reduction in both stroke volume and heart rate, reflecting loss of the inotropic and chronotropic effects of thyroid hormones. Peripheral vascular resistance at rest is increased, and blood volume is reduced. These hemodynamic alterations cause narrowing of pulse pressure, prolongation of circulation time, and decrease in blood flow to the tissues.[51- 54] The reduction in cutaneous circulation is responsible for the coolness and pallor of the skin and the sensitivity to cold. In most tissues, the decrease in blood flow is proportional to the decrease in oxygen consumption, so the arteriovenous oxygen difference remains normal. The hemodynamic alterations at rest resemble those of congestive heart failure. However, in hypothyroidism, cardiac output increases and peripheral vascular resistance decreases normally in response to exercise, unless the hypothyroid state is severe and of long standing. In severe primary hypothyroidism, the cardiac silhouette is enlarged, and the heart sounds are diminished in intensity.[55] These findings are largely the result of the effusion into the pericardial sac of fluid rich in protein and glycosaminoglycans, but the myocardium may also be dilated. Pericardial effusion is rarely of sufficient magnitude to cause tamponade. Angina pectoris may first appear or worsen during treatment of the hypothyroid state with thyroid hormone, although most patients with hypothyroidism and

coronary artery disease have no change, or improvement, in anginal symptoms with T4 treatment.[56] Electrocardiographic changes include sinus bradycardia, prolongation of the PR interval, low amplitude of the P wave and QRS complex, alterations of the ST segment, and flattened or inverted T waves. Pericardial effusion is probably responsible for the low amplitude in severe hypothyroidism. Systolic time intervals are altered; the pre-ejection period is prolonged, and the ratio of pre-ejection period to left ventricular ejection time is increased. Echocardiographic studies have revealed resting left ventricular diastolic dysfunction in overt hypothyroidism and, in some studies, subclinical hypothyroidism.[54] These findings normalize when the hypothyroidism is treated. Serum levels of homocysteine, creatine kinase, aspartate aminotransferase, and lactate dehydrogenase may be increased in hypothyroidism. [51, 57]

Typically, the isoenzyme patterns suggest that the source of the increased creatine kinase and lactate dehydrogenase is skeletal muscle, not cardiac muscle. All levels return to normal with therapy. Sequential cardiac biopsies in a hypothyroid patient with heart failure showed that messenger RNA (mRNA) levels from genes regulated by thyroid hormone that are important for the strength of myocardial contraction were normalized after T4 treatment.[58]The combination of large heart, hemodynamic and electrocardiographic alterations, and the described serum enzyme changes has been termed myxedema heart. In the absence of coexisting organic heart disease, treatment with thyroid hormone corrects the hemodynamic, electrocardiographic, and serum enzyme alterations of myxedema heart and restores heart size to normal. Hypothyroidism is consistently associated with elevations of total and low-density lipoprotein (LDL) cholesterol, which improve with T4 replacement.[59]

In a study of a cohort from the Framingham population with short-term hypothyroidism, serum TSH in women was positively correlated with total cholesterol and LDL, but the

elevation was from the less atherogenic large LDL particles.[60] The higher the original serum TSH concentration and elevation of serum LDL, the greater the magnitude of reduction in LDL cholesterol after T4 therapy. A subset of younger concentrations of serum triglycerides and C-reactive protein that improve with T4 treatment.[61] Most studies have shown that serum high-density lipoprotein (HDL)levels are not influenced by thyroid status. Hypothyroidism has been shown to be a risk factor for atherosclerosis and cardiovascular disease in several studies, although others have not shown this association. A prospective study from Japan showed an increased risk of ischemic heart disease in men, but not in women, with subclinical hypothyroidism.[62] The Whickham study showed no increase in cardiovascular mortality among patients with subclinical hypothyroidism who were followed up for more than 20 years.[63] A prospective study in the United States that monitored men and women age 65 or older for more than 10 years showed no influence of hypothyroidism (overt or subclinical) on cardiovascular outcome or mortality.[64] Cardiovascular outcome studies suggest that improvement from treatment of hypothyroidism, specially subclinical hypothyroidism, occurs primarily among those patients who are in middle age and not in older individuals (>65 years of age).[59]

Respiratory System

Pleural effusions usually are evident only on radiologic examination, but in rare instances they may cause dyspnea. Lung volumes are usually normal, but maximal breathing capacity and diffusing capacity are reduced. In severe hypothyroidism, myxedematous involvement of respiratory muscles and depression of both the hypoxic and the hypercapnic ventilatory drives can cause alveolar hypoventilation and carbon dioxide retention, which in turn can contribute to the development of myxedema coma. Obstructive sleep apnea is common but is reversible with restoration of a euthyroid state.

Alimentary System

Although most patients experience a modest gain in weight, appetite is usually reduced. The weight gain that occurs is caused partly by retention of fluid by the hydrophilic glycoprotein deposits in the tissues and does not exceed 10% of body weight. Peristaltic activity is decreased and, together with the decreased food intake, is responsible for the frequent complaint of constipation. The latter may lead to fecal impaction (myxedema megacolon). Gaseous distention of the abdomen (myxedema ileus), if accompanied by colicky pain and vomiting, can mimic mechanical ileus.[65] Elevations in the serum level of carcino-embryonic antigen, which may occur on the basis of hypothyroidism alone, add to the impression that an obstruction is present. Ascitis in the absence of another cause is unusual in hypothyroidism, but it can occur, usually in association with pleural and pericardial effusions. Like pericardial and pleural effusions, the ascitic fluid is rich in protein and glycosaminoglycans.

Achlorhydria after maximal histamine stimulation may be present in patients with primary hypothyroidism. Circulating antibodies against gastric parietal cells have been found in about one third of patients with primary hypothyroidism and may be secondary to atrophy of the gastric mucosa. Hypothyroid patients with positive parietal cell antibodies have a higher T4 requirement compared with antibody-negative patients.[66] Among Swedish patients with celiac disease, there was a 4.4-fold increased risk for Hypothyroidism, compared to the general population.[67]

Overt pernicious anaemia is reported in about 12% of patients with primary hypothyroidism. The coexistence of pernicious anaemia and other autoimmune diseases with primary hypothyroidism reflects the fact that autoimmunity plays the central role in the pathogenesis of these diseases. Hypothyroidism has complex effects on intestinal absorption. Although the rates of absorption for many substances are decreased, the total amount absorbed may be

normal or even increased because the decreased bowel motility allows more time for absorption. Malabsorption is occasionally overt. Liver function test results are usually normal, but levels of amino-transaminases may be elevated, probably because of impaired clearance.[68] The gall bladder contracts sluggishly and may be distended. In a population study of patients without diagnosed thyroid disease, men, but not women, with an elevated TSH had a 3.8-fold increased risk of cholelithiasis.[69] Atrophy of the gastric and intestinal mucosa and myxedematous infiltration of the bowel wall may be demonstrated on histologic examination. The colon may be greatly distended, and the volume of fluid in the peritoneal cavity is usually increased. The liver and pancreas are normal.

Central and Peripheral Nervous System

Thyroid hormone is essential for the development of the central nervous system.[122,70] Deficiency in fatal life or at birth leads to impaired neurologic development, including hypoplasia of cortical neurons with poor development of cellular processes, retarded myelination, and reduced vascularity.[122,67]If the deficiency is not corrected in early postnatal life, the damage is irreversible. Deficiency of thyroid hormone beginning in adult life causes less severe manifestations that usually respond to treatment with the hormone. Cerebral blood flow is reduced, but cerebral oxygen consumption is usually normal; this finding is in accord with the conclusion that the oxygen consumption of isolated brain tissue in vitro, unlike that of most other tissues, is not stimulated by administration of thyroid hormones.

In severe cases, decreased cerebral blood flow may lead to cerebral hypoxia. All intellectual functions, including speech, are slowed in thyroid hormone deficiency.[71] Loss of initiative is present, and memory defects are common; lethargy and somnolence are prominent, and dementia in elderly patients may be mistaken for senile dementia. Positron emission tomography (PET) brain scans of hypothyroid patients before and after T4 therapy

demonstrate reversible reduced glucose uptake in specific brain areas, such as the limbic system, which also correlates with behavioral and psychiatric symptoms.[72]

Psychiatric disorders are common and are usually of the paranoid or depressive type and may induce agitation (myxedema madness).[71] Headaches are frequent. Cerebral hypoxia due to circulatory alterations may predispose to confusional attacks and syncope, which may be prolonged and can lead to stupor or coma. Other factors predisposing to coma in hypothyroidism include exposure to severe cold, infection, trauma, hypoventilation with carbon dioxide retention, and depressant drugs. Epileptic seizures have been reported and tend to occur in myxedema coma. Night blindness is caused by deficient synthesis of the pigment required for dark adaptation. Hearing loss of the perceptive type is frequent due to myxedema of the eighth cranial nerve and serous otitis media. Perceptive deafness may also occur in association with a defect in the organic binding of thyroidal iodide (Pendred's syndrome) , but in these instances it is not a result of hypothyroidism per se. Thick, slurred speech and hoarseness are caused by myxedematous infiltration of the tongue and larynx, respectively.

Body movements are slow and clumsy, and cerebellar ataxia may occur. Numbness and tingling of the extremities are frequent; in the fingers, these symptoms may be caused by compression from glycosaminoglycan deposits in and around the median nerve in the carpal tunnel (carpal tunnel syndrome).[73] The tendon reflexes are slow, especially during the relaxation phase, producing the characteristic "hung-up reflexes"; this phenomenon is caused by a decrease in the rate of muscle contraction and relaxation rather than a delay in nerve conduction. The presence of extensor plantar responses or diminished vibration sense should alert the physician to the possibility of coexisting pernicious anemia with combined systemic disease. Electroencephalographic changes include low alpha-wave activity and general loss

of amplitude. The concentration of protein in the cerebrospinal fluid is often increased, but cerebrospinal pressure is normal. Histopathologic examination of the brain in patients with untreated hypothyroidism reveals that the nervous system is edematous with mucinous deposits in and around nerve fibers. In patients with cerebellar ataxia, neural myxedematous infiltrates of glycogen and mucinous material are present in the cerebellum. There may be foci of degeneration and an increase in glial tissue. The cerebral vessels may show atherosclerosis, but this is much more common if the patient has had coexistent hypertension.

Hypothyroidism has been associated with several neurologic conditions, although a strong etiological link has not been established. Epidemiologic studies have shown an association between Alzheimer's disease and hypothyroidism.[74] It is difficult to convincingly demonstrate this association, because the incidence of thyroid disease in the elderly population is high and, like that of dementia, increases with age. A mechanistic link is suggested by the observation of amyloid deposition in Down syndrome, a condition that is associated with an increased incidence of Hashimoto's disease, and the fact that thyroid hormone regulates amyloid gene processing in a number of cellular and animal models. However, subclinical hyperthyroidism has also been associated with Alzheimer's disease.[75] There is an increase in the cerebrospinal fluid concentration of reverse T3 in Alzheimer's disease patients who have normal circulating thyroid hormone levels, suggesting the potential for altered thyroid hormone metabolism in the brain.[76] However, the impact of normalizing T3 levels in the brain is not known. A corticosteroid-responsive encephalopathy is associated with chronic Hashimoto's thyroiditis but may be linked to autoimmunity rather than a process mediated specifically by low thyroid hormone levels or thyroid autoantibodies.[77]

Muscular System

Stiffness and aching of muscles are common and are worsened by cold temperatures. Delayed muscle contraction and relaxation cause the slowness of movement and delayed tendon jerks. Muscle mass may be reduced or enlarged due to interstitial myxedema. Muscle mass may be slightly increased, and the muscles tend to be firm. Rarely, a profound increase in muscle mass with slowness of muscular activity may be the predominant manifestation (Kocher-Debré-Sémélaigne syndrome or Hoffmann syndrome). Myoclonus may be present. The electromyogram may be normal, or it may exhibit disordered discharge, hyperirritability, and polyphasic action potentials. On histopathological examination, the muscles appear pale and swollen. The muscle fibers may show swelling, loss of normal striations, and separation by mucinous deposits. Type I muscle fibers tend to predominate.

Skeletal System: Calcium and Phosphorus Metabolism

Thyroid hormone is essential for normal growth and maturation of the skeleton. Growth failure in thyroid deficiency is caused by impaired general protein synthesis, reduced growth hormone, and especially reduced insulin-like growth factor-1.[78] The thyroid hormone receptor isoforms α and β have specific roles in bone maturation. Urinary excretion of calcium is decreased, as is the glomerular filtration rate, whereas fecal excretion of calcium and urinary and fecal excretion of phosphorus are variable. Calcium balance is also variable, and any changes are slight. The exchangeable pool of calcium and its rate of turnover are reduced, reflecting decreased bone formation and resorption. Because levels of parathyroid hormone are often slightly increased, some degree of resistance to its action may be present; levels of $1,25(OH)_2D$ (dihydroxy vitamin D) are also increased. Levels of calcium and phosphorus in serum are usually normal, but calcium may be slightly elevated.

Renal Function: Water and Electrolyte Metabolism

Renal blood flow, glomerular filtration rate, and tubular reabsorptive and secretory maxima are reduced. Blood urea nitrogen and serum creatinine levels are normal, but uric acid levels may be increased. Urine flow is reduced, and delay in the excretion of a water load may result in reversal of the normal diurnal pattern of urine excretion. The delay in water excretion appears to be due to decreased volume delivery to the distal diluting segment of the nephron as a result of the diminished renal perfusion; evidence supporting inappropriate secretion of vasopressin (syndrome of inappropriate antidiuretic hormone secretion) is less compelling.[79] These changes are reversed by treatment with thyroid hormone. The ability to concentrate urine may be slightly impaired. Mild proteinuria may occur. The impaired renal excretion of water and the retention of water by the hydrophilic deposits in the tissues result in increase in total body water, even though plasma volume is reduced. This increase accounts for the hyponatremia in some patients, because of increased level of exchangeable sodium. The amount of exchangeable potassium is usually normal in relation to lean body mass. Serum magnesium concentration may be increased, but exchangeable magnesium levels and urinary magnesium excretion are decreased.

Hematopoietic System

In response to the diminished oxygen requirements and decreased production of erythropoietin, the red blood cell mass is decreased; this is evident in the mild normocytic, normochromic anemia that often occurs. Less commonly, the anemia is macrocytic, sometimes from deficiency of vitamin B_{12}. Reference has already been made to the high incidence of pernicious anemia (and of achlorhydria and vitamin B_{12} deficiency without overt anemia) in primary hypothyroidism. Conversely, overt and subclinical hypothyroidism is present in 12% and 15% of patients, respectively, with pernicious anemia. Folate deficiency from malabsorption or dietary inadequacy may also cause macrocytic anemia. The frequent

menorrhagia and the defective absorption of iron resulting from achlorhydria may contribute to a microcytic, hypochromic anemia. The total and differential white blood cell counts are usually normal, and platelets are adequate, although platelet adhesiveness may be impaired. If pernicious anemia or significant folate deficiency is present, the characteristic changes in peripheral blood and bone marrow will be found. The intrinsic clotting mechanism may be defective because of decreased concentrations in plasma of factors VIII and IX, and this, together with an increase in capillary fragility and the decrease in platelet adhesiveness, may account for the bleeding tendency that sometimesoccurs.[65, 79]

Pituitary and Adrenocortical Function

In long-standing primary hypothyroidism, hyperplasia of the thyrotropes may cause the pituitary gland to be enlarged. This feature can be detected radiologically as an increase in the volume of the pituitary fossa.[80] Rarely, the pituitary enlargement compromises the function of other pituitary cells and causes pituitary insufficiency or visual field defects. Patients with severe hypothyroidism may have increased serum prolactin levels, stimulated by the elevation in thyrotropin-releasing hormone (TRH) and proportional to the level of serum TSH elevation, and galactorrhea may develop in some patients. Treatment with thyroid hormone normalizes the serum prolactin and TSH levels and causes disappearance of galactorrhea, if present. In rodents, thyroid hormone directly regulates growth hormone synthesis. Growth hormone is not directly regulated by thyroid hormone in humans, but thyroid status influences the growth hormone axis.[81]

In severe, long-standing primary hypothyroidism, pituitary and adrenal function may be secondarily decreased, and adrenal insufficiency may be precipitated by stress or by rapid replacement therapy with thyroid hormone.[81]The rate of turnover of aldosterone is decreased, but the plasma level is normal. Plasma renin activity is decreased, and sensitivity to

angiotensin II is increased, which may contribute to the association of hypertension with hypothyroidism.

Reproductive Function

In both sexes, thyroid hormones influence sexual development and reproductive function.[82] Infantile hypothyroidism, if untreated, leads to sexual immaturity, and juvenile hypothyroidism causes a delay in the onset of puberty followed by anovulatory cycles. Paradoxically, primary hypothyroidism may also rarely cause precocious sexual development and galactorrhea, presumably due to "spillover" of elevated TSH stimulating the luteinizing hormone (LH) receptor [83] and elevated TRH initiating excess prolactin release. In adult women, severe hypothyroidism may be associated with diminished libido and failure of ovulation. Secretion of progesterone is inadequate, and endometrial proliferation persists, resulting in excessive and irregular breakthrough menstrual bleeding. These changes may be due to deficient secretion of LH or pulse frequency and amplitude, or both. Rarely, in primary hypothyroidism, secondary depression of pituitary function may lead to ovarian atrophy and amenorrhea. Fertility is reduced, and there is an increase in spontaneous abortion and preterm delivery, although many pregnancies are successful.[84] Pregnancy complications are associated with both overt and subclinical hypothyroidism, although the impact has varied among different studies.[85]

A randomized prospective study of levothyroxine treatment in pregnant women with thyroid peroxidase (TPO) antibody positivity and normal range TSH showed that the increased incidences of preterm delivery and spontaneous abortion were reversed by treatment, although this finding remains to be confirmed.[86] Primary ovarian failure can also be seen in patients with Hashimoto's thyroiditis as part of autoimmune polyendocrine syndrome.[51] Hypothyroidism in men may cause diminished libido, erectile dysfunction, and oligospermia.

A significant fraction of men with hypothyroidism or hyperthyroidism have moderate to severe erectile dysfunction that improves with treatment of the thyroid disease.[87] Values for plasma gonadotropins are usually in the normal range in primary hypothyroidism. Among postmenopausal women, levels are usually somewhat lower than in euthyroid women of the same age but are nevertheless within the menopausal range. This provides a valuable means of differentiating primary from secondary hypothyroidism. The metabolism of both androgens and estrogens is altered in hypothyroidism. Secretion of androgens is decreased, and the metabolism of testosterone is shifted toward etiocholanolone rather than androsterone. With respect to estradiol and estrone, hypothyroidism favors metabolism of these steroids via 16α-hydroxylation rather than 2-oxygenation, with the result that formation of estriol is increased and that of 2-hydroxyestrone and its derivative, 2-methoxyestrone, is decreased.

Estriol appears to have both estrogenically antagonistic and agonistic effects. When given alone, it generally exerts an estrogenic effect, the strength of which depends on the dosage. When given in conjunction with estradiol, it appears to exert antagonistic effects. [123] Researchers have also found estriol to be an estrogen antagonist when given as a short burst bolus.[124] Others have found estriol to be a short-acting agonist when administered in a single dose. In addition, *in vitro* studies have found estriol competes with estradiol binding, offering a mechanism for its antagonistic effects. [125]

The sex hormone–binding globulin in plasma is decreased, with the result that plasma concentrations of both testosterone and estradiol are decreased, but the unbound fractions are increased. The alterations in steroid metabolism are corrected by restoration of the euthyroid state.[88]

Catecholamines

The plasma cyclic adenosine monophosphate (cAMP) response to epinephrine is decreased in hypothyroidism, suggesting a state of decreased adrenergic responsiveness. The fact that the responses of plasma cAMP to glucagon and parathyroid hormone are also decreased suggests that thyroid hormones have a general modulating influence on cAMP generation.[89] The reduced adrenergic responsiveness associated with hypothyroidism has been linked to all steps of catecholamine signaling, including receptor and post receptor actions, resulting in an impaired cAMP response. Direct measurement of norepinephrine in abdominal fat of hypothyroid patients shows reduced levels, and there is reduced production of glycerol in response to adrenergic agonist stimulation.[90] Augmentation of α2-receptor signaling has also been proposed as a factor reducing catecholamine responsiveness.

Energy Metabolism: Protein, Carbohydrate, and Lipid Metabolism

The decrease in energy metabolism and heat production is reflected in the low basal metabolic rate, decreased appetite, cold intolerance, and slightly low basal body temperature.[91] Both the synthesis and the degradation of protein are decreased, the latter especially so, with the result that nitrogen balance is usually slightly positive. The decrease in protein synthesis is reflected in retardation of both skeletal and soft tissue growth. Permeability of capillaries to protein is increased, accounting for the high levels of protein in effusions and in cerebrospinal fluid. In addition, the albumin pool is increased because of the greater decrease in albumin degradation compared with albumin synthesis. A greater than normal fraction of exchangeable albumin is in the extra-vascular space. The total concentration of serum proteins may be increased. Hypothyroidism is associated with a reduction in the disposition of glucose to skeletal muscle and adipose tissue.[92] Thyroid hormone has been shown to stimulate expression of the insulin-sensitive glucose transporter (GLUT4), and the levels of this transporter are reduced in hypothyroidism. However,

hypothyroidism is also associated with reduced gluconeogenesis, and the net effect of these influences is usually a minimal effect of hypothyroidism on serum glucose levels. Thyroid hormone down regulates expression of prohormone processing enzymes, which, therefore, have increased activity in hypothyroidism.

Degradation of insulin is slowed, and the sensitivity to exogenous insulin may be increased. In a patient with preexisting diabetes mellitus who develops hypothyroidism, insulin requirements may be reduced. A further influence on glucose uptake may occur at the tissue level. Polymorphisms in the 5′-deiodinase type 2 (D2) gene, which may affect local T3 production, have shown to be associated with impaired glucose disposal.[93] Both the synthesis and the degradation of lipid are depressed in hypothyroidism. However, degradation is reduced to a greater extent, with a net effect of accumulation of LDL and triglycerides.[91] The decrease in the lipid degradation rate may reflect the decrease in post heparin lipolytic activity as well as reduced LDL receptors. Plasma free fatty acid levels are decreased, and the mobilization of free fatty acids in response to fasting, catecholamines, and growth hormone is impaired. Impaired lipolysis of white fat in hypothyroid patients at baseline and in response to catecholamine reflects impaired free fatty acid mobilization.[43,90] All of these abnormalities are relieved by treatment.

A correlation was shown between total cholesterol and serum TSH levels in hypothyroid individuals identified from among 25,862 participants in a health fair, including those not aware of being hypothyroid and those on T4 replacement.[94] An elevation in serum LDL-cholesterol has-been associated, in most studies, with overt and subclinical hypothyroidism.[60]According to most studies, serum HDL and triglycerides levels are not influenced by hypothyroidism.[60,92] The reduction in LDL with T4 therapy is generally related to the original magnitude of LDL and TSH elevation: the higher the initial levels, the greater

the observed reduction in LDL.[60] A typical reduction in LDL is 5% to 10% of the original level.

DIAGNOSIS OF HYPOTHYROIDISM

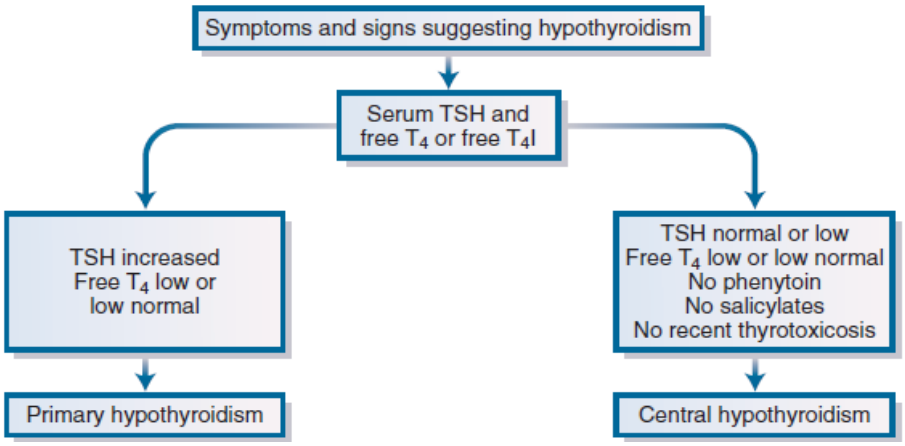

Figure 2: Diagnosis of hypothyroidism[28]

TSH level is the best screening test for detecting hypothyroidism. A normal TSH rules out primary hypothyroidism in asymptomatic patients. Abnormal TSH should be followed by determination of thyroid hormone levels. The measurement of thyroid auto-antibodies helps in diagnosing autoimmune process. However, this test should be ordered only if it will influence the decision to treat.

Screening

- TSH is a sufficient test for screening asymptomatic patients.
- We consider screening for:
    - Women and men older than 35 years, every 3 years
    - Women older than 60 years, every year
    - Persons with family history of spontaneous hypothyroidism and who are older than 35 years, once a year
    - Patients with personal history of another autoimmune disease, once a year
    - Routine screening of women planning pregnancy is not recommended

- TSH and thyroid hormone levels (preferably free thyroid hormone) should be ordered in all patients suspected to have thyroid disorder and in pregnant women.[95,96]

Table 2: Biomarkers in Hypothyroidism[97]

Biomarkers in Hypothyroidism[97]			
TSH Level	**Free T4 level**	**Free T3 level**	**Likely Diagnosis**
High	Low	Low	Primary Hypothyroidism
High (>10 mU per L)	Normal	Normal	Subclinical Hypothyroidism with high risk for further development overt hypothyroidism
High (6-10 mU per L)	Normal	Normal	Subclinical hypothyroidism with low risk for further development of overt hypothyroidism
High	High	Low	Congenital absence of T4-T3 converting enzymes. Amiodarone effect on T4-T3 conversion
High	High	High	Peripheral thyroid hormone resistance
Low	Low	Low	Pituitary thyroid deficiency or recent withdrawal of thyroxine after excessive replacement therapy.

Biochemical Markers of Altered Thyroid Status

Occasionally, a diagnosis of thyroid dysfunction is first suspected because of an abnormality in a laboratory result performed during an evaluation for an unrelated medical problem. Classic examples are a markedly elevated creatine kinase MM isoenzyme or low-density lipoprotein (LDL) cholesterol leading to the recognition of hypothyroidism (Table 3).[10] These tests are not useful in the diagnosis of thyroid disease, but some, such as sex hormone–binding globulin, ferritin, or LDL cholesterol, have been used as end points in clinical studies of the responsivity of the liver to thyroid hormone in patients with thyroid hormone resistance.

Table 3: Biochemical Markers of Thyroid Status.[10]

Biochemical Markers of Thyroid Status[10]
Thyrotoxicosis
Increased Osteocalcin Urine pyridinium collagen cross links Alkaline phosphatise (bone or liver) Atrial natriuretic hormone Sex hormone binding globulin Ferritin Von Willebrands factor **Decreased** Low density lipoprotein cholesterol Lipoprotein (a)
Hypothyroidism
Increased Creatinine kinase (MM isoform) Low density lipoprotein cholesterol Lipoprotein (a) Plasma Norepinephrine **Decreased** Vasopressin

TREATMENT OF HYPOTHYROIDISM

Hypothyroidism, either primary or central, is gratifying to treat because of the ease and completeness with which it responds to thyroid hormone. Treatment is almost always with levothyroxine, and the proper use of this medication has been reviewed extensively.[98 – 100] A primary advantage of levothyroxine therapy is that the peripheral de-iodination mechanisms can continue to produce the amount of T3 required in tissues under the normal physiologic control.[121] If one accepts the principle that replicating the natural state is the goal of hormone replacement, it is logical to provide the prohormone and allow the peripheral tissues to activate it by physiologically regulated mechanisms.

Pharmacologic and Physiologic Considerations

Levothyroxine has a 7-day half-life. About 80% of the hormone is absorbed relatively slowly and equilibrates rapidly in its distribution volume, so large post-absorptive perturbations in fT4 levels are avoided. Because of its long half-life, omission of a single day's tablet has no significant effect, and the patient may safely take an omitted tablet on the following day. In fact, the levothyroxine dosage can be calculated almost as satisfactorily on a weekly as on a daily basis. Although T4 is well absorbed and does not require fasting, regular ingestion of levothyroxine on an empty stomach results in the least variation in serum TSH concentration.[101]

The U.S. Food and Drug Administration (FDA) has issued standards for single-dose bioequivalence studies in normal volunteers to assess and compare T4 products in the United States.[102] The area under the curve (AUC) confidence interval must fall within 80% to 125% of the comparison product for a preparation to be considered equivalent. The desirability of a pharmacotherapeutic measurement such as TSH as an end point has been suggested by many professional organizations.[102] Recent regulations have narrowed the guidelines for measured T4 content, from between 90% and 110% to between 95% and 105% of the stated tablet dose,

with that content maintained for the entire shelf-life.[103] The availability in many countries of a multiplicity of tablet strengths with content ranging from 25 to 300 μg allows precise titration of the daily levothyroxine dosage for most patients with a single daily tablet, improving compliance significantly. The typical dose of levothyroxine, approximately 1.6 to 1.8 μg per kilogram of ideal body weight per day (0.7 to 0.8 μg/pound), usually results in the prescription of 75 to 112 μg/day for women and 125 to 200 μg/day for men. Replacement doses need not be adjusted upward in obese patients and should be based on lean body mass.[104] This dosage is about 20% greater than the T4 production rate because of incomplete absorption of the levothyroxine. In patients with primary hypothyroidism, these amounts usually result in serum TSH concentrations that are within the normal range. Because of the 7-day half-life, approximately 6 weeks is required before there is complete equilibration of the fT4 and the biologic effects of levothyroxine.

Accordingly, with rare exceptions (e.g., pregnancy), assessments of the adequacy of a given dose or of the effects of a change in dosage should not be made until this interval has passed. By and large, levothyroxine products are clinically equivalent, although problems do occur.[105] The variation in tablet content permitted by the FDA can result in slight variations in serum TSH in patients with primary hypothyroidism, even when the same brand is used. Using levothyroxine from a single manufacturer reduces variability that may be relevant for patients in whom close titration is required, such as the elderly, pregnant women, and thyroid cancer patients.[106] Although the serum TSH level is an indirect reflection of the levothyroxine effect in patients with primary hypothyroidism, it is superior to any other readily available method of assessing the adequacy of therapy. Return of the serum TSH level to normal is therefore the goal of levothyroxine therapy in patients with primary hypothyroidism. Some patients require slightly higher or lower doses than are generally used, owing to individual variations in absorption, and a number of conditions or associated medications may change

levothyroxine requirements in patients with established hypothyroidism. In decades past, desiccated thyroid was successfully employed for the treatment of hypothyroidism, and it still accounts for a small fraction of the prescriptions written for thyroid replacement in the United States. Although this approach was successful, desiccated thyroid preparations contain thyroid hormone derived from animal thyroid glands that have significantly higher ratios of T3 to T4 than the 1 : 11 value in normal human thyroid gland.[107] Accordingly, these unnatural preparations may lead to supraphysiologic levels of T3 in the immediate post-absorptive period (2 to 4 hours) due to the rapid release of T3 from Tg, its immediate and almost complete absorption, and the 1-day period required for T3 to equilibrate with its 40-L volume of distribution.[108] Mixtures of liothyronine and levothyroxine (*liotrix*) contain in a 1-grain (64-mg) equivalent tablet (Thyrolar-1 in the United States) the amounts of T3 (approximately 12.5 μg) and T4 (approximately 50 μg) present in the most popular desiccated thyroid tablet.[109] The levothyroxine equivalency of a 1-grain desiccated thyroid tablet or its liotrix equivalent can be estimated as follows. The 12.5 μg of liothyronine (T3) is completely absorbed from desiccated thyroid or from liotrix tablets.[108] Levothyroxine is approximately 80% absorbed,[110] and about 36% of the 40 μg of levothyroxine absorbed is converted to T3, with the molecular weight of T3 being 84% that of T4.

Accordingly, a 1-grain tablet should provide about 25 μg of T3, which would be approximately equivalent to that obtained from 100 μg of levothyroxine. This equivalency ratio can be used as an initial guide when switching patients from desiccated thyroid or liotrix to levothyroxine. Although levothyroxine is absorbed in the stomach and small intestine, normal gastric acid secretion is required for complete absorption.[111] Patients with impaired acid secretion on levothyroxine therapy require a 22% to 34% higher dose of levothyroxine to maintain the desired serum TSH. In those patients in whom acid secretion was normalized

therapeutically, the levothyroxine dose returned to baseline.[111] As indicated earlier, the use of levothyroxine for thyroid hormone replacement is a compromise with the normal pathway of T3 production, in which about 80% of T3 is derived from T4 5′-monodeiodination and approximately 20% (about 6 μg) is secreted directly from the thyroid gland.[121] Studies in thyroidectomized rats, for example, show that it is not possible to normalize T3 simultaneously in all tissues by an intravenous infusion of T4.[112] However, it should be recalled from the earlier discussion of T4 deiodination that the ratio of T3/T4 in the human thyroid gland is about 0.09, but in the rat thyroid gland it is 0.17.[121] Therefore, about 40% of the rat's daily T3 production is derived from the thyroid, compared with about 20% in humans.[121] Accordingly, the demonstration that T4 alone cannot provide normal levels of T3 in all tissues in the rat is of interest but is not strictly applicable to thyroid hormone replacement in humans. Nonetheless, the ratio of T3 to T4 in the serum of a patient receiving levothyroxine as the only source of T3 must be about 20% lower than that in a normal individual. Although serum T3 was the same level before and after thyroidectomy, a higher serum T4 concentration was necessary when the patient was on T4 replacement to maintain the same serum T3 level.[113] Although this may, to some extent, compensate for the lack of T3 secretion, the fact that T4 has an independent mechanism for TSH suppression, owing to the intracellular generation of T3 in the hypothalamic-pituitary-thyroid axis, means that a portion of the feedback regulation is independent of the plasma T3 concentration.

Although the concept of combined T4/T3 therapy has been recognized for many years, a positive study published in 1999 generated a great deal of interest in this approach.[114] Patients received 12.5 μg of T3 as a substitution for 50 μg of their levothyroxine preparation and scored, on average, somewhat higher on tests of mood than when they were taking levothyroxine alone. The dosage of thyroid hormone used in these studies was excessive, as judged by the fact that 20% of the group had serum TSH values below normal on either

regimen, and the test period was only a few months. Since this report, a large number of studies using a wide range of replacement strategies and relative T4/T3 content have been performed in different populations, and none has shown an advantage for combination therapy over T4 alone.[115] On the other hand, another study showed that the fT4 index correlated as closely as TSH levels with the resting energy expenditure in a group of patients in whom small supplements or decrements in their ideal replacement levothyroxine dosage were made.[116] The correlation with serum T3 was not statistically significant, suggesting that in humans, perhaps because of differences in peripheral metabolism of T4 compared with rodents, the fT4 index may be as accurate as the TSH value as an index of satisfactory thyroid hormone replacement. The practical difficulty with the design of tablets providing combinations of T3 and T4 is that the approximate dose of 6 μg of T3 provided would need to be released in a sustained fashion over 24 hours, which is quite different from the rapid absorption of T3 (with a peak at 2 to 4 hours) when given in its conventional form. Therefore, it appears that the current approach to thyroid replacement using levothyroxine alone, although not a perfect replication of the normal physiology, is satisfactory for most patients. A sustained-release T3 preparation has been developed and produces more stable levels of serum T3.[117] The clinical consequence of this more "physiologic" replacement profile is not known.

Institution of Replacement Therapy

The initial dose of levothyroxine prescribed depends on the degree of hypothyroidism and the age and general health of the patient. Patients who are young or middle age and otherwise healthy with no associated cardiovascular or other abnormalities and mild to moderate hypothyroidism (TSH concentrations, 5 to 50 mU/L) can be given an initial complete replacement dose of about 1.7 μg per kilogram of ideal body weight. The resulting increase in

serum T4 concentration to normal requires 5 to 6 weeks, and the biologic effects of T3 are sufficiently delayed so that these patients do not experience adverse effects. At the other extreme, an elderly patient who has heart disease, particularly angina pectoris, without reversible coronary lesions should be given a small initial dose of levothyroxine (25 μg/day), and the dosage should be increased in 12.5-μg increments at 2- to 3-month intervals with careful clinical and laboratory evaluation.

The goal in the patient with primary hypothyroidism is to return serum TSH concentrations to normal, reflecting normalization of that patient's thyroid hormone supply. This usually results in a mid-normal to high-normal serum fT4 concentration. The serum TSH should be evaluated 6 weeks after a theoretically complete replacement dose has been instituted, followed by minor adjustments to optimize the individual dose.[118] In patients with central hypothyroidism, serum TSH is not a reliable index of adequate replacement and the serum fT4 should be restored to a concentration in the upper half of the normal range. T4 dosing based on body weight and a serum fT4 in the upper reference range was found to improve markers of thyroid hormone action and was superior to replacement with a combination of T4/T3.[119] Patients with central hypothyroidism should also be evaluated and treated for glucocorticoid deficiency, if necessary, before institution of thyroid replacement.

Although the adverse effects of the rapid institution of therapy are unusual, pseudotumor cerebri has been reported in profoundly hypothyroid juveniles (8 and 12 years of age) who were given even modest initial levothyroxine replacement.[120] This complication appears 1 to 10 months after initiation of treatment and responds to treatment with acetazolamide and dexamethasone. The interval between initiation of treatment and the first evidence of improvement depends on the strength of dose given and the degree of the deficit. The serum sodium level increases even sooner if hyponatremia was present initially. Thereafter, pulse rate and pulse pressure increase, appetite improves, and constipation may disappear. Later,

psychomotor activity increases and the delay in the deep tendon reflex disappears. Hoarseness abates slowly, and changes in skin and hair do not disappear for several months. In individuals started on a complete replacement dose, the serum fT4 level should normalize after 6 weeks; a somewhat longer period may be necessary for serum TSH levels to return to normal, perhaps up to 3 months. In some cases, it is clinically appropriate to alleviate hypothyroidism rapidly. For example, patients with severe hypothyroidism withstand acute infections or other serious illnesses poorly, and myxedema coma may develop as a complication. In such circumstances, rapid near-repletion of the peripheral hormone pool in the average adult can be accomplished by a single intravenous dose of 500 mcg levothyroxine.

Alternatively, because of its rapid onset of action, liothyronine (25 mcg orally every 12 hours) may be administered if the patient can take medication by mouth. With both approaches, an initial biologic effect is achieved within 24 hours. Parenteral therapy with levothyroxine is then continued with a dose that is 80% of the appropriate oral dose but not in excess of 1.4 mcg/kg of ideal body weight. Because of the possibility that rapid increases in metabolic rate will overtax the existing pituitary-adrenocortical reserve, supplemental glucocorticoid (intravenous hydrocortisone, 5 mg/hour) should also be given to patients with severe hypothyroidism who are receiving high initial doses of thyroid hormones.

Finally, in view of the tendency of hypothyroid patients to retain free water, intravenous fluids containing only dextrose should not be given. When replacement therapy is withdrawn for short periods (4 to 6 weeks) for the purpose of evaluating therapy for thyroid cancer, rapid reinstitution of levothyroxine using a loading dose of three times the daily replacement dose for 3 days can usually be given, unless other complicating medical illnesses are present.

When hypothyroidism results from administration of iodine-containing or anti-thyroid drugs, withdrawal of the offending agent usually relieves both the hypothyroidism and the accompanying goiter, although it is appropriate to provide interim replacement until the gland recovers its function. This is especially true for amiodarone, which can remain in tissues for up to 1 year.

METHODOLOGY

This study was a comparative case control study conducted in the R.L.Jalappa Hospital and Research centre during the years 2011- 2012. The study was done on 70 hypothyroid patients attending outpatient clinic and those admitted in the medical wards.

The study group consisted of patients above the age of 18 years. All newly detected and old cases of hypothyroidism, irrespective of duration of hypothyroidism and type of treatment receiving were taken into the study. They were made into two groups. A control group of 54 euthyroid individuals without any previous thyroid diseases were taken with age and sex matched.

The exclusion criteria were all cases of organophosphorus poisoning, severe anemia (Hb < 7.0g/dl)[126], cardiac failure, uremia, cirrhosis of liver, vysya community, third trimester of pregnancy, previously diagnosed cases of malignancies including head, neck, lung, cervix and colon.

Ethical clearance was taken from the institution prior to commencement of the study.

History, physical examination, Thyroid function test, Pseudocholinesterase level, ECG, hemoglobin, blood urea, AST/ALT, serum bilirubin, ALP and stool occult blood were done for all patients.

The following clinical information was obtained, apart from investigations.

1. Duration of hypothyroidism
2. Dosage of thyroxine supplementation
3. Standard cuff blood pressure in supine position
4. Cardiovascular System examination

- Character of heart sounds

- Presence of abnormal heart sounds and murmurs.

5. Respiratory System Examination

6. Gastrointestinal Tract Examination

- Splenomegaly
- Liver span
- Shifting Dullness

7. Nervous System Examination

- Higher mental Examination
- Cranial Nerve Examination
- Motor System Examination
- Sensory System Examination

8. Investigations:

- Hemoglobin
- Blood Urea
- AST/ALT/Serum bilirubin/ALP
- Stool Occult Blood
- ECG
- Thyroid Function Test
- Pseudocholinesterase Measurement

Thyroid Function Test:

In the present study for the measurement of TSH, T4 and T3, chemiluminescence assay was employed.

TSH estimation

The VITROS TSH test is performed using the VITROS TSH Reagent Pack and the VITROS TSH Calibrators on the VITROS Immunodiagnostic System using Intellicheck® Technology.

An immunometric immunoassay technique is used, which involves the simultaneous reaction of TSH present in the sample with a biotinylated antibody (mouse monoclonal anti-whole TSH) and a horseradish peroxidase (HRP)-labeled antibody conjugate (mouse monoclonal anti-TSH β-subunit). The antigen-antibody complex is captured by streptavidin on the wells. Unbound materials are removed by washing.

The bound HRP conjugate is measured by a luminescent reaction.[127] A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system. The amount of HRP conjugate bound is directly proportional to the concentration of TSH present.

T4 estimation

The VITROS TT4 test is performed using the VITROS Total T4 Reagent Pack and the VITROS Total T4 Calibrators on the VITROS Immunodiagnostic System using Intelli-check® Technology. A competitive immunoassay technique is used, which depends on a competition between T4 present in the sample with a horseradish peroxidase (HRP)-labeled T4 conjugate for a limited number of binding sites on a sheep anti-T4 antibody presented in the liquid phase. Binding protein effects are eliminated by use of an appropriate buffer containing displacement agent. The antigen-antibody complex is captured by a donkey anti-sheep second antibody coated on the wells. Unbound materials are removed by washing.

The bound HRP conjugate is measured by a luminescent reaction.[127] A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide)

increases the level of light produced and prolongs its emission. The light signals are read by the system. The amount of HRP conjugate bound is indirectly proportional to the concentration of T4 present.

T3 estimation

The VITROS Total T3 test is performed using the VITROS Total T3 Reagent Pack and the VITROS Total T3 Calibrators on the VITROS Immunodiagnostic System using Intellicheck® Technology. A competitive immunoassay technique is used, which depends on a competition between T3 present in the sample with a horseradish peroxidase (HRP)-labeled T3 conjugate for a limited number of binding sites on a sheep anti-T3 antibody presented in the liquid phase. Binding proteins effects are eliminated by use of an appropriate buffer and blocking agent. The antigen-antibody complex is captured by a donkey anti-sheep second antibody coated on the wells. Unbound materials are removed by washing.

The bound HRP conjugate is measured by a luminescent reaction.[127] A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system. The amount of HRP conjugate bound is indirectly proportional to the concentration of T3 present.

Pseudocholinesterase Measurement

The VITROS Cholinesterase Slide method is performed using the VITROS cholinesterase slides and the VITROS Chemistry products calibrator kit 6. The VITROS Cholinesterase slide is a multilayered, analytical element coated on a polyester support. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. Cholinesterase hydrolyzes butyrylthiocholine to thiocholine. The liberated

thiocholine reduces potassium hexacyanoferrate III (potassium ferricyanide) to potassium hexacyanoferrate II (potassium ferrocyanide). The rate of color loss is monitored by reflectance spectrophotometry. The rate of change in reflection density is proportional to the cholinesterase activity in the sample.

Statistical Methods

Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance.

Analysis of variance (ANOVA) has been used to find the significance of study parameters between three or more groups of patients , Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups Inter group analysis) on metric parameters. Levene's test for homogeneity of variance has been performed to assess the homogeneity of variance. Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two or more groups. ROC curve analysis is performed to prove the Pseudocholinesterase (U/L) as diagnostic marker.

1. Sample Size estimation

Mean Known Population size

$n = \{ z^2 * \sigma^2 * [N / (N - 1)] \} / \{ ME^2 + [z^2 * \sigma^2 / (N - 1)] \}$

Mean Unknown population size

$n = (z^2 * \sigma^2) / ME^2$

ME: is the margin of error, measure of precision.

and Z is 1.96 as critical value at 95%CI

N: population size

n: Sample size

σ: Standard deviation

z: Critical value based on Normal distribution at 95% Confidence Interval

Standard deviation: $SD = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$

2. Analysis of Variance: F test for K Population means

Objective: To test K samples from K Populations with the same mean.

The mathematical model that describes the relationship between the response and treatment for the one-way ANOVA is given by

$$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$$

where Y_{ij} represents the *j*-th observation (j = 1, 2, ...n_i) on the *i*-th treatment (i = 1, 2, ..., k levels)

Limitations: It is assumed that populations are normally distributed and have equal variance. It is also assumed that samples are independent of each other.

Method. Let the j^{th} sample contain n_j elements(j=1,2,…K). Then the total number of elements is

$$N = \sum nj \qquad x.j = \sum \frac{xij}{nj}$$

$$S_1^{\,2} = \frac{\sum \sum_{i-1}^{n1} (x1 - \overline{x.j})^2}{N - K} \qquad S_2^{\,2} = \frac{\sum_{i-1}^{n1} nj(\overline{x.j} - \overline{x..})^2}{K - 1}$$

$F=S_2^{\,2}/S_1^{\,2}$ Which follows F distribution (K-1, N-K)

3. Chi-Square Test: The chi-square test for independence is used to determine the relationship between two variables of a sample. In this context independence means that the two factors are not related. In the chi-square test for independence the degree of freedom is equal to the number of columns in the table minus one multiplied by the number of rows in the table minus one.

$\chi^2 = \frac{\sum (Oi - Ei)^2}{Ei}$, Where Oi is Observed frequency and Ei is Expected frequency

With (n-1) degree of freedom.

The Assumptions of Chi-square test

The chi square test, when used with the standard approximation that a chi-square distribution is applicable, has the following assumptions:

- Random sample – A random sampling of the data from a fixed distribution or population.

- Sample size (whole table) – A sample with a sufficiently large size is assumed. If a chi square test is conducted on a sample with a smaller size, then the chi square test will yield an inaccurate inference. The researcher, by using chi square test on small samples, might end up committing a Type II error.

- Expected Cell Count – Adequate expected cell counts. Some require 5 or more, and others require 10 or more. A common rule is 5 or more in all cells of a 2-by-2 table, and 5 or more in 80% of cells in larger tables, but no cells with zero expected count. When this assumption is not met, Fisher Exact test or Yates' correction is applied.

4. **Fisher Exact Test:** The Fisher Exact Test looks at a contingency table which displays how different treatments have produced different outcomes. Its null hypothesis is that treatments do not affect outcomes-- that the two are independent. Reject the null hypothesis (i.e., conclude treatment affects outcome) if *p* is "small".

The usual approach to contingency tables is to apply the χ^2 statistic to each cell of the table. One should probably use the χ^2 approach, unless you have a special reason. The most common reason to avoid χ^2 is because you have small expectation values.

	Class1	Class2	Total
Sample1	a	b	a+b
Sample2	c	d	c+d
Total	a+c	b+d	n

2x2 Fisher Exact Test statistic= $\sum p = \frac{(a+b)!(c+d)!(a+c)!(b+d)!}{n!}\frac{1}{\sum a!b!c!d!}$

1: Fisher Exact test (rxc tables)

Let there exist two such variables X and Y, with m and n observed states, respectively. Now form an $m \times n$ matrix in which the entries a_{ij} represent the number of observations in which $x = i$ and $y = j$. Calculate the row and column sums R_i and C_j, respectively, and the total sum

$$N = \sum_i R_i = \sum_j C_j$$

of the matrix. Then calculate the conditional probability of getting the actual matrix given the particular row and column sums, given by -

$$P_{\text{cutoff}} = \frac{(R_1!\,R_2!\cdots R_m!)\,(C_1!\,C_2!\cdots C_n!)}{N!\,\prod_{i,j} a_{ij}!},$$

which is a multivariate generalization of the hyper-geometric probability function.

5. Student t test (Two tailed, independent)

Assumptions: Subjects are randomly assigned to one of two groups. The distributions of the means being compared are normal with equal variances.

Test: The hypotheses for the comparison of two independent groups are:

H_o: $u_1 = u_2$ (means of the two groups are equal)

H_a: u_1 u_2 (means of the two group are not equal)

The test statistic for is t, with $n_1 + n_2 - 2$ degrees of freedom, where n_1 and n_2 are the sample sizes for groups 1 and 2. A low p-value for this test (less than 0.05 for example) means that there is evidence to reject the null hypothesis in favour of the alternative hypothesis or there is evidence that the difference in the two means is statistically significant.

The test statistic is as follows:

t-Test: Two-Sample Assuming Equal Variances

$$S_p = \sqrt{\frac{(n_1-1)S_1^2 + (n_2-1)S_2^2}{n_1+n_2-2}}$$

In all work with two-sample t-test the degrees of freedom or df is:

$$df = n_1 + n_2 - 2$$

The formula for the two sample t-test is:

$$T = \frac{\overline{X} - \overline{Y}}{S_p\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

Pre-test: Test for variance assumption: A test of the equality of variance is used to test the assumption of equal variances. The test statistic is F with n_1-1 and n_2-1 degrees of freedom.

t-Test: Two-Sample Assuming Unequal Variances

$$T = \frac{\overline{X} - \overline{Y}}{\sqrt{\frac{S_X^2}{n_1} + \frac{S_Y^2}{n_2}}}$$

Note in this case the Degree of Freedom is measured by

$$df' = \frac{\left(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}\right)^2}{\frac{\left(\frac{s_1^2}{n_1}\right)^2}{n_1 - 1} + \frac{\left(\frac{s_2^2}{n_2}\right)^2}{n_2 - 1}}$$

and round up to integer.

Results of the t-test: If the p-value associated with the t-test is small (< 0.05), there is evidence to reject the null hypothesis in favour of the alternative. In other words, there is evidence that the means are significantly different at the significance level reported by the p-value. If the p-value associated with the t-test is not small (> 0.05), there is not enough evidence to reject the null hypothesis, and you can conclude that there is evidence that the means are not different.

6. Diagnostic statistics (Table 4)

	Disease						
Test	**Present**	**n**		**Absent**	**n**		**Total**
Positive	True Positive	*a*		False Positive	*c*		*a* + *c*
Negative	False Negative	*b*		True Negative	*d*		*b* + *d*
Total		*a* + *b*			*c* + *d*		

The following statistics can be defined:

- Sensitivity: probability that a test result will be positive when the disease is present (true positive rate, expressed as a percentage).

  = a / (a+b)

- Specificity: probability that a test result will be negative when the disease is not present (true negative rate, expressed as a percentage).

  = d / (c+d)

- Positive predictive value: probability that the disease is present when the test is positive (expressed as a percentage).

  = a / (a+c)

- Negative predictive value: probability that the disease is not present when the test is negative (expressed as a percentage).

  = d / (b+d)

- Accuracy is the sum of true positive and true negative divided by number of cases

7. Diagnostic values based on Area under curve

0.9-1.0 Excellent test

0.8-0.9 Good test

0.7-0.8 Fair test

0.6-0.7 Poor test

0.5-0.6 Fail

8. Significant figures

+ Suggestive significance (P value: $0.05<P<0.10$)

* Moderately significant (P value:$0.01<P \leq 0.05$)

** Strongly significant (P value : $P \leq 0.01$)

Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1 , Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data. Microsoft word and Excel have been used to generate graphs, tables**.** [128-132]

Observation and Results

Table 5: Age distribution of patients studied

Age in years	Hypothyroid (Newly detected)		Hypothyroid (On Treatment)		Controls	
	No	%	No	%	No	%
19-20	2	5.4	0	0.0	4	7.4
21-30	9	24.3	10	30.3	7	12.9
31-40	11	29.7	12	36.36	16	29.6
41-50	5	13.5	5	15.15	14	25.9
51-60	5	13.5	3	9.09	6	11.1
>60	5	13.5	3	9.09	7	12.9
Total	37	100.0	33	100.0	54	100.0
Mean ±SD	41.29±16.22		39.24±13.70		43.29±14.16	

Samples are age matched with P = 0.622

Among the 70 hypothyroid patients studied, age distribution showed 32.85% (n=23) were in the age group of 31-40 years, 27.14% (n=19) were in the age group of 21-30 years and 11.42% (n=8) were above 60 years of age. The mean age (in years) was found to be 40.33±15.01 among hypothyroid patients.

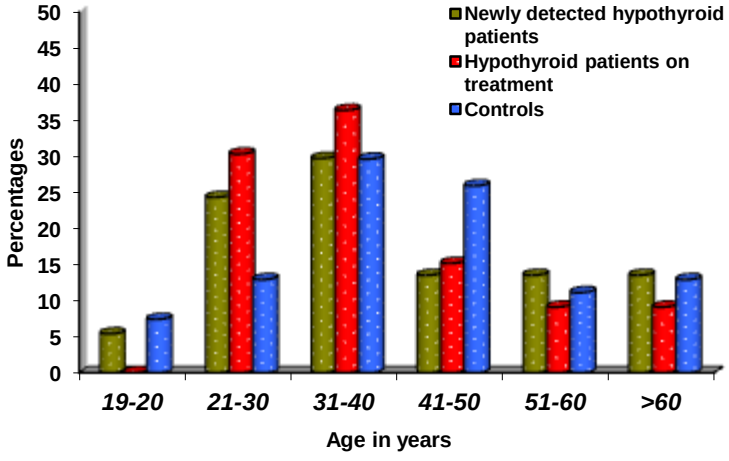

Figure 3: Age distribution of patients studied

Table 6: Gender distribution of patients studied

Gender	Hypothyroid (Newly detected)		Hypothyroid (on treatment)		Controls	
	No	%	No	%	No	%
Male	3	8.1	1	3.0	4	7.4
Female	34	91.9	32	97.0	50	92.6
Total	37	100.0	33	100.0	54	100.0

Samples are gender matched with p=0.704

Gender distribution showed 5.7% (n=4) were males and 94.3% (n= 66) were females among the hypothyroid patients (Table 6) resulting in male: female ratio of 1:16.5.

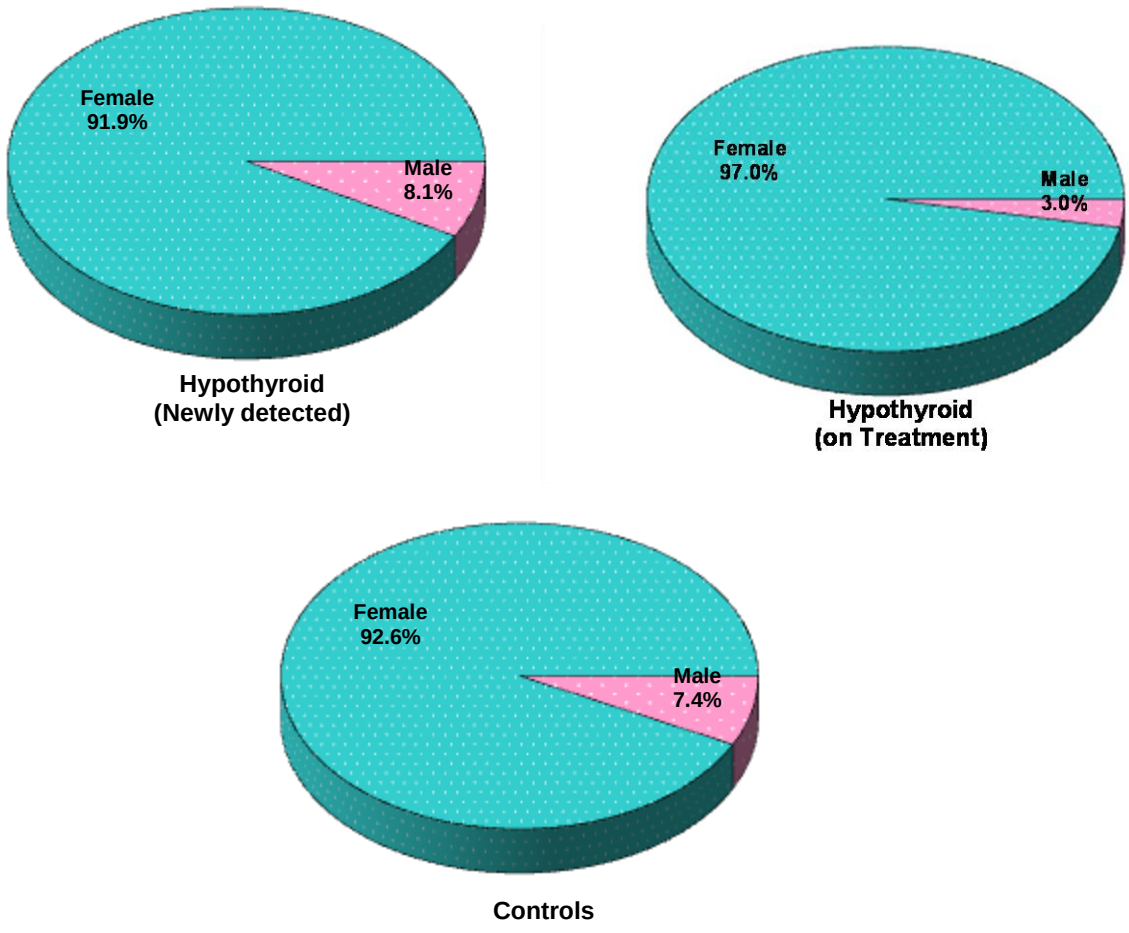

Figure 4: Gender distribution of patients studied

Table 7: Thyroxine supplementation in the hypothyroid group (on treatment)

Treatment (Thyroxine in micrograms)	Number of patients	%
<50	14	42.4
>50	19	57.5
Total	33	100.0

In the study group, 57.5% of patients on treatment were found to be on a daily thyroxine supplementation of more than 50 micrograms and were on a duration of treatment for 1-5 years.

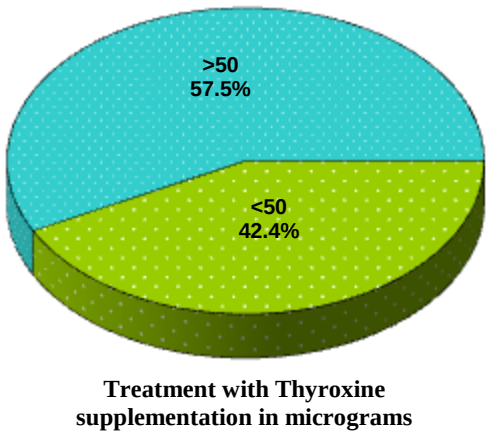

Figure 5: Thyroxine supplementation in the hypothyroid group (on treatment)

Table 8: Hypothyroid group on treatment with thyroxine (in years)

On Treatment (years)	Number of patients	%
< 1 year	9	27.2
1-5 years	19	57.5
>5 years	5	15.1
Total	33	100.0

In the study group of hypothyroid patients on treatment, 15.1% (n=5) were on thyroxine supplementation for greater than 5 years. Majority of the patients (57.5%) were receiving treatment for 1-5 years.

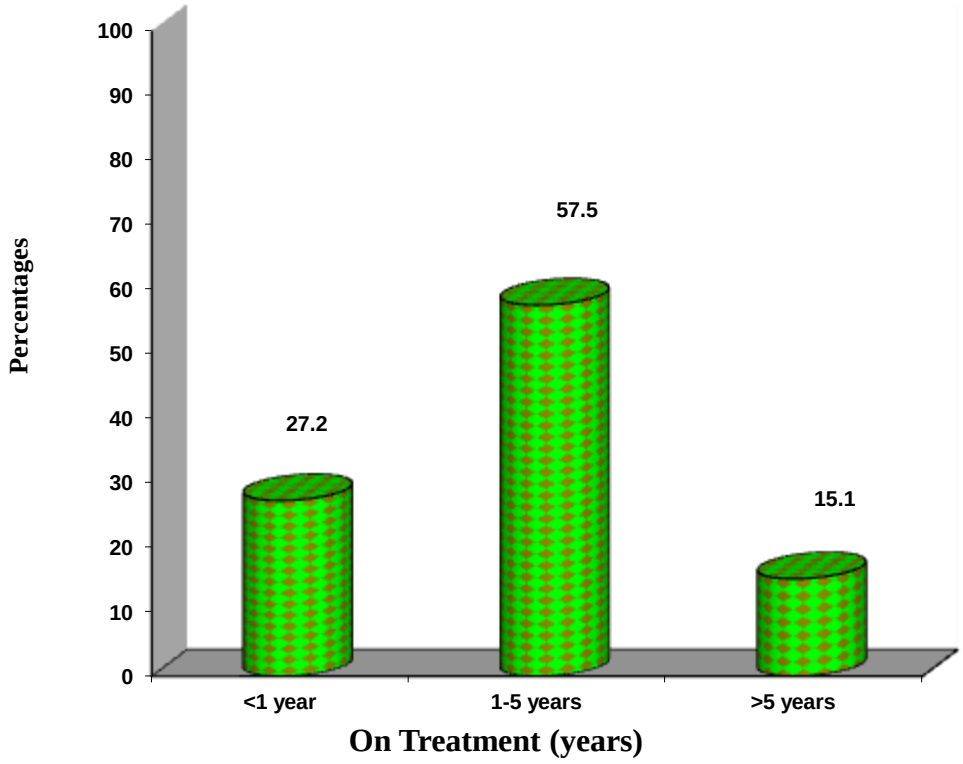

Figure 6: Hypothyroid group on treatment with thyroxine (in years)

Table 5: Comparison of study parameters of three groups of studied

Variables	Newly detected	Hypothyroid	Controls	P value
TSH(mcLU/ml)	52.22±41.62	14.02±22.18	2.23±1.44	<0.001**
T4 (mcg/dl)	5.23±3.11	8.22±3.07	8.86±2.04	<0.001**
T3(ng/ml)	0.93±0.53	1.38±1.10	1.14±0.21	0.024*

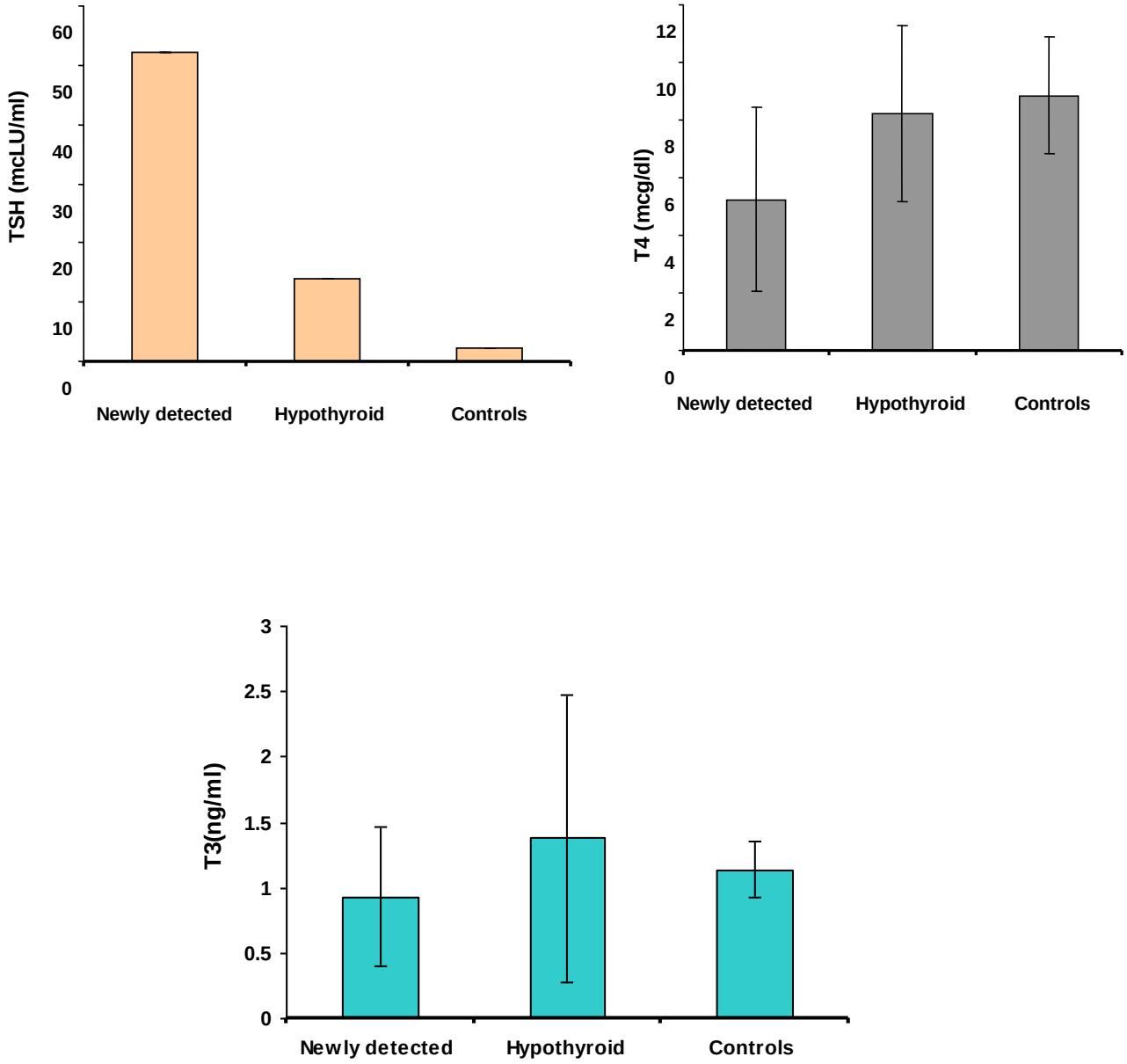

Figure 7: Comparison of study parameters among the three study groups.

Table 10: Thyroid parameters studied among the three study groups

Thyroid parameters	Hypothyroid (Newly detected)		Hypothyroid (on treatment)		Controls	
	No	**%**	**No**	**%**	**No**	**%**
TSH (mcLU/ml)	**(n=37)**		**(n=33)**		**(n=54)**	
• <0.5	0	0.0	1	3.0	2	3.7
• 0.5-4.7	0	0.0	8	24.2	48	88.9
• >4.7	37	100.0	24	72.7	4	7.4
T4 (mcg/dl)	**(n=37)**		**(n=31)**		**(n=54)**	
• <5.4	20	54.0	4	12.9	3	5.5
• 5.4-11.7	17	45.9	23	74.2	47	87.0
• >11.7	0	0.0	4	12.9	4	7.4
T3(ng/ml)	**(n=33)**		**(n=30)**		**(n=54)**	
• <2.3	32	96.9	27	90.0	54	100.0
• 2.3-4.2	1	3.1	1	3.3	0	0.0
• >4.2	0	0.0	2	6.6	0	0.0

Comparison of study parameters (Table 9 and Table 10) including TSH, T4, T3 among the study groups were found to be statistically significant. All newly detected hypothyroid patients (n=37) had a TSH level greater than 4.7 mcLU/ml. Mean TSH level was 52.22±41.62. 54% of patients (n=20) had a T4 level less than 5.4 microgram/dl. Mean T4 level was 5.23±3.11.

Among the study group of hypothyroid patients on treatment, 72.7% (n=24) had a TSH level greater than 4.7 mcLU/dl. Mean TSH level was 14.02±22.18. 74.2% of patients (n=23) had a T4 level in the range 5.4-11.7 microgram/dl. Mean T4 level was 8.22±3.07.

In the control group 88.9% of patients (n=48) had a TSH level in the range 0.5-4.7mcLU/ml. Mean TSH level was 2.23±1.44. 87% of the patients (n=47) had a T4 level in the range 5.4-11.7 microgram/dl. Mean T4 level was 8.86±2.04.

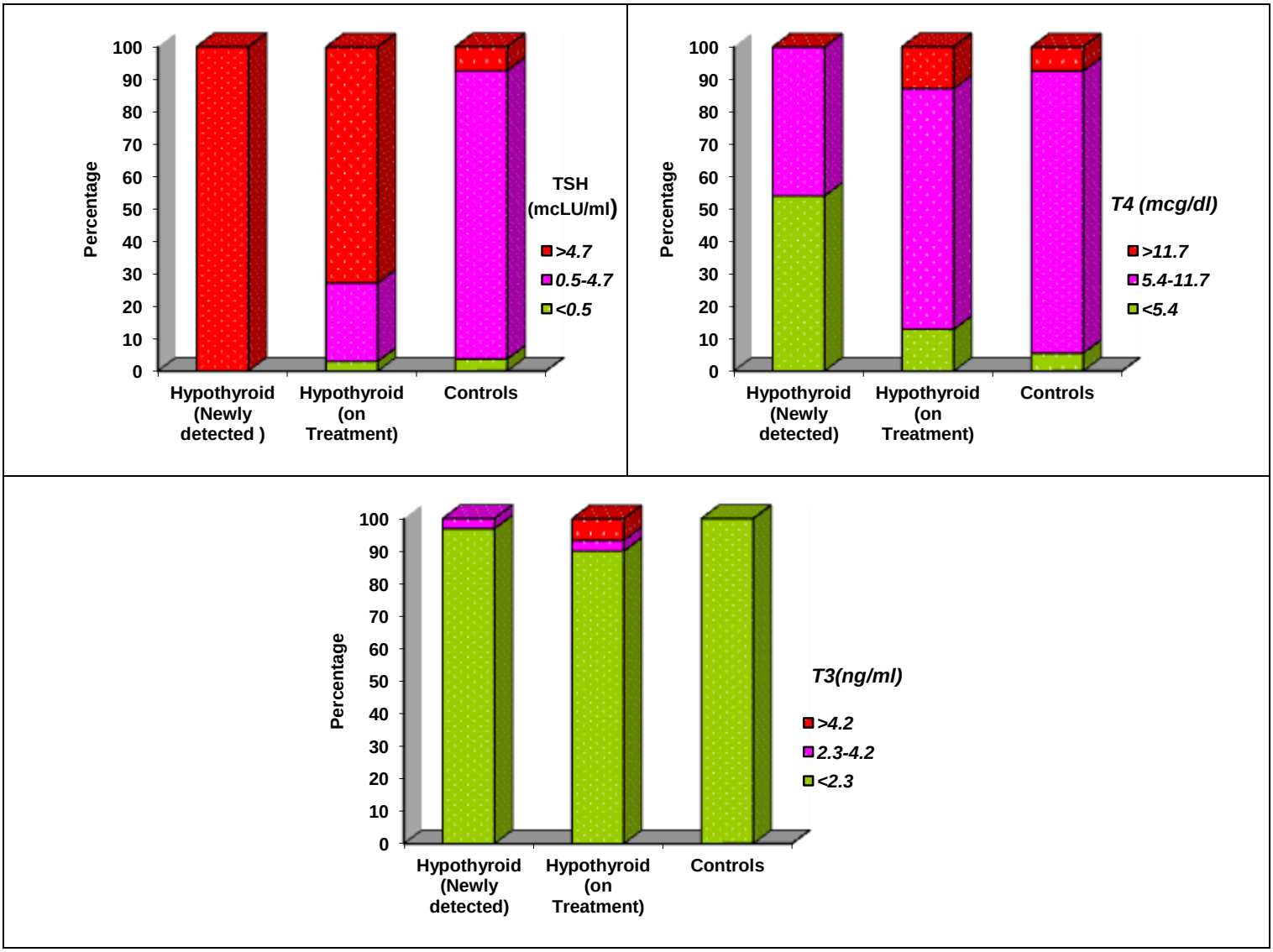

Figure 8: Thyroid parameters among the study groups

Table 11: Pseudocholinesterase (U/L) levels among the study groups

Pseudocholinesterase (U/L)	Hypothyroid (Newly detected)		Hypothyroid (on treatment)		Controls	
	No	%	No	%	No	%
<1000	2	5.4	0	0.0	0	0.0
1000-2000	11	29.7	0	0.0	0	0.0
2000-4000	23	62.1	8	24.2	0	0.0
4000-6000	1	2.7	18	54.5	34	62.9
6000-8000	0	0.0	5	15.1	17	31.4
>8000	0	0.0	2	6.0	3	5.6
Total	37	100.0	33	100.0	54	100.0
Mean ±SD	3344.18±1304.56		6103.67±1511.77		6753.20±1173.58	

The mean Pseudocholinesterase (U/L) level is significantly less in newly detected hypothyroidism cases with a mean activity of 3344.18 ± 1304.56(P <0.001**).There is a 50.48% decrease in mean level of pseudocholinesterase in the group as compared with random normal group. A 9.62% decrease in mean pseudocholinesterase activity was observed among hypothyroid patients on treatment as compared with random normal group.

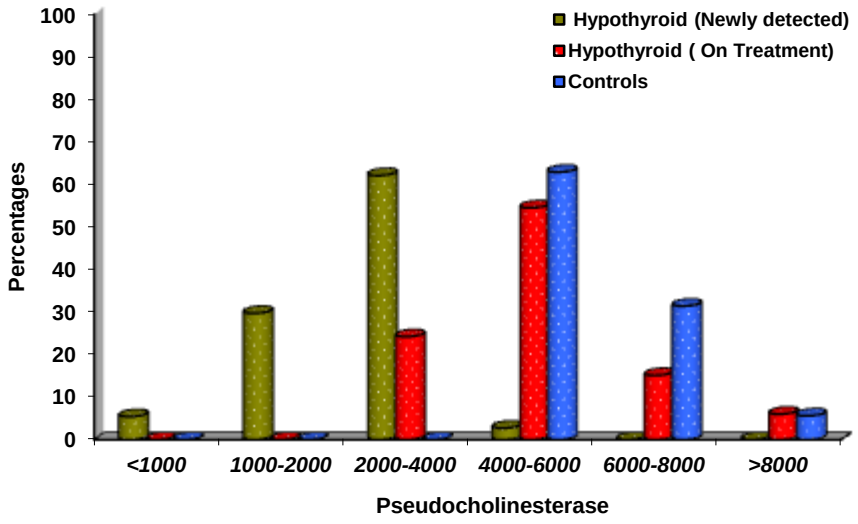

Figure 9: Pseudocholinesterase (U/L) levels among the study groups

Table 12: Frequency of Symptoms among the hypothyroid patients

Symptoms	Hypothyroid (Newly detected) (n=37)		Hypothyroid (On Treatment) (n=33)	
	No	%	No	%
1.Tiredness & weakness	37	100.0	12	36.3
2.Dry skin	33	89.1	6	18.1
3.Feeling cold	26	70.2	4	12.1
4.Hair loss	32	86.5	5	15.1
5.Difficulty in concentration & poor memory	10	27.0	1	3.0
6.Constipation	18	48.6	3	9.0
7.Weight gain with poor appetite	17	45.9	2	6.0
8.Dyspnea	1	2.7	0	0.0
9.Hoarse voice	8	21.6	1	3.0
10.Menorrhagia	5	13.5	0	0.0
11.Parasthesia	4	10.8	0	0.0
12.Impaired hearing	-	-	-	-

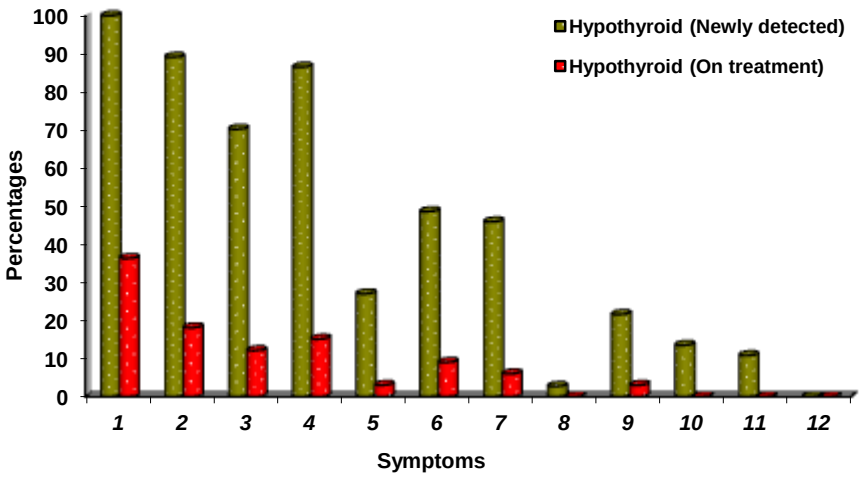

Figure 10: Frequency of Symptoms among the hypothyroid patients

The most common symptoms among newly detected hypothyroid patients (Table 12 and Figure 10) were tiredness and weakness (100%), Dry skin (89%) and hair loss (86.5%). This is followed by cold intolerance (70.2%), constipation (48.6%) and decreased appetite (45.9%).

Table 13: Frequency of Signs among the hypothyroid patients

Signs	Hypothyroid (Newly detected) (n=37)		Hypothyroid (on treatment) (n=33)	
	No	%	No	%
1.Dry coarse skin	32	86.4	5	15.1
2.Puffy face, hands & feet	10	27.0	2	6.0
3.Diffuse alopecia	18	48.6	2	6.0
4.Bradycardia	-	-	-	-
5.Peripheral oedema	3	8.1	0	0.0
6.Delayed tendon reflex relaxation	11	29.7	1	3.0
7.Carpal tunnel syndrome	-	-	-	-
8.Serous cavity effusions	-	-	-	-

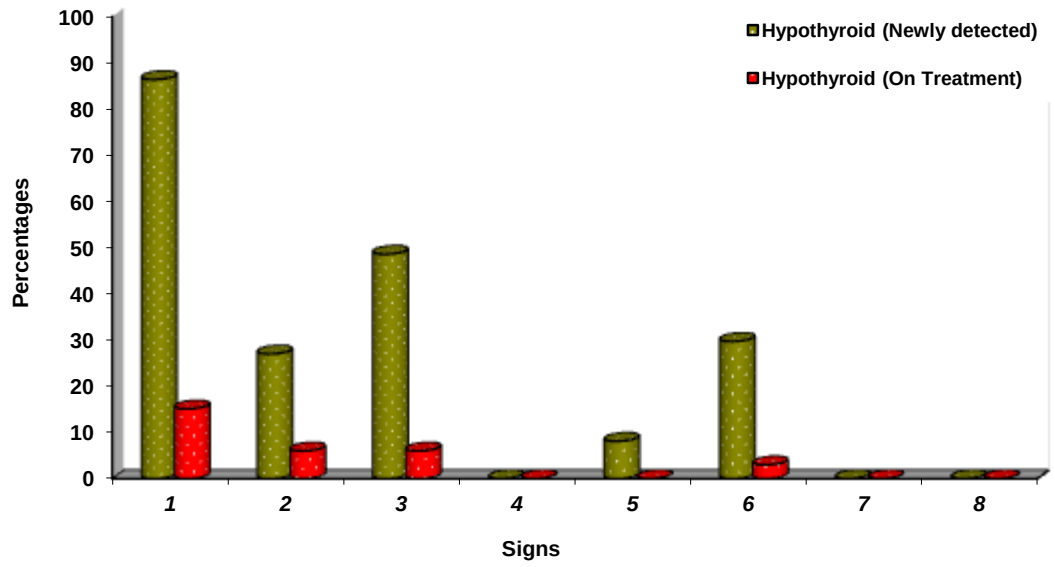

Figure 11: Frequency of Signs among the hypothyroid patients

The major signs on examination among the newly detected hypothyroid patients were dry coarse skin (86.4%), diffuse alopecia (48.6%), delayed tendon reflex relaxation (29.7%) and puffy face, hand and feet (27%).

Table 14: Comparison of study variables in cases and controls

Variables	Cases (n=70)	Controls (n=54)	P value
Age in years	40.33±15.01	43.29±14.16	0.265
Male	4(5.7%)	4(7.4%)	0.704
Female	66(94.3%)	50(92.6%)	
TSH (mcLU/ml)	24.45±9.91	2.01±0.23	<0.001**
T4 (mcg/dl)	6.63±3.45	8.86±2.04	<0.001**
T3(ng/ml)	1.14±0.87	1.15±0.22	0.984
Pseudocholinesterase (U/L)	4645.08±1967.9	6753.20±1173.6	<0.001**

Among the 70 hypothyroid patients included in the study, a 31.21% decrease in mean pseudocholinesterase level was observed as compared with random normal group. Mean level of Pseudocholinesterase in hypothyroid patients was 4645.08±1967.9 (P value <0.001**).

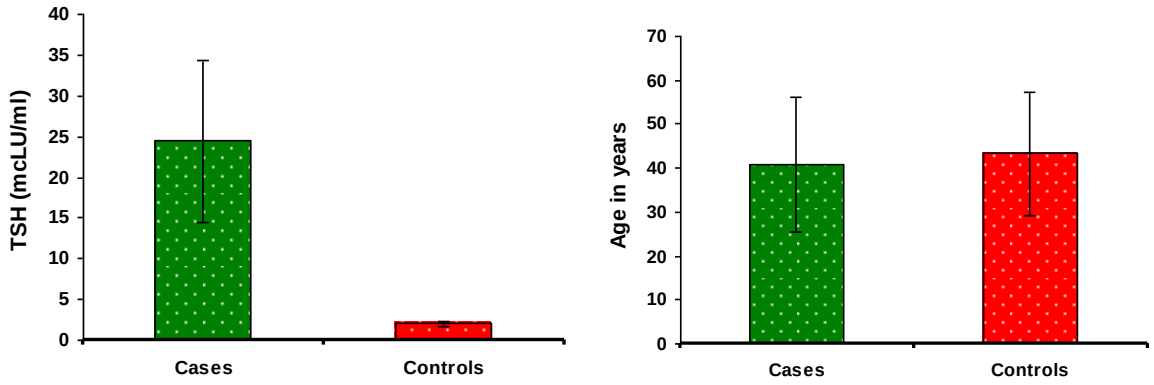

Figure 12: Comparison of study variables in cases and controls

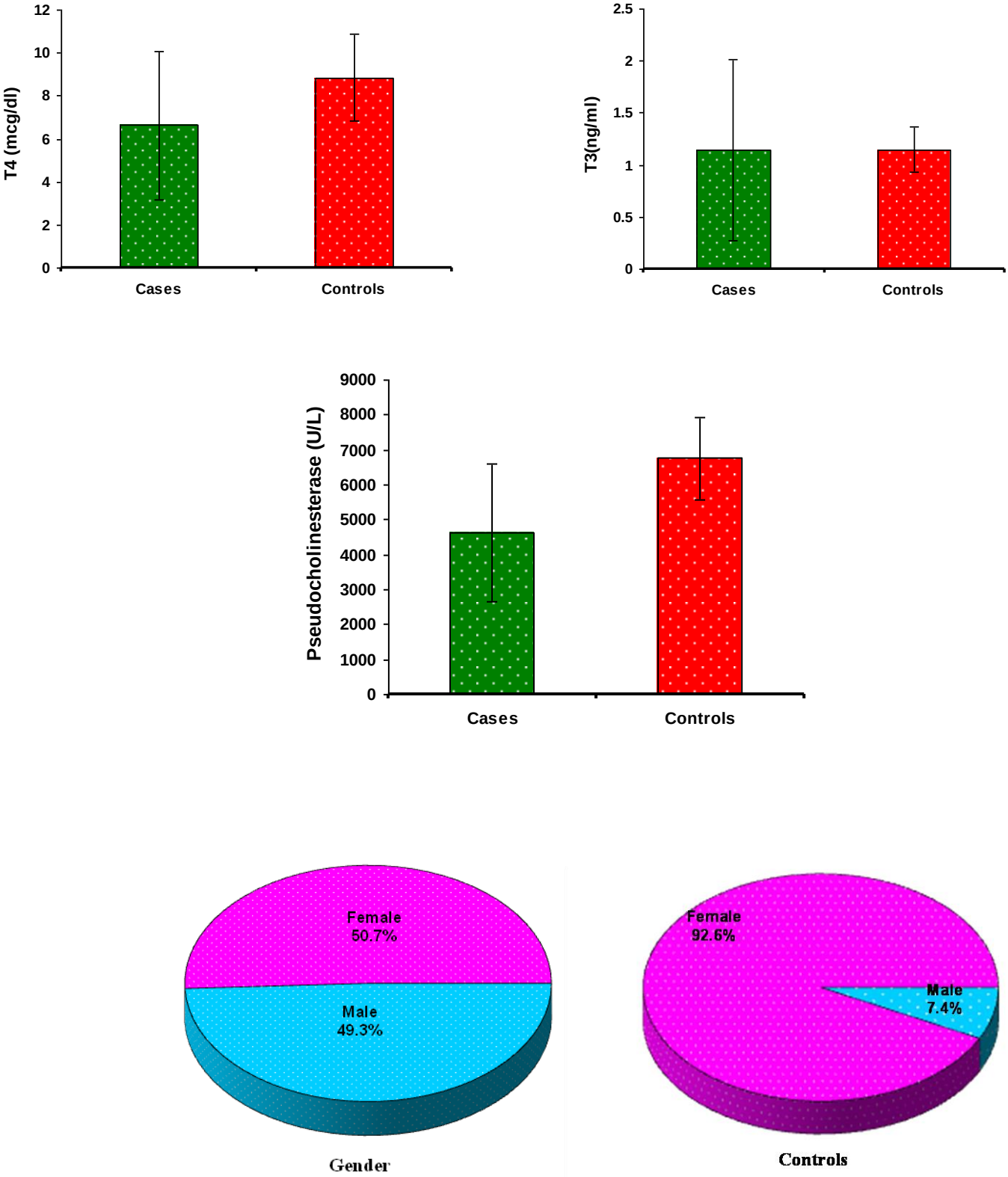

Figure 13: Comparison of study variables in cases and controls

Table 15: ROC curve analysis to predict Pseudocholinesterase (U/L) as a diagnostic marker for Hypothyroid cases.

	Cut-off	Sensitivity	Specificity	LR+	LR-	AUC	P value
Pseudocholinesterase (U/L)	<5290	71.43	94.44	12.88	0.30	0.837	<0.001**

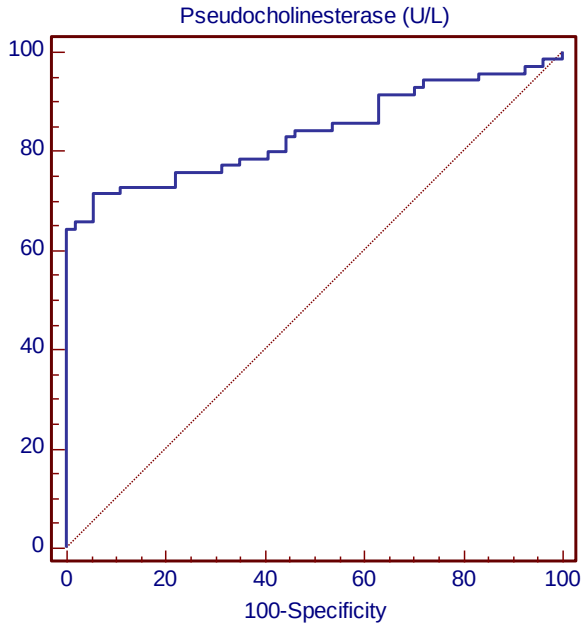

Figure 14: ROC curve analysis to predict Pseudocholinesterase (U/L) as a diagnostic marker for Hypothyroid cases.

Pseudocholinesterase (U/L) is a diagnostic marker with an area under ROC curve of 0.837, indicating that it is a good marker with moderate sensitivity (71.43%) and high specificity (94.44%).

Table 16: Evaluation of Pseudocholinesterase (U/L) as diagnostic and prognostic marker for Hypothyroid cases

Pseudocholinesterase (U/L)	Hypothyroid (Newly detected)	Hypothyroid (on treatment)	Controls
Min-max	558.00-5113.0	4135.0-10381.00	5104.00-9625.00
Mean ± SD	3344.68±1304.57	6103.67±1511.77	6753.21±1173.58
95%CI	2909.2-3779.15	5567.63-6639.72	6432.88-7073.53
Inference	Pseudocholinesterase (U/L) is significantly less in cases not on treatment with F=78.264, P<0.001**, However the cases on treatment are more close to controls with P=0.100 indicating this as a good prognostic factor		

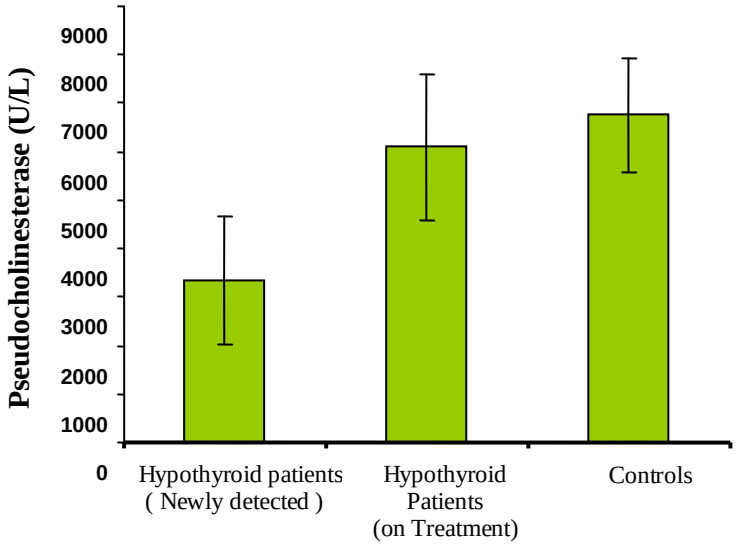

Figure 15: Evaluation of Pseudocholinesterase (U/L) as diagnostic and prognostic marker for Hypothyroid cases

Table 17: Pearson correlation of Pseudocholinesterase (U/L) with thyroid parameters

	Hypothyroid (Newly detected)		Hypothyroid (On treatment)		Controls	
	r value	p value	r value	p value	r value	p value
Pseudocholinesterase vs TSH	0.016	0.925	-0.436	0.011*	0.164	0.237
Pseudocholinesterase vs T4	-0.119	0.498	0.219	0.236	-0.196	0.157
Pseudocholinesterase vs T3	0.101	0.575	-0.005	0.977	0.309	0.023*

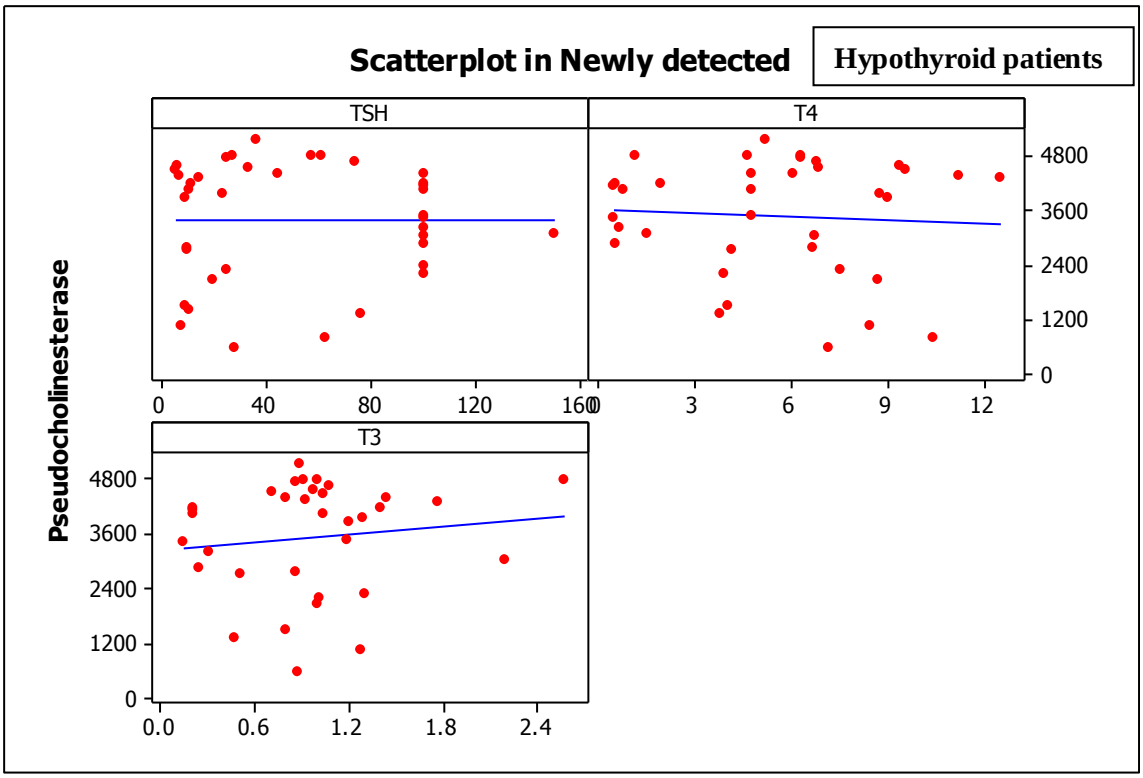

Figure 16: Scatter plot: Pseudocholinesterase with thyroid parameters in newly detected hypothyroid patients.

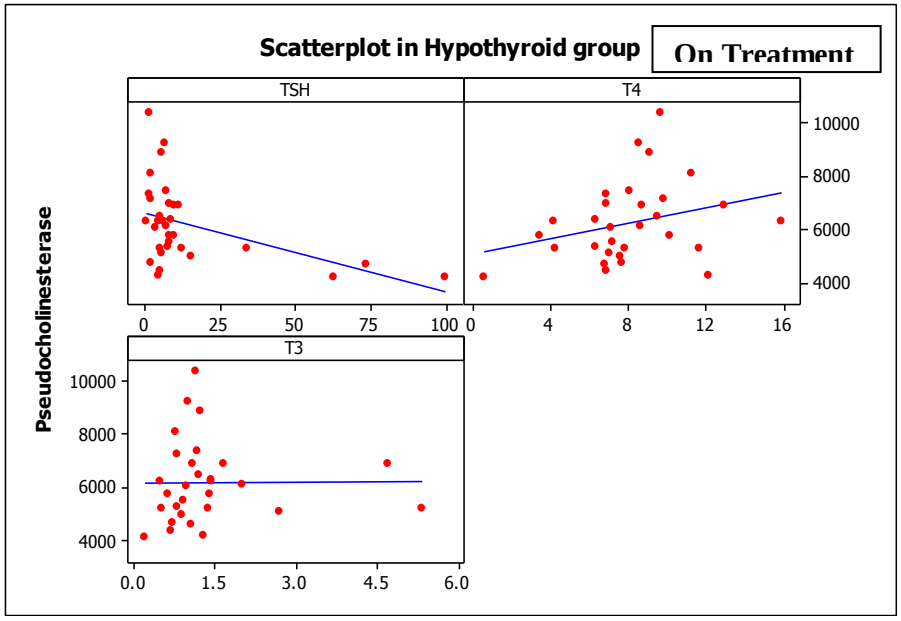

Figure 17: Scatter plot: Pseudocholinesterase with thyroid parameters in Hypothyroid patients on treatment.

There is convincing evidence from the present study showing that there is a significant negative correlation (**r value -0.436; p value 0.011*)** between pseudocholinesterase and TSH in hypothyroid patients on treatment.

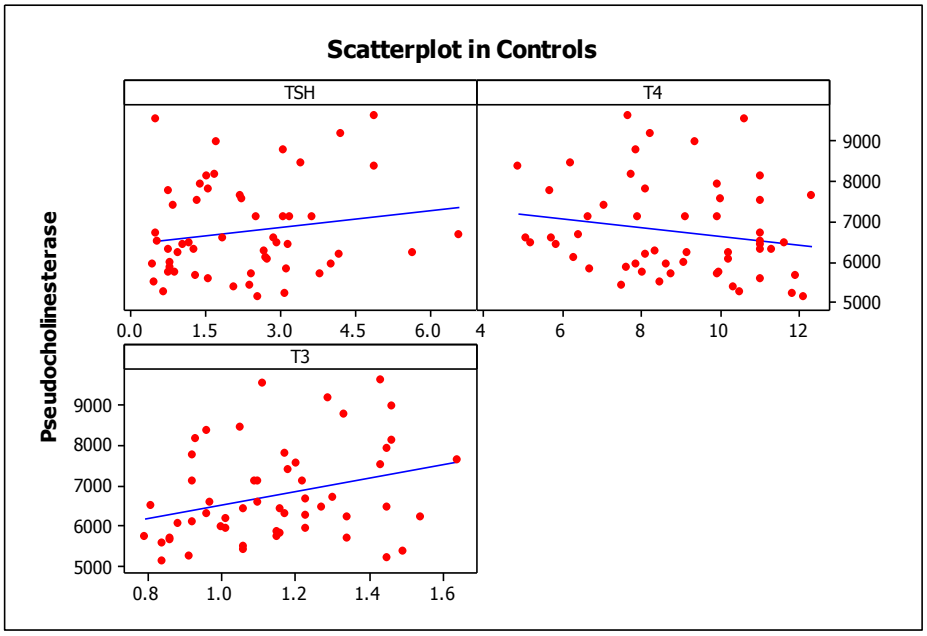

Figure 18: Scatter plot in Controls (Pseudocholinesterase with thyroid parameters)

DISCUSSION

Amidst few studies on Pseudocholinesterase activity in Hypothyroidism, the present comparative case control study tried to determine the same in hypothyroid patients attending a tertiary care teaching hospital in Kolar.

In the present study 37 newly detected hypothyroid patients had a 50.48% decrease in mean activity for pseudocholinesterase level than that found in the random normal group. When all hypothyroid patients (both newly detected and on treatment groups) were taken into consideration, a 31.21% decrease in mean activity for pseudocholinesterase level was found as compared with the random normal group. The Mean Pseudocholinesterase (U/L) is significantly less in newly detected hypothyroidism cases with a mean activity of 3344.18±1304.56 U/L (P <0.001**). As compared to a previous study by Thompson J.C et al, 12 patients with myxoedema had a 30 % decrease in mean activity for pseudocholinesterase level than that found in random normals. Even when myxoedema is not associated with a subnormal esterase activity, a rise in activity does occur when the patient becomes euthyroid. Thompson J.C et al found that treatment restores the esterase level to the average mean activity of random normal as the patient becomes euthyroid. There was no evidence in their investigation that any of the pseudocholinesterase variants modified the conclusions.[38] In the present study, we found that treatment with thyroxine supplementation restored the esterase level approximately near to the average mean activity of random normal as the patient became euthyroid.

Vlaicu R et al showed that decreased levels of Serum cholesterol and beta-lipoproteins occurring in hyperthyroidism were found to be accompanied by an enhanced activity of pseudocholinesterase, while in patients with myxedema, the pathologically increased levels of serum cholesterol and beta-lipoproteins were associated with diminished serum

pseudocholinesterase activity. The behavior of cholesterol and pseudocholinesterase activity and especially the ratio between these parameters might be used for the diagnosis of thyroid disease and for the control of therapeutic efficiency.[39] In the present study, pseudocholinesterase is found to be a diagnostic marker with an area under ROC curve of 0.837, indicating that it is a good marker with moderate sensitivity (71.43%) and high specificity (94.44%). Pseudocholinesterase is significantly less in cases not on treatment with F=78.264, P<0.001**, however the cases on treatment are more close to controls with P=0.100 indicating this as a good prognostic factor. In addition there is convincing evidence from our study showing that there is a significant negative correlation (r value -0.436; p value 0.011*) between pseudocholinesterase and TSH in hypothyroid patients on treatment.

Comparison of study parameters including TSH, T4, T3 and pseudocholinesterase among the study groups were found to be statistically significant (<0.001**,<0.001**,0.024* respectively). Among the 70 hypothyroid patients studied, age distribution showed 32.85% (n=23) were between the age of 31-40 years. Gender distribution showed 5.7% of hypothyroid patients were males and 94.3% were females when both groups were considered together. 57.5% of patients on treatment were found to be on more than 50 micrograms of thyroxine and were on a duration of treatment for 1-5 years.

The most common symptoms in the present study among hypothyroid patients were tiredness & weakness (70%), dry skin (55.71%) and hair loss (52.85%). The most common signs were dry coarse skin (52.8%), alopecia (28.57%) and puffy face, hands and feet (17.14%). In another study done by Larsen PR et al showed that of the symptoms, the most common were weakness (99%), dry skin (97%) and lethargy (91%). While the most common signs in their study were eyelid edema (90%), facial edema (79%) and coarse hair (76%) [28].

CONCLUSION

In this comparative case control study, the correlation between pseudocholinesterase and TSH activity in hypothyroidism was examined. There is convincing evidence from the present study showing that there is a negative correlation between pseudocholinesterase and TSH in hypothyroid patients who are on treatment. There is a mean decrease in pseudocholinesterase level among hypothyroid patients with a cut off level of less than 5290 U/L. The results from this study suggest that pseudocholinesterase can be used both as a diagnostic and prognostic indicator in hypothyroidism.

SUMMARY

This dissertation was conducted on 70 hypothyroid patients, with an objective to study Pseudocholinesterase as a diagnostic and prognostic indicator in hypothyroidism with special reference to pseudocholinesterase activity in hypothyroidism.

The summary of the study is given below:

Majority of the patients were in the age group of 31 to 40 years.

Mean age of patients was 40.33 ± 15.01 years.

The number of females in the study was 16.5 times the number of males with male to female ratio 1:16.5.

Most common symptoms were in the present study among hypothyroid patients were tiredness & weakness (70%), dry skin (55.71%) and hair loss (52.85%). The most common signs were dry coarse skin (52.8%), alopecia (28.57%) and puffy face, hands and feet (17.14%).

The mean Pseudocholinesterase (U/L) level is significantly less in newly detected hypothyroidism cases with a mean activity of 3344.18 ± 1304.56 U/L (P <0.001**).

There is a 50.48% decrease in mean level of pseudocholinesterase in the group of newly detected hypothyroid patients as compared with the random normal group.

A 9.62% decrease in mean pseudocholinesterase activity was observed among hypothyroid patients on treatment as compared with random normal group.

Among the 70 hypothyroid patients included in the study, a 31.21% decrease in mean pseudocholinesterase level was observed as compared with random normal group. Mean level of pseudocholinesterase was 4645.08 ± 1967.9 U/L (P value <0.001**).

There is convincing evidence from the present study showing that there is a significant negative correlation (**r value -0.436; p value 0.011*)** between pseudocholinesterase and TSH in hypothyroid patients on treatment.

Pseudocholinesterase is a diagnostic marker with an area under ROC curve of 0.837, indicating that it is a good marker with moderate sensitivity (71.43%) and high specificity (94.44%).

Pseudocholinesterase is significantly less in cases not on treatment with F=78.264, P<0.001**, However the cases on treatment are more close to controls with P=0.100 indicating this as a good prognostic factor.

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PROFORMA

PSEUDOCHOLINESTERASE AS A DIAGNOSTIC AND PROGNOSTIC INDICATOR IN HYPOTHYROIDISM

NAME

AGE

SEX

ADRESSS

OCCUPATION

TELEPHONE NUMBER

PRESENTING COMPLAINTS

Regarding Hypothyroidism

Puffiness of face

Peripheral Edema

Generalized Weakness and Lethargy

Hoarseness of Voice

Intolerance to cold

Decreased appetite

Weight Gain

Dyspnea

Constipation

Reduced Urine Output

Dryness of Skin

Falling of Hairs

Palpitation

Deafness

Menorrhagia

PAST MEDICAL/ SURGICAL HISTORY

Known Case of Hypothyroidism

On treatment or not

Any head and neck surgeries

MENSTRUAL HISTORY

Menstrual cycles – Regular

Irregular

Duration

Quantity

FAMILY HISTORY

Any history of medical illness in family

TREATMENT HISTORY

PERSONAL HISTORY

ALCOHOL INTAKE

GENERAL EXAMINATION:

Appearance

Built

Nourishment

Pallor

Icterus

Clubbing

Pedal edema

Lymphadenopathy

Hair texture- normal/ dry

Hair loss – present/ Absent

Skin- Rough/Dry/ Scaly

Signs of Hyperlipidemia – Xanthelessma

Xanthoma

Arcus senilis

THYROID SWELLING Present/ Absent

HEART RATE

BLOOD PRESSURE

RR-

Temp-

SYSTEMIC EXAMINATION

NERVOUS SYSTEM-

Higher Mental Function

Level of Consciousness

Orientation

Impairment of memory

Slowing of Intellectual Functions

Cranial Nerves Examination

Motor System

		Right	Left
Tone	Upper limb		
	Lower Limb		
Power	Upper limb		
	Lower Limb		
Deep Tendon Reflexes			
	Upper limb		
	Lower limb		

CVS: JVP S1 S2 S3 S4 PERICARDIAL RUB

RESPIRATORY SYSTEM:

Abdomen :

Liver Span Shifting Dullness

INVESTIGATIONS

1. Serum Pseudocholinesterase

2. Thyroid function test

   TSH: T4: T3:

3. Hb

4. Blood Urea

5. AST: ALT: Serum Bilirubin : ALP:

6. Stool occult blood:

7. ECG

Treatment

Signature of Guide Signature of Candidate

KEY TO MASTER CHART

SL.NO	SERIAL NUMBER
MRD	MEDICAL RECORDS DEPARTMENT NUMBER
PF	PUFFINESS OF FACE, HANDS AND FEET
DS	DRYNESS OF SKIN
L	LETHARGY
DA	DECREASED APPETITE
HL	HAIR LOSS
H/O	HISTORY OF
H	HYPOTHYROIDISM
TH	THYROXINE SUPPLEMENTATION
NS	NECK SURGERY
PA	PALLOR
O	OEDEMA
SC	SKIN CHANGES
HC	HAIR CHANGES
G	GOITER
SH	SIGNS OF HYPERLIPIDEMIA
PU	PULSE
PS	PSEUDOCHOLINESTERASE (U/L)
BILI	BILIRUBIN
BU	BLOOD UREA
OB	STOOL OCCULT BLOOD
Hb	HEMOGLOBIN
N	NEGATIVE
P	PRESENT
A	ABSENT

MASTER CHART: HYPOTHYROID CASES

SL.NO	AGE	sex	MRD	Symptomatology					Past H/O			General Physical Examination								THYROID FUNCTION TEST			PSEUDO							
				PF	DS	L	DA	HL	H	T	NS	P	O	SC	HC	G	SH	Pu	BP	TSH	T4	T3	PS	AST	ALT	ALP	Bili	BU	Hb	OB	ECG
1	50	F	674514	P	P	P	P	P	A	A	A	P	P	P	P	A	A	62	100/50	100	3.89	1.01	2175	17	10	98	0.96	27	10.7	N	LVC
2	25	F	689860	A	P	P	A	P	A	A	A	-	P	A	A	P	A	82	120/70	62.36	10.41		768	26	18	110	0.82	18	13.2	N	NORMAL
3	36	F	690550	P	P	P	A	P	A	A	A	P	A	P	P	A	A	80	140/90	27.53	7.12	0.87	558	31	40	117	0.43	11	10.4	N	NORMAL
4	65	F	688498	P	P	P	P	P	A	A	A	P	P	P	P	A	A	92	140/90	9.15	4	0.8	1478	13	27	284	0.4	30	8.6	N	NORMAL
5	37	M	617508	A	P	P	P	P	A	A	A	-	A	P	P	A	A	84	120/80	100	0.44	0.2	4139	20	24	110	1	26	12	N	LVC
6	21	F	772568	A	P	P	A	P	A	A	A	-	A	P	A	A	A	84	110/80	24.3	7.49	1.3	2270	20	28	94	0.7	22	12	N	NORMAL
7	20	F	678589	A	A	P	P	A	A	A	A	-	A	A	A	A	A	84	110/80	10.58	4.75	1.03	4050	22	26	116	0.8	24	12	N	NORMAL
8	38	F	610991	P	P	P	P	A	A	A	A	P	A	P	P	A	A	82	120/70	19.31	8.65	1	2036	18	20	90	0.8	16	10.1	N	NORMAL
9	35	F	767242	A	P	P	A	P	A	A	A	-	A	P	A	A	A	80	120/70	150	1.5	52NG	3065	18	24	96	0.84	26	13	N	LVC
10	50	F	625892	A	P	P	P	P	A	A	A	-	A	P	P	A	A	74	120/80	9.54	4.13	0.5	2727	28	34	92	0.82	26	11	N	NORMAL
11	40	F	710910	A	P	P	A	P	A	A	A	-	A	P	P	A	A	82	130/90	100	0.77	0.2	4038	44	32	74	0.48	31	11	N	LVC
12	50	F	717238	A	P	P	A	P	A	A	A	-	A	P	A	A	A	80	120/82	35.91	5.21	0.89	5113	22	35	100	0.64	24	12	N	NORMAL
13	65	M	634201	A		P	A	A	A	A	A	-	A	P	A	A	A	84	120/76	6.5	11.17	0.92	4340	30	34	110	1	30	12	N	NORMAL
14	25	F	713390	A	P	P	A	P	A	A	A	P	A	P	A	A	A	74	110/70	10.96	1.92	1.4	4160	12	16	125	0.6	19	8	N	NORMAL
15	70	F	711158	P	P	P	P	P	A	A	A	P	P	P	P	A	A	82	140/80	9.26	6.67	0.86	2752	35	55	186	0.74	40	10	N	LVC
16	65	F	615727	A	P	P	P	P	A	A	A	-	A	P	A	A	A	76	120/80	100	0.53	0.24	2838	24	30	100	0.8	33	11	N	LVC
17	21	F	690594	A	A	P	A	A	A	A	A	-	A	A	A	P	A	78	130/70	7.35	8.45	1.28	1028	67	30	98	0.93	17	12.6	N	NORMAL
18	33	F	702127	A	A	P	A	A	A	A	A	P	A	A	A	A	A	78	130/80	10.2	5.37		1417	79	29	163	0.68	12	9.3	N	NORMAL
19	35	F	708257	P	P	P	P	P	A	A	A	-	A	P	A	A	A	80	120/80	100	4.74	0.8	4383	37	40	51	1	14	13	N	LVC

SL.NO	AGE	sex	MRD	Symptomatology					Past H/O			General Physical Examination								THYROID FUNCTION TEST			PSEUDO							
				PF	DS	L	DA	HL	H	T	NS	P	O	SC	HC	G	SH	Pu	BP	TSH	T4	T3	PS	AST	ALT	ALP	Bili	BU	Hb	OB	ECG
20	55	F	720147	P	P	P	A	P	A	A	A	P	A	P	P	A	A	90	140/100	76.12	3.76	0.47	1312	54	41	114	0.81	14	9.2	N	NORMAL
21	37	F	773206	A	P	P	P	P	A	A	A	P	A	P	P	A	A	80	110/80	100	0.44	0.138	3406	20	24	124	0.9	12	9.4	N	LVC
22	27	F	745427	A	P	P	A	P	A	A	A	-	A	P	P	A	A	80	114/80	26.65	6.28	0.91	4780	24	20	108	1	24	12	N	NORMAL
23	35	F	660351	A	P	P	A	P	A	A	A	-	A	P	A	A	A	68	130/90	100	6.73	2.2	3030	25	30	118	0.86	24	11	N	LVC
24	60	F	729895	P	P	P	P	P	A	A	A	P	A	P	P	A	A	72	100/60	100	0.65	0.3	3200	26	20	104	0.8	20	10	N	LVC
25	59	F	450034	A	P	P	A	P	A	A	A	-	A	P	A	P	A	76	130/80	24.51	6.3	0.86	4750	24	30	108	1	22	11.4	N	NORMAL
26	53	F	693682	A	P	P	A	P	A	A	A	-	A	P	A	A	A	78	122/82	33.08	6.86	0.71	4530	28	36	160	0.74	28	10	N	NORMAL
27	29	F	783602	A	P	P	A	P	A	A	A	-	A	P	A	A	A	80	120/80	100	5.1		2383	22	24	112	1	20	12	N	LVC
28	21	F	744683	A	P	P	A	P	A	A	A	-	A	P	P	A	A	80	120/84	44.28	6.01	1.44	4384	18	25	170	0.6	24	12	N	NORMAL
29	19	F	751085	A	P	P	P	P	A	A	A	-	A	P	P	A	P	74	100/70	100	4.76	1.19	3450	24	22	110	1	22	11	N	LVC
30	45	F	795090	A	P	P	A	P	A	A	A	-	A	P	P	A	A	80	124/84	60.62	4.61	1	4783	24	28	100	1	24	12	N	LVC
31	33	F	636112	P	P	P	P	P	A	A	A	-	A	P	P	A	A	84	130/70	73.84	6.76	1.07	4652	18	14	84	0.8	20	11	N	NORMAL
32	80	F	706320	A	P	P	A	P	A	A	A	-	A	P	P	A	A	82	130/70	100	0.49	0.2	4145	26	22	116	0.92	18	10	N	LVC
33	25	F	808532	A	P	P	P	P	A	A	A	-	A	P	A	A	A	80	100/70	6	9.33	0.97	4574	26	35	272	0.61	18	15.8	N	LVC
34	24	F	812931	A	P	P	P	P	A	A	A	P	A	P	A	A	A	86	120/70	8.96	8.97	1.2	3844	32	25	206	0.7	25	8.7	N	NORMAL
35	55	F	810847	P	P	P	P	P	A	A	A	-	A	P	P	A	A	82	120/80	56.9	1.12	2.58	4773	22	27	230	0.8	14	10.3	N	LVC
36	50	F	809014	A	P	P	P	P	A	A	A	-	A	P	A	A	A	86	128/74	5.38	9.54	1.04	4449	14	16	84	1	38	11.1	N	NORMAL
37	40	F	811882	A	P	P	A	P	A	A	A	-	A	A	A	A	A	74	130/84	23.2	8.74	1.29	3955	24	18	120	1	22	13.2	N	NORMAL

SL.NO	AGE	sex	MRD	Symptomatology					Past H/O			General Physical Examination								THYROID FUNCTION TEST			PSEUDO							
				PF	DS	L	DA	HL	H	T	NS	P	O	SC	HC	G	SH	Pu	BP	TSH	T4	T3	PS	AST	ALT	ALP	Bili	BU	Hb	OB	ECG
38	25	F	655018	A	A	A	A	A	P	A	A	-	A	A	A	A	A	76	126/74	14.1	11.8	1.77	4308	12	9	166	0.53	28	118	N	NORMAL
39	46	F	684269	A	A	A	A	A	P	A	A	P	A	A	A	A	A	72	114/76	2.06	10.6	0.77	8089	17	34	178	0.66	21	10.6	N	NORMAL
40	60	F	836324	P	P	P	P	A	P	P	A	-	A	P	A	A	A	70	118/88	100	10.9	0.2	4215	19	27	121	0.5	17	10.9	N	LVC
41	21	F	841783	A	P	P	A	P	P	P	A	-	A	P	P	A	A	68	130/86	74	11.4	1.07	4582	21	26	108	0.64	18	11.4	N	NORMAL
42	60	F	720842	A	A	A	A	A	P	P	A	-	A	A	A	A	A	74	124/80	15.45	12	0.88	4967	25	20	104	1	20	12	N	NORMAL
43	34	F	725507	A	A	A	A	A	P	P	A	-	A	A	A	A	A	80	120/80	3.53	11	0.99	6047	28	30	119	0.9	24	11	N	NORMAL
44	50	F	710732	A	P	P	P	A	P	P	A	-	A	P	A	A	A	80	124/90	6.88	12.3	1	9222	30	28	144	1	24	12.3	N	NORMAL
45	67	M	639783	A	P	P	A	P	P	P	A	P	A	P	P	A	A	80	110/80	63	10		4135	24	28	110	0.8	30	10	N	NORMAL
46	30	F	726924	A	A	A	A	A	P	P	A	-	A	A	A	A	A	78	120/80	9.97	12	1.1	6879	26	20	106	1	24	12	N	NORMAL
47	61	F	760452	P	P	P	A	P	P	P	A	-	A	P	A	A	A	84	110/80	33.8	12	0.509	5250	17	32	114	0.8	20	12	N	NORMAL
48	34	F	715494	A	A	A	A	A	P	P	A	-	A	A	A	A	A	78	130/80	5.34	11	5.34	5236	24	20	100	1	24	11	N	NORMAL
49	35	F	660351	A	A	P	A	A	P	P	A	-	A	A	A	A	A	70	120/90	10			5742	25	30	118	0,86	24	11	N	NORMAL
50	50	F	674514	A	A	A	A	A	P	P	A	-	A	A	A	A	A	76	128/78	4.71		1.16	4225	17	10	98	0.96	27	10.7	N	NORMAL
51	32	F	680994	A	A	A	A	A	P	P	A	P	A	A	A	A	A	80	120/70	0.4	10	1.45	6277	28	20	120	1	26	10	N	NORMAL
52	21	F	664396	A	A	P	A	A	P	P	A	P	A	A	A	A	A	80	130/80	11.44	10	4.71	6884	22	36	142	0.9	26	10	N	NORMAL
53	36	F	690550	A	A	A	A	A	P	P	A	P	A	A	A	A	A	72	134/84	5.07			6462	31	40	117	0.43	11	10.4	N	NORMAL
54	45	F	711075	A	A	A	A	A	P	P	A	P	A	A	A	A	A	82	120/76	7.52	10	2.01	6108	18	27	120	0.83	22	10	N	NORMAL
55	35	F	682568	A	A	A	A	A	P	P	A	-	A	A	A	A	A	76	120/70	8.05	12.9	1.68	6920	19	12	82	0.47	20	12.9	N	NORMAL
56	40	F	754724	A	A	P	A	P	P	P	A	-	A	A	A	A	A	84	120/80	8.51	13.9	0.907	5520	31	14	78	1	26	13.9	N	NORMAL

SL.NO	AGE	sex	MRD	Symptomatology					Past H/O			General Physical Examination								THYROID FUNCTION TEST			PSEUDO							
				PF	DS	L	DA	HL	H	T	NS	P	O	SC	HC	G	SH	Pu	BP	TSH	T4	T3	PS	AST	ALT	ALP	Bili	BU	Hb	OB	ECG
57	36	F	799824	A	A	P	A	P	P	P	A	-	A	A	A	A	A	74	120/80	12.5	12	1.37	5250	20	26	124	1	24	12	N	NORMAL
58	50	F	707084	A	A	A	A	A	P	P	A	-	A	A	A	A	A	74	120/80	4.61	12.4	0.492	6244	16	25	122	0.6	24	12.4	N	NORMAL
59	30	F	805748	A	A	A	A	A	P	P	A	P	A	A	A	A	A	76	122/80	5.44	7.2	0.69	4410	30	21	104	0.5	38	7.2	N	NORMAL
60	30	F	811454	A	A	A	A	A	P	P	A	P	A	A	A	A	A	84	120/80	7.8	9.1	0.81	5290	18	27	140	0.55	16	9.1	N	NORMAL
61	65	F	813368	A	A	P	A	A	P	P	A	-	A	A	A	A	A	84	128/80	5.48	11.9	2.7	5095	21	21	90	0.5	6	11.9	N	NORMAL
62	40	F	822038	A	A	A	A	A	P	P	A	-	A	A	A	A	A	88	126/84	5.61		1.25	8892	18	22	100	0.8	18	12	N	NORMAL
63	40	F	720080	A	A	A	A	A	P	P	A	-	A	A	A	A	A	76	128/74	1.83			10381	24	16	98	0.68	20	11.4	N	NORMAL
64	59	F	721883	A	A	A	A	A	P	P	A	-	A	A	A	A	A	78	120/80	7.09	11	1.17	7404	28	30	98	0.9	24	11	N	NORMAL
65	25	F	689860	A	A	A	A	A	P	P	A	-	A	A	A	A	A	80	122/84	1.7			7281	26	18	110	0.82	18	13.2	N	NORMAL
66	21	F	709571	A	A	P	A	A	P	P	A	-	A	A	A	P	A	84	120/84	8.56	10.8	1.44	6308	23	32	162	0.72	32	10.8	N	NORMAL
67	21	F	772568	A	A	A	A	A	P	P	A	-	A	A	A	A	A	76	122/86	8.04			5742	20	28	94	0.7	22	12	N	NORMAL
68	34	F	709822	A	A	P	A	A	P	P	A	-	A	A	A	A	A	82	120/80	6.35	12		6276	20	28	90	1	23	12	N	NORMAL
69	24	F	812931	A	A	A	A	A	P	P	A	P	A	A	A	A	A	82	116/74	2.03			4691	32	25	206	0.7	25	8.7	N	NORMAL
70	38	F	817744	A	A	A	A	A	P	P	A	-	A	A	A	A	A	76	110/70	2.1			7089	20	22	160	0.8	24	12	N	NORMAL

MASTER CHART: CONTROL CASES

SL.NO	AGE	sex	MRD	Symptomatology					Past H/O			General Physical Examination								THYROID FUNCTION TEST			PSEUDO							
				PF	DS	L	DA	HL	H	T	NS	P	O	SC	HC	G	SH	Pu	BP	TSH	T4	T3	P	AST	ALT	ALP	Bili	BU	Hb	OB	ECG
1	30	F	738510	A	A	A	A	A	A	A	A	P	A	A	A	A	A	92	130/80	1.55	8.11	1.17	7804	18	22	202	0.84	24	7.6	N	NORMAL
2	70	F	788693	A	A	A	A	A	A	A	A	-	A	A	A	A	A	82	130/90	4.18	8.11	1.01	6151	21	24	102	0.7	27	11.8	N	NORMAL
3	35	F	787462	A	A	A	A	A	A	A	A	-	A	A	A	A	A	80	110/90	0.77	9.07	1	5951	28	20	114	0.8	10	12.4	N	NORMAL
4	45	F	661693	A	A	A	A	A	A	A	A	-	A	A	A	A	A	76	112/74	4.2	8.21	1.29	9173	20	24	124	0.54	19	13.1	N	NORMAL
5	60	F	761146	A	A	A	A	A	A	A	A	-	A	A	A	A	A	80	110/70	1.82	5.73	0.97	6563	15	22	106	-	18	12	N	NORMAL
6	65	F	808662	A	A	A	A	A	A	A	A	-	A	A	A	A	A	70	130/70	0.5	10.6	1.11	9536	25	32	132	0.68	32	12.1	N	NORMAL
7	65	F	799535	A	A	A	A	A	A	A	A	P	A	A	A	A	A	80	120/70	3.11	6.68	1.16	5814	26	30	200	0.86	23	10.1	N	NORMAL
8	40	F	806951	A	A	A	A	A	A	A	A	-	A	A	A	A	A	76	110/70	0.78	7.62	1.15	5844	20	18	124	0.92	20	9,8	N	NORMAL
9	50	F	809742	A	A	A	A	A	A	A	A	-	A	A	A	A	A	80	120/80	4.88	7.67	1.43	9625	24	28	106	0.9	24	12	N	NORMAL
10	30	F	809896	A	A	A	A	A	A	A	A	-	A	A	A	A	A	74	110/70	1.17	11.6	1.45	6446	22	20	114	0.78	10	11.6	N	NORMAL
11	50	F	806830	A	A	A	A	A	A	A	A	-	A	A	A	A	A	84	110/70	2.05	10.3	1.49	5374	28	32	136	0.82	25	12.3	N	NORMAL
12	45	F	788137	A	A	A	A	A	A	A	A	-	A	A	A	A	A	80	120/80	3.16	5.83	1.16	6417	18	24	96	0.64	28	12.3	N	NORMAL
13	24	F	789675	A	A	A	A	A	A	A	A	-	A	A	A	A	A	72	110/80	2.39	7.49	1.06	5405	14	20	176	0.69	27	13.4	N	NORMAL
14	65	F	814572	A	A	A	A	A	A	A	A	P	A	A	A	A	A	70	120/80	2.73	10.2	0.88	6069	23	34	92	0.19	39	9.7	N	NORMAL
15	50	F	815307	A	A	A	A	A	A	A	A	-	A	A	A	A	A	72	124/84	2.22	9.99	1.2	7559	22	27	93	0.5	14	13.4	N	NORMAL
16	50	F	812433	A	A	A	A	A	A	A	A	P	A	A	A	A	A	78	126/80	2.51	9.12	1.09	7095	25	30	112	0.8	21	10.1	N	NORMAL
17	55	F	790490	A	A	A	A	A	A	A	A	-	A	A	A	A	A	80	120/70	4.88	4.88	0.96	8350	24	26	174	0.82	27	11.7	N	NORMAL
18	20	F	812832	A	A	A	A	A	A	A	A	-	A	A	A	A	A	84	110/70	2.54	12.1	0.84	5104	23	28	142	0.72	9	11.3	N	NORMAL
19	60	F	811853	A	A	A	A	A	A	A	A	-	A	A	A	A	A	88	140/90	5.64	10.2	1.54	6231	25	30	123	0.74	27	11.8	N	NORMAL
20	67	F	811167	A	A	A	A	A	A	A	A	-	A	A	A	A	A	80	130/70	0.74	8	0.79	5724	30	24	154	0.7	21	12.7	N	NORMAL
21	60	F	674200	A	A	A	A	A	A	A	A	-	A	A	A	A	A	82	132/82	3.4	6.19	1.05	8430	22	19	137	0.9	16	11.4	N	NORMAL
22	50	F	811524	A	A	A	A	A	A	A	A	-	A	A	A	A	A	80	110/70	2.2	12.3	1.64	7612	32	26	164	0.6	25	14	N	NORMAL

SL.NO	AGE	sex	MRD	Symptomatology					Past H/O			General Physical Examination								THYROID FUNCTION TEST			PSEUDO							
				PF	DS	L	DA	HL	H	T	NS	P	O	SC	HC	G	SH	Pu	BP	TSH	T4	T3	P	AST	ALT	ALP	Bili	BU	Hb	OB	ECG
23	19	F	811859	A	A	A	A	A	A	A	A	-	A	A	A	A	A	76	110/70	0.87	9.95	1.15	5735	20	30	-	0.62	25	12.3	N	NORMAL
24	38	F	820737	A	A	A	A	A	A	A	A	-	A	A	A	A	A	78	110/74	0.639	10.5	0.907	5218	27	32	108	0.7	22	12.6	N	NORMAL
25	43	F	818917	A	A	A	A	A	A	A	A	-	A	A	A	A	A	80	120/80	3.17	6.63	0.92	7099	30	46	68	0.3	24	10.7	N	NORMAL
26	40	F	819371	A	A	A	A	A	A	A	A	P	A	A	A	A	A	76	124/70	2.7	6.27	0.922	6102	22	27	98	0.8	19	9.8	N	NORMAL
27	30	F	819358	A	A	A	A	A	A	A	A	-	A	A	A	A	A	80	130/70	3.08	11.8	1.45	5178	26	32	168	0.84	24	10.8	N	NORMAL
28	38	F	821193	A	A	A	A	A	A	A	A	P	A	A	A	A	A	82	134/80	1.37	9.93	1.45	7906	23	27	98	0.6	16	10.4	N	NORMAL
29	67	F	819604	A	A	A	A	A	A	A	A	-	A	A	A	A	A	84	120/80	4.03	7.86	1.01	5932	16	25	108	0.76	26	10	N	NORMAL
30	45	F	820237	A	A	A	A	A	A	A	A	-	A	A	A	A	A	80	124/70	1.26	11.3	1.17	6275	19	22	110	0.66	28	11.4	N	NORMAL
31	20	F	821080	A	A	A	A	A	A	A	A	P	A	A	A	A	A	76	130/70	1.29	11.9	0.856	5647	27	20	120	0.69	21	4.4	N	NORMAL
32	35	F	820979	A	A	A	A	A	A	A	A	-	A	A	A	A	A	78	120/80	3.79	9.91	0.86	5689	24	28	122	0.8	13	14.1	N	NORMAL
33	40	F	820981	A	A	A	A	A	A	A	A	P	A	A	A	A	A	72	110/70	1.03	11	1.06	6410	18	20	104	0.66	29	9.4	N	NORMAL
34	40	F	820975	A	A	A	A	A	A	A	A	-	A	A	A	A	A	74	140/90	1.33	11	1.43	7502	17	18	102	-	18	13.8	N	NORMAL
35	40	F	821138	A	A	A	A	A	A	A	A	P	A	A	A	A	A	68	130/90	2.87	5.06	1.1	6581	24	27	-	0.7	22	10.6	N	NORMAL
36	25	F	821078	A	A	A	A	A	A	A	A	-	A	A	A	A	A	84	110/80	1.54	11	0.84	5560	14	18	108	-	34	11.2	N	NORMAL
37	45	F	821002	A	A	A	A	A	A	A	A	-	A	A	A	A	A	70	100/60	0.437	8.46	1.06	5479	36	27	-	0.82	36	12.6	N	NORMAL
38	45	F	822873	A	A	A	A	A	A	A	A	-	A	A	A	A	A	76	100/80	2.94	5.19	1.27	6435	24	21	110	-	26	10	N	NORMAL
39	32	F	814629	A	A	A	A	A	A	A	A	-	A	A	A	A	A	80	110/84	0.51	11	0.805	6494	28	20	-	0.6	20	9.4	N	NORMAL
40	50	F	808852	A	A	A	A	A	A	A	A	-	A	A	A	A	A	86	140/70	6.57	6.4	1.23	6657	38	16	92	0.74	16	12.1	N	NORMAL

SL.NO	AGE	sex	MRD	Symptomatology					Past H/O			General Physical Examination								THYROID FUNCTION TEST			PSEUDO							
				PF	DS	L	DA	HL	H	T	NS	P	O	SC	HC	G	SH	Pu	BP	TSH	T4	T3	P	AST	ALT	ALP	Bili	BU	Hb	OB	ECG
41	20	F	815366	A	A	A	A	A	A	A	A	P	A	A	A	A	A	90	140/80	0.421	8.62	1.23	5930	24	20	120	-	11	7.8	N	NORMAL
42	60	F	814646	A	A	A	A	A	A	A	A	-	A	A	A	A	A	90	150/80	0.5	11	1.3	6714	20	16	84	-	49	14.9	N	NORMAL
43	40	F	814279	A	A	A	A	A	A	A	A	-	A	A	A	A	A	88	110/80	0.73	11	0.96	6307	30	26	110	-	24	9.8	N	NORMAL
44	25	F	797400	A	A	A	A	A	A	A	A	-	A	A	A	A	A	82	120/80	2.67	8.33	1.23	6263	28	18	92	0.64	11	13.2	N	NORMAL
45	38	F	794691	A	A	A	A	A	A	A	A	-	A	A	A	A	A	80	100/70	0.726	5.69	0.922	7739	18	14	100	0.8	10	12.9	N	NORMAL
46	21	F	809353	A	A	A	A	A	A	A	A	-	A	A	A	A	A	88	140/90	2.42	8.75	1.34	5673	34	10	69	1	20	13.3	N	NORMAL
47	45	F	820268	A	A	A	A	A	A	A	A	-	A	A	A	A	A	86	110/70	3.63	9.92	1.22	7121	28	36	138	0.9	24	10	N	NORMAL
48	34	F	786193	A	A	A	A	A	A	A	A	-	A	A	A	A	A	84	114/80	1.52	11	1.46	8132	27	24	130	0.9	25	14	N	NORMAL
49	60	F	811853	A	A	A	A	A	A	A	A	-	A	A	A	A	A	80	120/84	0.94	9.14	1.34	6231	17	26	121	0.62	27	11.8	N	NORMAL
50	45	F	794026	A	A	A	A	A	A	A	A	P	A	A	A	A	A	84	110/70	3.04	7.89	1.1	7113	18	15	125	0.7	12	10.2	N	NORMAL
51	35	M	823762	A	A	A	A	A	A	A	A	-	A	A	A	A	A	84	124/80	1.71	9.35	1.46	8984	35	24	126	-	34	14.1	N	NORMAL
52	35	M	827367	A	A	A	A	A	A	A	A	-	A	A	A	A	A	80	120/80	3.05	7.86	1.33	8767	36	38	78	0.23	22	16.2	N	NORMAL
53	35	M	827617	A	A	A	A	A	A	A	A	P	A	A	A	A	A	98	110/80	0.841	7.05	1.18	7386	33	43	119	1.3	22	8.3	N	NORMAL
54	67	M	827364	A	A	A	A	A	A	A	A	-	A	A	A	A	A	80	140/90	1.66	7.73	0.931	8137	40	41	166	0.55	29	11.6	N	NORMAL