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Relationship between Thyroid Function and Lipid Profile In Thyroid Disorder

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RELATIONSHIP BETWEEN THYROID FUNCTION AND LIPID PROFILE IN THYROID DISORDER

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DECLARATION

I, hereby, declare that, the research work as a dissertation entitled **"Relationship between thyroid function and lipid profile in thyroid disorder"** submitted to the Institute of Biological Sciences, University of Rajshahi, Rajshahi, Bangladesh for the degree of Doctor of Philosophy (Ph.D) is the result of the original research work carried out by me under the supervision of Dr. Parvez Hassan, Professor, Institute of Biological Sciences, University of Rajshahi, Rajshahi, Bangladesh.

I, further, declare that, this dissertation or part thereof has not been the basis for the award of any degree, diploma or associate ship of any other similar title.

Signature of the candidate

11.6.2012

(Mirza Md. Nazrul Islam)

CERTIFICATE

We do hereby certify that **Mirza Md. Nazrul Islam** is the sole author of the dissertation entitled **"Relationship between thyroid function and lipid profile in thyroid disorder"**. This dissertation or part thereof has not been previously submitted for the award of any degree, diploma or associate ship of any other similar title.

We are forwarding this dissertation to be examined for the degree of Doctor of Philosophy to the Institute of Biological Sciences, University of Rajshahi, Rajshahi, Bangladesh. Mirza Md. Nazrul Islam has fulfilled all the requirements according to the rules of the University for submission of a dissertation for the PhD degree.

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DEDICATED TO

My parents I owe my life and basic learning

My wife for her great loses and sacrifices

My children, my inheritance and future

My brothers and sisters for their continuous support and inspiration

My relatives for their love and affections

My neighbors and villagers for whom I am a social being

My colleagues and fellows I feel always

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And mankind whom I interacted in different spheres of life.

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The author

ABSTRACT

Background:

Thyroid disease, one of the major health hazards in Bangladesh, is mainly seen in the Northern part, along the belt of the River Brahmaputra and the Jamuna. Thyroid disorders are the second endocrine problem, next to Diabetes Mellitus (DM) in Bangladesh, with no age barrier, mostly prevalent with manifestations among the middle aged people and more among females. Thyroid hormones influence all major metabolic pathways with specific regard to lipid metabolism affecting synthesis, mobilization and degradation of lipids.

Dyslipidemia, a disorder of lipoprotein metabolism may be manifested either by elevation of total cholesterol, Low Density Lipoprotein Cholesterol, (LDL-C) triglyceride or a decrease in High Density Lipoprotein Cholesterol (HDL-C) in the blood. It is a major risk factor for atherosclerotic cardiovascular diseases, leading to Coronary Heart Disease (CHD) that is Ischemic Heart Disease (IHD) in particular.

In the present study thyroid function and lipid profile have been investigated in a population that is the patients with thyroid disorder which is the representative of peoples living in hyper endemic iodine deficient zone in the Northern part of Bangladesh.

Objectives:

To evaluate relation between thyroid function and dyslipidemia in patients with thyroid disorders.

Study design:

A total of Five hundred thirty three (533) patients clinically suspected with thyroid disorders were included in the study. Among them 365 were females and 168 were males. They were both from rural and urban areas and from low and high socio-economic groups in the Northern part of Bangladesh.

Materials and Methods:

Five hundred thirty three (533) patients clinically suspected with thyroid disorders consented to both general physical examination including neck and systemic examination. 5ml of venous blood was drawn from each for estimation of thyroid hormones levels (T3, T4 and TSH). Each was asked to come the next day morning after over night fasting. Another 5ml of blood was taken from each for estimation of lipid profile. The study was done in the Institute of Biological Sciences, University of Rajshahi using the laboratory facilities of Centre for Nuclear Medicine and Ultrasound, Mymensingh and Rangpur and the Department of Biochemistry, Banga Bandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh during the period of July 2004 to June 2009.

Outcome and Results:

Among the study population 207 had normal thyroid function i.e. were euthyroid and 326 had abnormal thyroid function. Out of total 325 patients having abnormal thyroid function; 162 had hyperthyroidism and 164 had hypothyroidism. Among the hyperthyroid patients 123 were female and 39 were male; and 118 females and 46 males were in hypothyroid group. Most of the study populations were in the middle age group (30-39 years). There was a high prevalence of mild to moderate thyroid enlargement among the study population.

Patients with hyperthyroidism were associated clinically with hot intolerance, increased appetite, weight loss, decreased sleep, palpitation and tremor where as patients with hypothyroidism were associated clinically with cold intolerance, decreased appetite, increased sleep, weight gain, hoarseness of voice and pretibial myxoedema. Both groups showed weakness, but more marked in hypothyroidism. Also both groups showed features of heart failure, that is, shortness of breath with no significant difference.

This study showed significant elevation of total cholesterol (TC, $p<0.001$), LDL cholesterol (LDL-C, $p<0.001$), Triglycerides (TG, $p<0.001$) and no significant change of high density lipoprotein cholesterol (HDL-C, $p>0.077$) in patients with hypothyroidism; with no significant abnormalities in lipid profile relating to dyslipidemia in hyperthyroid patients. There were positive and linear associations between serum TSH levels and TC, LDL-C and TG, and negative and linear correlation

of TSH with HDL-C ($r = 0.28$, $p<0.01$; $r = 0.23$, $p<0.01$; $r = 0.14$, $p <0.01$ and $r = -0.15$, $p< 0.01$ respectively). The more increase in TSH levels, the more was the degree of hypothyroidism and more there was dyslipidemia with risk of atherosclerosis.

Conclusion:

Hypothyroidism relates to dyslipidemia. So, measures should be taken to screen people particularly living in the Northern part of Bangladesh for thyroid disorders and to evaluate thyroid function in these peoples. Appropriate treatment and proper preventive measures should be taken for this groups with specific attention to sub-clinical hypothyroidism to avoid the harmful effects of hypothyroidism (overt/sub-clinical) leading to abnormalities in lipid profile, that is, dyslipidemia and its consequences to the development of atherosclerotic cardiovascular diseases with Ischemic Heart Disease (IHD) in particular.

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CHAPTER-1

INTRODUCTION

1.0. INTRODUCTION

Thyroid gland through its secreted hormones maintains the level of metabolism in the tissue that is optimal for their normal functioning. No tissue in the body is spared from its action. Iodine is essential in small amount for the synthesis of the thyroid hormone, which is necessary for normal growth, development and well being of all humans and animals (Hetzel 1991).

Iodine deficiency disorders remain a global public health problem of major importance. Globally, nearly one billion people are living in iodine deficient areas; of these individuals, 190 million to 655 million have goiter and 3 million to 20 million are suffering from thyroid disorders (Hetzel 1988). According to WHO, UNICEF and ICCID (1993) about 75% of the total human beings suffering from iodine deficiency disorders live in the less developed countries.

The deficiency of iodine has several important health consequences which are collectively known as iodine deficiency disorders (IDDs). The cumulative consequences in iodine deficient populations spell diminished performance for the entire economy of the affected areas. The spectrum and prevalence of thyroid disorders are known to be influenced by environment factors, especially by iodine intake (Wang and Crapo 1997). Indeed, iodine deficiency is regarded as the most common cause by thyroid disorders World wide (Dunn *et al.* 1986).

In certain geographical areas remote from the sea, diet is deficient in iodine. Reportedly, south East Asia {which includes Bangladesh, India and Indonesia} and Western Pacific (including China) accounts for more than 50% the world's population at risk of thyroid disorders including IDD. Thyroid disease, one of the major health hazards in Bangladesh, is mainly seen in the northern part and along the belt of the Brahmaputra and the Jamuna River. Thyroid disorders are the second most common endocrine problem in Bangladesh (Moslem *et al.* 2000).

Thyroid disorders may occur in the form of abnormality in size, shape, histological structure or function of the gland. Endemic goiter may reach enormous size and complications may be seen. Among the consequences are thyrotoxicosis and malignancies. The condition is essentially preventable. Iodine deficiency is the most

common cause of hypothyroidism world wide. In persons living in iodine replete areas causes are congenital; spontaneous because of chronic auto immune disease (atrophic autoimmune thyroiditis or goitreaous autoimmune thyroiditis (Hashimoto's thyroiditis), iatrogenic because of goitrogens drugs or destructive treatment of thyrotoxicosis (Vander pump *et al.* 1995).

Thyroid hormones influence all major metabolic pathways. Their most obvious and well known action is an increase in basal expenditure obtained acting on protein, carbohydrate and lipid metabolism. With specific regard to lipid metabolism thyroid hormones affect synthesis, mobilization and degradation of lipids, although degradation of lipids is influenced more than synthesis. The best known effects on lipid metabolism include:

a. Enhanced utilization of lipid synthesis;

b. Increase in synthesis and mobilization of triglycerides in adipose tissue;

c. Increase in the concentration of non-esterified fatty acids (NEFA);

d. Increase in the lipoprotein lipase activity (Pucci *et al.* 2000).

Thyroid hormones increase the fractional clearance of LDL by increased number of LDL receptors in the liver; also regulate the activity of cholesteryl ester transfer protein (CETP) and more specifically hepatic lipase (HL).

Cholesterol and triglycerides are two main lipids in blood; these are carried in blood by a specific transport protein called apo-proteins, which are together known as lipoproteins are –

1. Very low density lipoprotein (VLDL)
2. Low density lipoprotein (LDL)
3. High density lipoprotein (HDL) and another is
4. Chylomicrons.

Elevated serum total cholesterol, LDL cholesterol and low serum HDL cholesterol are the major independent risk factors of CHD (Coronary heart disease). Epidemiologic observations, angiographic studies and lipid lowering trials, as well as experimental studies, confirm the importance of LDL as a cause of atherosclerosis in both man and woman with or without symptoms of CHD.

An elevated LDL cholesterol level appears to be the primary CHD risk factors, and the higher the total cholesterol and LDL cholesterol levels are the greater the risk of an atherosclerotic events. A strongly positive relation exists between serum cholesterol level and CHD world wide, and no threshold has been identified below which a lower blood cholesterol is not associated a lower the risk of CHD; the lower the cholesterol the risk of CHD, even in Chinese populations WHO, by western standards, have a low cholesterol concentration (Chen *et al.* 1991).

Many studies in laboratory animals indicate that rising serum level of LDL and related lipoproteins will indicate and sustain atherogenesis (Traub *et al.* 1998). Moreover, human with genetic form of severely elevated LDL exhibit premature atherosclerotic diseases (Tabas 1999). Both of these examples demonstrate that elevated LDL alone, without the need for other CHD risk factors, is independently atherogenic.

For many years, it was believed that major action of LDL was merely to deposit its cholesterol within the arterial wall. More recently, LDL has found to be a pro-inflammatory agent (Schwartz 1999). It sets into motion to chronic inflammatory response that is the hall mark of the atherosclerotic lesion. Elevated LDL appears to be involved in all stages of atherogenesis: Endothelial dysfunction, and thrombosis. Elevated cholesterol levels in the plasma lead to increased retention of LDL particles in the arterial wall, their oxidation and secretion of various inflammatory mediators and chemo attractants (Witztum and Palinsky 1999).

One sequelae of this is the disruption of endothelial cell function by oxidized LDL (Witztum and Palinsky 1999), with subsequent loss of production of nitric oxide. Treatment of elevated LDL cholesterol levels has been shown to re-establish normal coronary vasodilatory response to acetylcholine. Links low LDL is also a potent mitogle. In different populations, the risk for CHD is positively correlated with serum total cholesterol; the total cholesterol level in turn is highly correlated with LDL cholesterol levels. The association between serum cholesterol levels and risk is curvilinear (or long-linear) (Nielsen 1996). This observation strongly suggests that elevated LDL cholesterol is the primary risk factors for atherosclerosis.

A low level of HDL cholesterol is a potent individual predictor for CHD in population in which average cholesterol levels are relatively high, but it may not hold as a predictor in population in which mean levels of serum total cholesterol and LDL cholesterol levels are low. In this regard, low HDL cholesterol resembles the other independent major risk

factors (Smoking, hypertension and diabetes): it appears to promote coronary atherosclerosis when a high LDL level is present but not when it is absent (Grundy *et al.* 1990). Thus, low cholesterol and non lipid risk factor aggravate the effect of LDL cholesterol, especially when total and LDL cholesterol are only moderately elevated (Wood *et al.* 1998).

Many prospective epidemiological studies have reported a positive relationship between serum triglycerides and incidence of CHD (Austin *et al.* 1998, Assman *et al.* 1998).

Non lipid risk factors of obesity, hypertension, diabetes and cigarette smoking are also interrelated with triglycerides (Grundy 1998). Raised triglycerides are in fact an independent risk for CHD (Austin *et al.* 1998, Assman *et al.* 1998). This independence suggests that some triglycerides rich lipoproteins (TGRLP) are atherogenic. The most likely candidate for atherogenic TGRLP is remnant lipoproteins which include very low density lipoproteins (VLDL) and intermediate density lipoprotein (IDL). They are cholesterol enriched particles and have many properties of LDL. Their elevation emerge a strong predictors of coronary atherosclerosis or CHD (Tatami *et al.* 1981, Steiner *et al.*1987, Krauss *et al.* 1987, Phillips *et al.* 1993, Tornvall *et al.* 1993, Koren *et al.* 1996, Karpe *et al.* 2001, Takeichi *et al.* 1999, Thompson *et al.* 1998, Sacks *et al.* 2002) .

Dyslipidemia is a disorder of lipoprotein metabolism that may be manifested either by elevation of total cholesterol, Low Density Lipoprotein Cholesterol, (LDL-C) triglyceride or a decrease in High Density Lipoprotein Cholesterol (HDL-C) in the blood. It is a major risk factor for atherosclerotic cardiovascular diseases, leading to Coronary Heart Disease (CHD) that is Ischemic Heart Disease (IHD) in particular.

Thyroid hormones play an important role in regulating lipid metabolism, and thyroid dysfunction can result in lipid abnormalities which increase the risk of endothelial dysfunction, hypertension and cardiovascular disease. Thyroid dysfunction produces multiple alterations in composition and transport of lipoproteins.

In the present study, thyroid function and lipid profile have been investigated, in a population that is in the patients with thyroid disorder which is the representative of peoples living in hyper endemic iodine deficient zone in the Northern part of Bangladesh. The main objective of the study was to evaluate relation between thyroid function and dyslipidemia in patients with thyroid disorders.

CHAPTER-2

RATIONALE, HYPOTHESIS, AIMS AND OBJECTIVES

2 RATIONALE, HYPOTHESIS, AIMS AND OBJECTIVES.

2.1. RATIONALE:

Dyslipidemia is a disorder of lipoprotein metabolism including lipoprotein over production or deficiency. It may be manifested by elevation of total cholesterol (TC) low density lipoprotein cholesterol (LDL-C) and triglycerides (TG) concentrations, and a decrease in the high density lipoprotein cholesterol (HDL-C) in the blood. It is a major risk factor for atherosclerotic cardiovascular disease, the most common cause of death in the USA and most European countries. The frequency of Ischaemic Heart Disease (IHD) is on a rapid rise in the developing countries including Bangladesh. It is predicted that in the very near future it will be the number one killer disease. Siddique *et al.* (2005) working on the spectrum of thyroid disorders in Bangladesh observed that more than 50% patients with unstable angina were dyslipidemic.

Changes in composition of lipoproteins occur in hypothyroidism and hyperthyroidism (Muls *et al.* 1984, Friis *et al.* 1987). Iodine deficiency is the most common cause of hypothyroidism world wide, and is more prevalent in Bangladesh particularly its Northern part which is the hyper endemic Iodine deficient zone. In recent years, more and more evidence demonstrated that hypothyroidism is associated with increased prevalence of Coronary Heart Disease (CHD) (Cappola *et al.* 2003, Neves *et al.* 2008). This association is due to dyslipidemia. Lipid is usually normal in sub-clinical/overt hyperthyroidism (Duntas 2002).

No such study to find out the link between thyroid function and dyslipidemia has yet been done in Bangladesh. The present prospective study was undertaken with the view to evaluate this association in the context of Bangladesh with particular emphasis to the population from the Northern part along the belt of the river Brahmaputra and the river Jamuna. The observations (information) and results emerging from the study is expected to be useful in the treatment as well as in the prevention of atherosclerotic cardiovascular diseases.

2.2. RESEARCH HYPOTHESIS:

Hypothyroidism relates to dyslipidemia.

2.3. AIMS AND OBJECTIVES:

1. To evaluate thyroid status by measuring T3, T4 and TSH in patients clinically suspected with thyroid disorders.
2. To measure lipid profile (Total cholesterol, LDL-C, HDL-C and Triglyceride) for each of the above patients after overnight fasting.
3. To find out relation between thyroid function and dyslipidemia.

CHAPTER-3

REVIEW OF LITERATURE

3.0. REVIEW OF LITERATURES

THYROID GLAND

3.1.1. (a) Anatomy of the thyroid gland

A thyroid gland is the largest endocrine gland of the body. It is very vascular organ, surrounded by a sheath derived from the pre-tracheal layer of deep cervical fascia. The sheath attaches the gland to the larynx and the trachea (Snell 1992),

The thyroid gland is a discrete organ, situated in the anterior neck, immediately in front of the trachea, and below the thyroid cartilage weighing between 20- 25 gm. It is always asymmetric, with the right lobe often twice the size of the left lobe, the two lobes united by isthmus. The thyroid gland is usually larger in women than men (O' Riordan *et al.* 1982).

The blood flow to the thyroid gland is about 5 ml /gm /min. In hyperthyroidism the flow to the gland is markedly increased, and a whistling sound, or bruit, may be heard over the lower pole of the gland and may even be felt in the same areas as a vibration or thrill (Greenspan 1983).

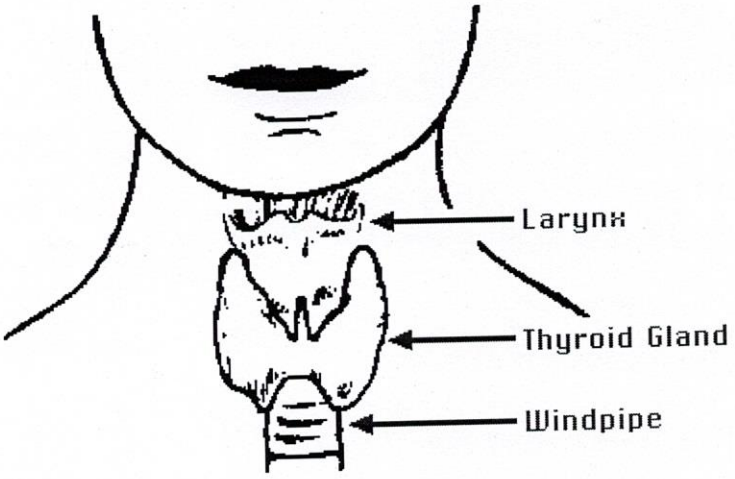

Fig.1. Anatomy of the thyroid gland

3.1.1.(b) Histology

Thyroid component

A connective tissue capsule surrounds the thyroid gland. The thyroid follicle is the functional unit of the thyroid gland and contains thyroglobulin, which is an exocrine secretion product of follicular epithelial cells. Thyroglobulin is a glycoprotein and contains 120 tyrosyl residues. It serves as a matrix in which thyroid hormone is formed and also is the storage form of thyroid hormone.

Follicular epithelial cells are the predominant type of cells in thyroid tissue. The ultra structure of follicular epithelial cells reflects their secretory function. A round basal nucleus, numerous mitochondria and apical Golgi apparatus are scattered throughout the cytoplasm. Their apical surface is covered by numerous microvilli, which increase under the effect of TSH.

Parafollicular cells or c cells are the larger cells compared to follicular cells which exists between thyroid follicles and secretes calcitonin.

Thyroid gland secretes two hormones:

Thyroxine (T_4) (3, 5, 3i, 5i tetraiodothyroxine) and T_3 (triiodothyronine)

The ratio of T_3 / T_4 secretion is (10-20) / 1 and the blood concentration ratio of free

T_4 /T_3 are 2/1.

Thyroid cells uptake inorganic iodide from the plasma to make thyroid hormones.

Iodide intake: Dietary iodide intake is 500 micro gm / day in the U.S. Thyroid iodide. The thyroid gland contains 5.7 mg of iodide stored in the thyroglobulin, which is the storage form of thyroid hormone. The storage form of thyroid hormone in plasma is thyroxine binding protein (TBG), triiodothyronine binding protein (TBPA) and albumin (T_4 storage).

Spheroidal follicles are formed by acini lined by cuboidal epithelium. The cells possess microvilli that project into the colloid. The colloid material in follicles contains thyroglobulin, which is the carrier molecule for T3 and T4. The microvilli are believed to participate in the mobilization of thyroid hormones from Thyroglobulin, by the enzymatic cleavage of T3/T4 from Thyroglobulin.

Thyroid hormone affects cellular oxidative processes throughout the body.

3.1.1. (c) Embryology:

Development of thyroid gland starts at the 4th week of intra-uterine life and is functioning at the end of 12 week (Langman 1981).

Embryologically, the gland has a dual origin. One part that is the para follicular cells, derive from an inevagination of pharyngeal endoderm in the vertex of the laryngeal pouch. The other part, that is follicular cells, derive from bilateral ectodermal buds from the last branchial cleft (Ultimobranchial body), which ultimately fuse in the midline.

The thyroid cells derived from ectoderm form the follicular cells and are responsible for the production of the thyroid hormones. The cells arising from endoderm give rise to Para follicular cells or clear cells, whose principal product is thyrocalcitonin.

Cell remnants along the tract of the lingual inevagination often connect with the thyroid to produce pyramidal lobe. Occasionally a lingual thyroid may persist, but it is almost never active in the presence of normal thyroid function. The same is true of thyroglossal duct remnant (Herbert 1996).

3.1.1. (d) Physiology:

The thyroid hormones are unique in that they contain 59-65% of the trace element iodine (Greenspan, 1983). The thyroid gland secretes predominantly thyroxine (T4), and only a small amount of triiodothyronine (T3); approximately 85% of T3 is produced by mono-deiodination of T4 in other tissues such as liver, muscles and kidneys. T4 is probably not metabolically active until converted to T3 and may be regarded as a pro-hormone (Edwards *et al.* 1995).

Factors associated with decreased conversion of T4 to T3 are as follows:

1) Fetal life.

2) Calorie restrictions.

3) Hepatic diseases.

4) Major systemic illness.

5) Drugs.

- Propylthiouracil

- Glucocorticoids
- Propanolol
- Iodinated X-ray contrast agents (Iopanoic acid, ipodate sodium Amiodarone).

6) Selenium deficiency (Greenspan, 1983).

A biologically inactive iodothyronine called reverse-T3 is also found in significant concentration in human serum (O'Riordan *et al.* 1982).

Ingested iodine is rapidly reduced to iodide in the upper intestine and 90% is absorbed in the first 60 minutes after ingestion (Herbert 1996).

3.1.1. (e) Biology of iodine:

Iodine is an essential trace element for normal growth and development of humans and animals. It is present in the body in a minute amount-about 0.00004% of body weight (15-23 mg) or less than a hundredth of the amount of iron in a healthy human adult. Over three fourths of this iodine is concentrated in a single tissue, the thyroid gland, which uses it in the synthesis of the thyroid hormones- triiodothyronine (T_3) and thyroxine (T_4).

3.1.1. (f) Sources of iodine:

The ocean is the main natural source of iodine. It was present during the primordial development of the earth, but large amount were leached from the surface soil by glaciations, snow, and rain and were carried by wind, rivers, and floods into the sea (Hetzel 1989). Iodine occurs in the deeper layers of the soil and is found in oil-well effluent. In general, the older an exposed soil surface the more likely it is to be leached of iodine. Iodine occurs in the soil and in the sea as iodine. Iodine ions are oxidized by sunlight to elemental iodine, which is volatile. Every year some 400,000 tones of iodine escape from the surface of the sea. The concentration of iodine in the sea water is about 50-60 μgm/litre and in air it is approximately 0.4 μgm/cubic meter. Iodine content in water of less than 2 μgm/litre, or 2 ppb, is associated with environmental iodine deficiency (EID). The atmosphere absorbs iodine from the sea which then returns through the rain and snow. It is then carried by rivers to the lower hills and plains, eventually returning to the sea. High rainfall, snow and flooding increase the loss of iodine from soil. The return of iodine to the soil is slow and small in amount compared to the original loss. Hence no natural correction can take place, and iodine deficiency (ID) persists in the soil

indefinitely. All crops grown in iodine deficient soils are deficient in iodine. As a consequence, humans and animals that are totally dependent on food grown in such soil easily become iodine deficient.

The iodized salt, seafood, drinking water and milk are the dietary source of iodine (Matheson and Krukowski 1996). Thyroid is the principal gland that takes up iodine and uses it to make thyroid hormones.

3.1.2. Metabolism and Synthesis of thyroid hormone (Herbert 1996).

It is difficult to formulate a compartmental model of iodine metabolism that on the one hand is simple and clinically useful and on other hand permits a quantitative reconstruction of the metabolic cycle although a compromise does show the interrelationships among extra-thyroidal, intra-thyroidal and hormonal iodine. The fractions lost transplacentally and by mammary excretion are excluded because of their transitory nature.

Iodine trapped and organified by the thyroid gland is utilized in the synthesis of thyroid hormones. Iodine also is liberated by the intra-thyroidal de-iodination of amino acids not utilized in hormone production. This compartment is large and has a slow turnover.

The thyroid gland syntheses the hormone thyroxin from tyrosine Iodine always reaches the thyroid gland in the form of iodide. The thyroid gland has a special affinity for this element and is able to trap transport and concentrate it for the synthesis of thyroid hormone (Graham and Blizzard 1973).

Iodine is utilized by thyroid glands as follows:

a) Iodide trapping:

The thyroid gland concentrates iodide by active transporting it from the circulation to the colloid. The transport mechanism is frequently called the "Iodide pump" or Iodide Trapping mechanism". The trapping mechanism depends on oxidative phosphorylation. Iodide trapping is inhibited by anoxia, hypothermia, cyanides, dinitrophenol etc. and blocked by perclorate, thiocyanate, pertechnetate.

b) Oxidation of iodine:

In the thyroid gland iodide is oxidized to iodine by hydrogen peroxide.

c) Organification:

After oxidation, iodine is bound to the 3 position of tyrosine molecules attached to thyroglobulin. The enzyme responsible for the oxidation and binding of iodide is thyroid peroxidase, with hydrogen peroxide accepting the electrons. Mono-iodotyrosine (MIT) is next iodinated in the 5 position to form di-iodotyrosine (DIT).

d) Coupling:

Two DIT then undergo an oxidative condensation to form T4. This is called coupling. T3 is formed by condensation of MIT with DIT. These thyroid hormones remain bound to thyroglobulin (Tg) in the follicular lumen until needed. The thyroid-stimulating hormone (TSH) stimulates the coupling process.

f) Storage and release of thyroid hormones.

In order to release thyroid hormones from the thyroglobulin store at the center of the follicle, the glycoprotein must first be taken back into the thyroid cell where lysomal enzymes can release the iodo-thyronines for secretion into circulation (O' Riordan *et al.* 1982).

The normal thyroid gland contains at least one month's supply of thyroid hormone. Release is stimulated by TSH which acts upon the thyroid cell membrane, Adenyl cyclase is activated resulting in prompt rise in cyclic AMP (adenosine mono phosphate) hydrolysis to Tg and release of T3 and T4 occurs (Herbert 1996).

MIT and DIT are not liberated outside the gland in physiological conditions. Tri-iodothyronine is four times as active as thyroxine (Krishnamoorthy 1978).

The human thyroid secretes about 80 micro gram of T4 and 4 microgram of T3 per day. Once released in blood T3 and T4 are bound. The free thyroid hormones in plasma are in equilibrium with protein bound thyroid hormones in plasma and tissues. It is free thyroid hormones in plasma that are physiologically active and that inhibit pituitary secretion of TSH. The function of protein binding appears to be maintenance of a large pool of readily available free hormones. The plasma proteins that bind thyroid hormones are albumin, pre-albumin and thyroxine binding globulin (TBG). Most of the circulating T4 is bound to TBG. 99.98% of T4 is bound; the free T4 level is only about 2 ng/dl. 99.8% of T3 is protein bound, 46% to TBG and most of the remainder to albumin, with very little to pre-albumin. The range of normal total plasma T4 is approximately 64 to

175 nmol/L and that of plasma T3 approximately 0.8 to 3.16 nmol/L (Edwards *et.al.* 1995).

There are some differences of normal range of thyroid hormone level between adult and children because TBG levels are higher in newborn and children. Normal range of T3 and T4 are almost same for both male and female (Ishaque *et al.* 1987).

Age adjusted range of T3, T4 and TSH in different age groups (Sills *et al.* 1992).

Age group (In year)	Normal range.
T3 (nmol/L)	
1-15 Years	1.57 to 3.30
>15 Years	1.23 to 3.10
T4 (nmol/L)	
1-12 Years	70.8 to 160.9
>12 Years	58.0 to 154.4
TSH (m IU/L)	
For all ages	0.6 to 4.8

The hypothesis that the free hormone concentration determines the individual thyroid status stems from the observation of patients with alteration or absence of TBG. In these cases the thyroid status does not depend upon the level of total circulating hormone, but rather on the free hormone concentration (De Nayer 1982).

3.1.3. Control of Thyroid Function:

Pituitary Thyroid Axis

TSH regulates the synthesis and secretion of thyroxine (T4) and triiodothyronine (T3). TSH is a 28 k Da glycoprotein secreted by pituitary thyrotrophs and is composed of two subunits. Its alpha-subunit is identical to the alpha-subunit of LH and FSH and is devoid of biologic activity. The beta-subunit determines the biologic actions and immunologic characteristics of TSH. TSH circulates unbound in blood and has a half-life of approximately 50 minutes. It is secreted in a pulsatile fashion with the highest levels found between 0200 and 0400 hours. The major regulator of TSH secretion is negative

feedback control on thyrotrophs by thyroxine (T4) and triiodothyronine (T3). However, hypothalamic TRH is necessary for the normal maintenance of TSH secretion. TSH secretion in response to TRH stimulation is enhanced when thyroid hormone levels are low and blunted when thyroid hormone levels are high.

T4 and T3 circulate primarily in the protein bound state thyroxin-binding globulin (TBG), thyroxin-binding prealbumin circulated unbound -- it is this free fraction that is biologically active. T3 has a 3-fold greater biologic activity than T4. These hormones have many target tissues and increase metabolic activity and protein synthesis. Conditions, which affect TBG levels, affect the levels of total T4. For example, estrogens (oral contraceptives, pregnancy), genetically determined, and acute hepatitis may increase levels of TBG. Whereas, in the settings of glucocorticoid therapy, severe illness, or nephrotic syndrome TBG levels may be low.

3.1.4. Iodine deficiency and the thyroid hormones:

Functionally iodine is important because it is an essential component of thyroid hormones-T4 and T3. Where iodine deficiency prevails, the level of serum T4 falls, but serum T3 remains normal or rises due to increased production, to maintain clinical euthyroidism. As a part of the mechanism for compensating for ID, a small but definite increase in the TSH level has also been reported in areas of mild or moderate deficiency. Since iodine deficiency has been shown to increase the sensitivity of the thyroid to TSH, it is also possible that goitrogenesis can occur with TSH levels that are actually within the normal range (Degroot 1984).This TSH stimulates the thyroid gland to produce thyroid hormones. Continuous stimulation of thyroid gland by TSH result in enlargement of the gland through hypertrophy and hyperplasia of the thyroid parenchyma cells (Ganong 1995). This enlargement is known as goiter.

3.1.5. Thyroid Hormone Action:

Thyroid hormone interacts with nuclei of cells by binding to nuclear receptors and mitochondrial receptors with resulting increase of ATP.

3.1.6 Effects of thyroid hormone:

The thyroid hormones play upon a great multiplicity of metabolic processes, influencing the concentration and activity of numerous enzymes; the metabolism of substrates, vitamins, and minerals; the secretion and degradation rates of virtually all others

hormones; and the response of their target tissues to them. As a consequence, it can truly be said that no tissue or organ system escapes the adverse effects of thyroid hormone excess or insufficiency (Ingbar and Woeber 1981).

Some of the widespread effects of thyroid hormones in the body are secondary to stimulation of oxygen consumption, although the hormones also affect growth and development in mammals, help regulate lipid metabolism and increase the absorption of carbohydrate from the intestine (Ganong 1995).

Thyroid Hormones are essential for adequate fetal growth and the development of neural and skeletal systems. It also contributes to:

- BMR regulation
- O_2 consumption.
- Marked chrono- and inotropic effects on myocardium.
- Sympathetic effect on myocardium.
- Drive of CNS respiratory centers.
- Increased erythropoiesis.
- Increased steroid hormone clearance, and effects in controlling LH, FSH, and GH.

3.1.7. Thyroid status and Socio-economic Condition:

The widespread metabolic role of the thyroid hormones, the diverse processes involved in the synthesis, secretion, and metabolism of the hormone and the complex mode of regulation of thyroid function suggest that a great many factors could influence one or more aspects of thyroid hormone production. In general the factors can be considered in the following categories: endogenous variables, pharmacological agents, environmental alteration, and dysfunction or diseases of the other organs system (Ingbar and Woeber 1981).

Socio-economic status is a complex concept. First variable is income, wealth or standard of living. Second one is educational attainment.

Malnutrition may be due to inadequate food intake, defective absorption, poor dietary habit, food faddism etc. One important consequence of malnutrition is retardation in children's physical and mental growth and development (Krishnamoorthy 1978).

Under nutrition and malnutrition are the most serious human health and social problems that affect vast areas of the world, much more prevalent and endemic in developing countries. Malnutrition prevails in low socioeconomics condition especially when associated with iodine deficiency. These are likely to be more remote, suffer more social deprivation, and have a disadvantage in school facilities. All such factors have to be taken into account, apart from the problem of adapting tests developed in Western countries for use in Third World countries (Hetzel 1991).

A study on nutritional status of school children of Dhaka shows that only about 26.2% children are in the normal group, 47.3% wasted, 14.3% stunted & 12.2% are both wasted & stunted ,when both height & weight are taken into consideration. This data indicates that most of the children in our country are wasted compared to the western standard (Ahmed *et al.* 1990).

Experimental and clinical studies have shown that acute & chronic starvation and calorie restriction significantly alter the endocrine system in general and affect the thyroid gland in a number of ways. Thyroid gland weight, histological structure, and glandular function are known to change depending on nutritional state (Hetzel 1991).

Both macro and micronutrient deficiencies are a common occurrence in developing countries like Bangladesh. In developing countries like Bangladesh, discriminatory food practices against women are highly prevalent; actually beginning shortly after birth. The female child is given less food. A recent study showed that 92% of women are fed last, least and left over (BRAC 1999)

Protein calorie malnutrition often is present in low socio-economic conditions and may contribute to thyroid disorders (Gaitan *et al.* 1983).

3.1.8. Physiological and molecular basis of thyroid hormone action: (Paul 2001)

Thyroid hormones (THs) play critical roles in differentiation, growth, and metabolism. Indeed, TH is required for the normal function of nearly all tissues; with major effects on oxygen consumption and metabolic rate. Disorders of the thyroid gland are among the most common endocrine maladies. Furthermore, endemic cretinism due to iodine deficiency remains a public health problem in developing countries at the advent of the third millennium. Thus the study of TH action has important biological and medical implications.

3.1.9. Diseases of the Thyroid: (Mazzaferri *et al.* 1998).

Pathological lesions of various morphology, which can be divided into two types, affect the thyroid gland: Those that show a diffuse pattern and those that produce nodules.

Diffuse thyroid lesion: Diffuse thyroid lesions are those that are associated with non neoplastic conditions affecting the glands: hyperplasia and thyroiditis.

This category includes;

I. Thyroiditis

a) Acute thyroiditis: Occurs in young malnourished children, elderly debilitated adults, or immuno-compromised individuals. Grossly the thyroid may be focally or diffusely soften. Acute inflammation with micro abscess formation, necrosis and microorganism as is seen microscopically. The routes of infection to the thyroid are direct extension from a focus in the head and neck or hematogenous spread.

b) Granulomatous sub-acute thyroiditis: This probably represents the response of the thyroid to systemic viral infection; some authors suggest that it is an actual viral infection of the gland. The disease is not thought to be auto-immune in nature. Pathologically it is characterized by asymmetrical or diffuse involvement of the glands. Irregular white-tan lesions or several small poorly demarcated nodules may simulate carcinoma.

c) Palpation thyroiditis: It is found in most surgically resected thyroids and it probably represents the thyroid response to minor trauma. The histological feature of these lesions includes multiples isolated follicles or small groups of follicles that shows partial or circumferential loss of epithelial cells and replacement of the lost epithelium by inflammatory cells, predominantly, macrophages.

d) Chronic lymphocytic (Hashimoto's) thyroiditis: It represent a spectrum of autoimmune injury, the gland is firm and symmetrically enlarged, weighing from 25 gms to 250 gms. Normal thyroid lobulation is accentuated by interlobular fibrosis. The thyroid follicles are small and atritic. Colloid appears dense or may be absent. The follicular cell are metaplastic and includes oncocytic (Suen 1990).

e) Painless thyroiditis: Recent clinical, immunological and pathological studies have confirmed that painless thyroiditis is an autoimmune disease.

f) Riedel's thyroiditis: This type of chronic thyroiditis affects mostly adult and elderly females. There is fibrosing reaction that destroys the whole gland, resulting in a hard gland on examination. It also affects the surrounding muscles simulating carcinoma. Microscopically, fibrous tissue is hyalinised and completely replaces the area of gland which is involved (Rosai 1989)

g) Focal nonspecific thyroiditis: Lymphocytic infiltration of the thyroid glands is found more frequently. Iodide (Iodine) may combine with a protein, act as an antigen and evoke an immune response localized to the thyroid gland.

h) Fibrosing thyroid lesions: This category included the fibrosing variant of Hashimoto's thyroiditis, and fibrous atrophy. Grossly the fibrosis involves the all or part of the thyroid glands and is described as woody and very hard. Extension of fibrosis beyond the thyroid is characteristic.

II. Goiter:

The term goiter refers only to visible or palpable enlargement of the thyroid and infers no particular alteration in thyroid function. It takes many forms:

Physiological goiter:

Physiological goiter is the term applied to thyroid enlargement seen commonly in girls at the menarche or in women during pregnancy. The cause is unknown and normally the goiter regress in time. No treatment is required.

Endemic Goiter:

Goiter was once endemic in certain geographical areas, usually remote from the sea, where the diet is deficient in iodine. Goiter may reach enormous size and complication may be seen as consequence. This includes diseases such as thyrotoxicosis and malignancy. The condition is essentially preventable.

Sporadic Goiter:

Goiter may occur sporadically in non-goitrous regions and in the absence of iodine deficiency. The cause is uncertain but undoubtedly some are autoimmune in nature and due to the presence of specific thyroid growth –stimulating immunoglobulin.

Euthyroid state is normally maintained but such patients are more prone to develop secondary thyrotoxicosis and to have a higher than average risk of thyroid cancer.

III. Nodular lesions: Nodular lesions comprise those disorders that consist of non neoplastic hyperplasia as well as benign and malignant tumours.

This category includes the common thyroid lesions that present as

Solitary nodules

Multiple nodules

Benign nodular goiter

Toxic nodule

Benign and malignant neoplasm

IV. Neoplasm's of The Thyroid (Edwards *et al.*1995)

Thyroid neoplasm is classified as follows.

Benign.

Follicular Adenoma.

Malignant.

Primary.

Follicular epithelium: Differentiated

- Follicular.

- Papillary.

Follicular epithelium :Undifferentiated

- Anaplastic.

Lymphoid cells

- Lymphoma.

Parafollicular cell.

- Medullary.

Secondary.

Metastatic.

Local infiltration.

3.1.10. Causes of thyroid disorders

Causes of hyperthyroidism:

1. Grave's Disease
2. Multinodular Goiter.
3. Autunomously functioning solitary nodule.
4. Thyroiditis.
5. Iodine induced.
6. Extra-thyroidal source of thyroid hormone excess-Factitious hypethyroidism, Struma ovarii.
7. TSH induced –Inappropriate TSH secretion by pituitary, Choriocarcinoma,
8. Follicular Carcinoma.

(Edwards *et al.* 1995).

Causes of hypothyroidism:

1. Hypothyroidism resulting from loss or atrophy of the thyroid tissue.

a) Post ablative hypothyroidism.

b) Primary idiopathic hypothyroidism.

c) Sporadic athyreotic cretinism (Thyroid aplasia/dysplasia)

2. Hypothyroidism due to in sufficient stimulation of the intrinsically normal gland.

a) Shehan's syndrome

b) Infiltrative disorders of pituitary or hypothalamus.

3. Goitrous.

a) Hashimotos' thyroiditis.

b) Endemic iodine deficiency.

c) Antithyroid agents.

(Ingbar and Woeber 1981).

3.1.11. Sub-Clinical Thyroid Disease:

Sub-clinical thyroid disease is, by its very nature, a laboratory diagnosis. Patients with sub-clinical disease have few or no definitive clinical signs or symptoms of thyroid dysfunction. Thus, it is critically important that the normal reference range for TSH is standardized and that laboratories engage in appropriate quality control procedures to ensure that the results they report are accurate and reproducible (Spencer *et al.* 1996).

Sub-clinical hypothyroidism is defined as a serum TSH concentration above the statistically defined upper limit of the reference range when serum free T_4 (FT_4) concentration is within its reference range (Ross 2001). Other causes of an elevated serum TSH must be excluded, for example: recent adjustments in levothyroxine dosage with failure to reach a steady state (Surks and Oppenheimer 1973) particularly in poorly compliant patients; transient increase in serum TSH in hospitalized patients during recovery from severe illness (Wong *et al.* 1981) or during recovery from destructive thyroiditis, including postviral subacute thyroiditis and postpartum thyroiditis; untreated primary adrenal insufficiency (Gharib *et al.* 1972) patients receiving recombinant human TSH injections (Ladenson *et al.* 1997); and the presence of heterophilic antibodies against mouse proteins, which cause falsely high TSH concentrations in some assays. (Wood *et al.* 1991) Although central hypothyroidism (usually hypothalamic) may cause a mildly elevated serum TSH concentration (due to a circulating bioinactive TSH molecule), the serum FT_4 concentration is generally clearly low in these patients (Beck-Peccoz *et al.* 1985).

Some investigators suggest that the upper limit of normal for serum TSH concentration should be 2.5 mIU/L in a population rigorously screened to exclude thyroid disease or drugs that influence thyroid function. In support of this position is a higher rate of progression to overt hypothyroidism and a higher prevalence of antithyroid antibodies in individuals with serum TSH higher than 2.5 mIU/L compared with those with serum TSH

between 0.5 and 2.5 mIU/L (Vanderpump *et al.* 1995) Although a serum TSH concentration higher than 2.5 but less than 4.5 mIU/L may identify some individuals with the earliest stage of hypothyroidism and those suspect for Hashimoto thyroiditis, there is no evidence for associated adverse consequences. Furthermore, serum TSH concentrations between 2.5 and 4.5 mIU/L may be due to minor technical problems in the TSH assay, circulating abnormal TSH isoforms, or heterophilic antibodies; normal individuals with serum TSH concentrations in this range would be misidentified as having hypothyroidism. Given these concerns as well as the pulsatile nature and continuous distribution of serum TSH concentrations, the panel defined the reference range of normal serum TSH concentration as 0.45 to 4.5 mIU/L.

The panel evaluated the strength of the evidence for the association of untreated sub-clinical hyperthyroidism and the following clinical outcomes: progression to overt hyperthyroidism, adverse cardiac end points, atrial fibrillation, cardiac dysfunction, systemic and neuropsychiatric symptoms, reduced bone mineral density, and fractures (Rockel *et al.* 1987). The panel also assessed the strength of the association between the TSH level and the risks and benefits of treatment Similar to the approach taken in many reported studies, the panel classified patients with sub-clinical hyperthyroidism into 2 categories: those with mildly low but detectable serum TSH (0.1-0.45 mIU/L) and those with a clearly low serum TSH (<0.1 mIU/L). In all clinical settings, causes of subnormal serum TSH concentration other than sub-clinical hyperthyroidism must be excluded.

Screening for Sub-clinical Thyroid Diseases:

The rationale for population screening hinges on the high prevalence of sub-clinical thyroid dysfunction in the child population and on the potential health benefits and risks of detecting and treating these diseases. We used the US Preventive Services Task Force criteria (US Preventive Services Task Force 2002) for recommending a screening test, which requires evidence of effectiveness of early detection. One of the most important criteria for recommending a screening test is that screening asymptomatic persons and treating them for the condition should result in improved measurable and important health outcomes when compared with persons who are not screened and who present with signs or symptoms of the disease. An alternative to population screening is aggressive case finding, defined as the application of a test to a person presenting to a clinician for a

reason usually unrelated to the test being applied to determine the person's likelihood of having a particular disease or condition.

Thyroid dysfunction is more prevalent in certain population groups, including women older than 60 years, persons with previous radiation treatment of the thyroid gland (radioactive iodine or therapeutic external beam radiation), those who have had previous thyroid surgery or thyroid dysfunction, and those who have type 1 diabetes mellitus, a personal history of autoimmune disease, a family history of thyroid disease, or atrial fibrillation. The panel recommends aggressive case finding in these high-risk groups. The panel also endorses thyroid function testing (serum TSH measurement) for patients seeking medical care who have signs or symptoms suggestive of thyroid dysfunction (Ladenson *et al.* 2000) or those being evaluated for palpable thyroid abnormalities.

The panel recommends against population-based screening for thyroid disease. Case ascertainment in certain high-risk groups is encouraged. The panel finds the evidence insufficient to recommend for or against routine determination of TSH levels (screening) in pregnant women or women planning to become pregnant. It is reasonable to consider serum TSH measurement for women with a family history of thyroid disease, prior thyroid dysfunction, symptoms or physical findings suggestive of hypothyroidism or hyperthyroidism, an abnormal thyroid gland on examination, type 1 diabetes mellitus, or a personal history of an autoimmune disorder.

3.1.12. Thyroid Dysfunction:

A. Hypothyroidism.

When thyroid hormone concentrations are low many metabolic processes slow down. Typical presenting symptoms include: fatigue, slowing of physical and mental performance, hoarseness, cold intolerance, constipation, dry skin and coarse hair.

Secondary and tertiary hypothyroidisms are defined as pituitary TSH deficiency and hypothalamic TRH deficiency, respectively. Secondary hypothyroidism is likely, in the setting of low T4, with inappropriately low serum TSH levels that do not increase after TRH administration. This usually is associated with deficiencies of other anterior pituitary hormones; however, isolated thyrotrophic failure has also been reported. Tertiary hypothyroidism is suggested by inappropriately low plasma TSH levels, normal or exaggerated response to exogenous TRH, and imaging evidence of hypothalamic or

pituitary stalk disease. Secondary and tertiary hypothyroidisms are very rare compared to primary hypothyroidism.

Primary hypothyroidism

A low concentration of serum T4 and an increased level of TSH is common. Chronic lymphocytic thyroiditis (Hashimoto's) is the most common form (affecting approximately 2% to 4% of the population in the 5th decade) and frequently serum antibodies toward thyroglobulin or thyroid microsomal antigen can be found. When diagnosed, most patients with Hashimoto's thyroiditis are clinically and biochemically euthyroid a firm bosselated goiter usually leads to the biochemical confirmation. Stimulation testing is usually not needed in the evaluation of hypothyroidism.

B. Hyperthyroidism.

This clinical syndrome is due to excessive circulating thyroid hormone. The signs and symptoms are associated with increased tissue metabolic activity and apparent increased tissue sensitivity to catecholamines. The clinical presentation includes: nervousness, heat intolerance, weight loss despite increased appetite, warm moist skin, increased perspiration, palpitations, systolic hypertension, and onycholysis.

The most common spontaneous form of hyperthyroidism is Graves' disease. It is 6-fold more common in women than men and occurs with a frequency of 0.4% in the general population. This is an autoimmune of disorder associated with the generation of thyroid-stimulating Immunoglobulins (TSIS) that act as agonists at the TSH receptor. Two other components of Graves' disease are an infiltrative ophthalmopathy and dermopathy also presumably on an autoimmune basis. Other causes of hyperthyroidism include: hyperfunctioning solitary thyroid adenoma, toxic multinodular goiter, lymphocytic thyroiditis with low thyroid radioactive iodine uptake, TSH-producing pituitary tumor, trophoblastic tumor (e.g., hydatidiform mole), and struma ovarii (ovarian teratoma with follicular thyroid tissue).

The diagnosis of hyperthyroidism is usually straightforward: elevated serum T4 concentration and suppressed serum TSH concentration. The most common subtype, Graves' disease, can be confirmed with finding an elevated plasma TSI and high thyroid radioactive iodine uptake. The thyroiditis forms of hyperthyroidism are unique in that the

hyperthyroxinemia is partly due to damage to the thyroid gland and release of stored thyroid hormone thyroid radioactive iodine uptake in this circumstance is very low.

3.1.13. Distribution of Thyroid Disorders:

a) Global Distribution of IDD (Awwal 1999).

Worldwide endemic goiter is distributed in certain restricted localities, away from the sea, at the head of rivers and in isolated valleys with poor soil and high rainfall. In the tropics it is found in Africa, Central & South America & in Asia. Papua New Guinea has been the focus of much research.

In Africa it is a problem in the Atlas Mountain, the Nile valley and high land of Kenya, Rwanda, Burundi, Cameroon, Gambia, Congo and Nigeria. It is present in the Central & South America. In Asia it is found in the Himalayas-from the Pamirs to Kashmir, Nepal, Myanmar, China and also in Thailand, Vietnam and Malaysia (Cook 1996).

About 29% of the world population lives in iodine deficient areas. In the majority of these areas attempts to control IDD have not yet been undertaken. The most recent estimate shows a higher prevalence of goiter (about 12% of world's population) in spite of extensive iodination programs (WHO/UNICEF/ICCIDD 1993). Distribution of IDD in different continents is shown in table.

Table 1. The global distribution of goiter (WHO Region).

Regions	Population	At risk	TGR
Africa	550	181	86
America	727	168	63
Eastern Mediterranean	406	173	93
Europe	847	141	97
South East Asia	1355	486	176
Western Pacific	1553	423	141
Total	5438	1572	655

Numbers in millions. Source: WHO/UNICEF/ICCIDD 1993.

b) Distribution in ASIA:

The largest population suffering from IDD is found in Asia. A significant proportion of the people who live in China, India, and Indonesia are at risk of IDD (IDD News letter 1989, Hetzel 1989, WHO 1985). According to the national survey of Bhutan in 1983, 100% people of that country are at risk of IDD and the over all prevalence of cretinism is about 7% (Kochupillai *et al.* 1986).

In the South and South East Asian region alone, about 486 million people are at risk of IDD (WHO / UNICEF /ICCIDD 1993).In South East Asia, more than 176 million people have goiter, perhaps 6 million are cretins and another 36 million suffer from some degree of mental and motor impairment as a result of IDD (Heywood and Marks 1993, WHO/UNICEF/ICCIDD 1993). Researchers from India found 20% incidence of nerve deafness and borderline mental sub normality in more than half of the school children in their study population (Kochupillai *et al.*1986).

Table 2. The Prevalence of Goiter in eight countries of WHO South East Asia Region

Country	Total Population	% of population at risk	TGR
Bhutan	14.46	100.0	64.5
Nepal	16.386	100.0	57.6
Bangladesh	127.00	100.0	47.0
Sri-lanka	16.099	100.0	19.3
Thailand	52.708	100.0	15.0
Burma	39.92	100.0	14.3
India	746.00	100.0	7.3
Indonesia	161.003	100.0	6.3

Number in million. Source: WHO 1985 and Updated by Awwal 1999.

In the People's Republic of China, it has been estimated that more than 330 million people (30%) are living in iodine deficient area and therefore, exposed to the risk of IDD. Out of this 330 million at risk, some 14 million have goiter and there are over 200,000 cretins and many more are suffering from milder degree of iodine deficient mental and neurological retardation (Hetzel 1988).

A constant etiological factor for goiter in the countries of the central Asia and the Middle East is I D. In Iraq and Afghanistan, the prevalence of goiter is about 50%. Iran is also affected by ID.A goiter survey was done in school children near Teheran in 1982 and more than 85% of the school children were found to have goiter (Bastani 1985).

c. Thyroid Disorders in Bangladesh:

Endemic goiter owing to iodine deficiency remains a significant problem worldwide, especially in underdeveloped and developing countries Thyroid disorders such as goiter and cretinism, are recognized as a global public health problems.

The prevalence of thyroid disorders in Bangladesh is very high. These are broadly the problems of iodine deficiency disorders (IDD), which have no age barrier and entail lifelong follow-up and care. In childhood, thyroid disorders are easily detectable but on the other end of the spectrum the clinical presentation of thyroid diseases in the elderly is often undetected. The physicians must be continuously on guard for the wide range of signs and symptoms suggesting thyroid disorders. Bangladesh is the largest delta in the world, major rivers, The Ganges, The Bramaputtra, Tista and the Meghna form the huge delta. It is also situated in the monsoon zone in Asia.

The most important single factor, which contributes to the incidence of goiter, is the inadequate content of iodine in the food and water, resulting in insufficient dietary intake of iodine. An adult body contains 20-50 mg of iodine. About 10 mg of this amount is concentrated in the normal thyroid gland. In case of simple goiter this amount may be reduced to about 1 mg. The lack of iodine within the body creates a heavy burden on the thyroid gland to absorb more iodine for synthesis of the hormones essential for normal body metabolism.

The latest national iodine deficiency disorders surveys in 1993 conducted jointly By University of Dhaka, International Council for control of Iodine Deficiency Disorders

(ICCIDD) and UNICEF shows that 47.1% of the population of Bangladesh is suffering from Goiter.

Alam *et al.* (1995) in a hospital based study at the Institute of Nuclear Medicine (INM), Banghabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh found the spectrum of thyroid disorders as follows:

Simple Goiter	----------	35.36%
Solitary Nodule	----------	26.80%
Hyperthyroid	----------	23.57%
Hypothyroid	----------	10.12%
Thyroiditis	----------	1.56%
Ca.Thyroid	----------	2.58%.

Since patients are referred to INM from all over the country, the pattern of the thyroid disorders observed here may be considered as the spectrum of thyroid disorders in the country.

Siddique *et al.* (1992) in a similar type of study also done in INM and found spectrum of disorders as follows.

Diffuse Goiter.---------- 30.09%

Simple nodular Goiter.---------- 34.83%

Multinodular goiter.---------- 13.86%

Hyperthyroidism.----------5.09%

Hypothyridism.----------5.80%

Thyroiditis.---------- 1.30%.

Khalilullah *et al.* (1996) in another community-based study in greater Dinajpur District of Bangladesh found the prevalence of thyroid disorders as follows:

Goiter---------- 29.80%

Hyperthyroid.---------- 5.79%

Hypothyroid.---------- 2.02%

Subclinical hyper.---------- 12.09%

Subclinical hypo.---------- 15.37%

Thyroid disease profile at Nuclear Medicine center (NMC) Chittagong, Bangladesh was as follows:

Normal---------- 28.23%

Cretinism.---------- 0.90%

Diffuse Goiter.---------- 28.96%

IDG.---------- 3.27%

Subclinical hyper.---------- 0.16%

Subclinical hypo.---------- 1.22%

MNG.---------- 4.09%

Hyperthyroidism.---------- 11.37%

Hypothyroidism.----------5.15%

Cold nodular Goiter.---------13.42%

Thyroiditis.----------3.19%

Faisal Kabir *et al.* (1992) showed the thyroid disorders in greater Rajshahi District of Bangladesh as follows

Euthyroid with goiter---------- 61.85%

Hypothyroid.---------- 8.72%

Hyperthyroid.---------- 12%

IDG.---------- 13.49%

Paul *et al.* (1998) carried out a study in Greater Khulna district of Bangladesh and found the incidence of goiter as follows,

Diffuse goiter.---------- 41.60%

Hypothyroidism.---------- 34.11%

Solitary Cold nodule.----------11.40%

Hyperthyroidism.---------- 10.85%

Nontoxic multinodular goiter.---------2.01%

Thyroiditis.---------- 0.44%.

INM, Dhaka 2000 shows,

Simple goiter---------- 38.75%

Solitary nodule.---------- 19.66%

Hyperthyroid.----------22.30%

Hypothyroid.---------- 16.62%

Cancer thyroid.----------2.53%

Others.---------- 0.24%.

3.1.14. Clinical Presentation of Thyroid Dysfunction

a. Signs And Symptoms of Hypothyroidism.

(DiGeorge and Lafranchi, 1996.)

Ectodermal:

Poor growth

Dull faces-thick pale lips, large tongue, depressed nasal bridge, periorbital oedema.

Dry scaly skin.

Sparse brittle hair

Diminished sweating

Carotenemia

Vitiligo

Circulatory:

Sinus bradycardia / heart block.

Cold extremities

Cold intolerance

Pallor

ECG Changes

Low voltage QRS complex.

Neuromuscular:

Muscle weakness.

Hypotonia-constipation, potbelly

Myxedema coma

Pseudohypertrophy of muscles

Myalgia

Physical and mental lethargy

Delayed relaxation of reflexes

Paresthesis

Umbilical hernia

Cerebral atxsia

Metabolic:

Myxoedema

Serous effusion

(Pleural.pericardial)

Hoarseness of voice (Cry)

Weight gain

Menstrual irregularities

Arthralgia

b. Clinical Manifestation of Hyperthyroidism:

Increased catecholamine effect:

Nervousness,

Palpitation

Tachycardia

Atrial arrhythmias

Systolic hypertension

Tremor

Brisk reflex

Hypermetabolism:

Increased sweating

Shiny, smooth skin

Heat intolerance

Fatigue

Weight loss-increase appetite

Increase bowel movement

Myopathy:

Weakness

Periodic paralysis

Cardiac failure

Miscellaneous:

Proptosis

Stare, exopthalmos, lid lag

Hair loss

Inability to concentrate

Personality changes

Goiter

Thyroid bruit

Acute thyroid storm.

3.1.15. Methods of Evaluation of Thyroid Status:

a) Diseases of thyroid gland almost always manifest themselves through symptoms resulting from excessive or insufficient production of thyroid hormones or through local symptoms in the neck, particularly goiter or in the case of Grave's disease through exopthalmos. The physician's attention is directed initially at the major signs as seeks to establish both a functional and anatomical diagnosis. The functional diagnosis is based upon a carefully taken history, a through search for the physical signs of hypothyroidism or thyro-toxicosis, and an intelligent appraisal of the results of laboratory tests. The anatomical diagnosis will depend largely upon the examination of the thyroid gland itself.

The physician should first inspect the neck from the front and sides. The presence of old surgical scars, distended veins, and redness or fixation of the overlying skin should be noted. If a mass is present, attention should be directed to its location and whether or not it moves on swallowing. Movement on swallowing is a characteristic feature of the thyroid gland.

Palpation of the neck is best accomplished by standing behind the seated patient. Position of the cricoid cartilage is first noted, the examiner then attempts to outline the thyroid gland and to determine the limit of the lower border of the lateral lobes. While the patient swallows sips of water at appropriate intervals. A normal thyroid gland can usually be felt on palpation. The examiner should note the size, shape of the gland and consistency. A normal gland feels rubbery. Softer than normal in case of diffuse colloid goiter and the hyperplastic gland of Grave's disease, Stony hard in the case of carcinoma.

Auscultation of the neck should be performed since it gives some indication of the vascularity of the gland. A systolic or continuous bruit is commonly heard over a hyperplastic gland.

In addition to examination of the thyroid gland and regional lymph nodes, evidence of compression or displacement of adjacent structures should also be sought. Once you have examined the thyroid gland you should be able to draw one of the following conclusion about the gland and about its activity.

The gland	**Activity of gland.**
1. Contains one palpable nodule	1. Normal.
2. Contains more than one palpable nodule	2. Hyper secretion.
3. If diffusely enlarged	3. Hypo secretion.

(Browse 1985).

b. The function of the thyroid gland may be evaluated in many different ways.

1) Test of thyroid hormones in blood.
2) Evaluation of the hypothalamic-pituitary-thyroid axis.
3) Assessment of iodine metabolism.
4) Evaluation of thyroid gland size.
5) Thyroid biopsy.
6) Observation of the effects of thyroid hormones on peripheral tissues and
7) Measurement of thyroid auto-antibodies.

It is common to use more than one method to evaluate thyroid status. Most commonly clinical and biochemical assessment is done for determination of iodine status of an individual (Greenspan 1983).

c. Thyroid function tests:

Thyroid function tests are divided into *in vitro* and in *vivo* studies.

a) *In vitro* tests.

1) Hormones: T3
T4
FT4
FT3

TSH

RT3 (reverse T3)

2) Protein. Thyroglobulin

3) Anti-thyroid antibodies:

Anti-Tg antibody.

Anti-microsomal antibody.

Anti-TSH receptor antibody (Stimulating type)

Anti-TSH receptor antibody (Blocking type)

4) Urinary excretion of iodine.

b) *In vivo* tests.

1) Common.

a) I 131 uptake

b) Thyroid scan

c) Ultrasonography.

2) Uncommon.

TSH stimulation test.

T3 suppression test

Perchlorate washout test.

TRH stimulation test.

Several different laboratory tests are employed in the evaluation of thyroid function, but the more common immunoassays used for routine diagnostic purposes are for the two iodothyronine, T3 and T4 and also those for thyrotropin (THS) and free thyroxine (FT4). The physicians faced with overt symptoms of hypo or hyperthyroidism has little difficulty in making a diagnosis without confirmatory laboratory tests, although these are often useful in planning subsequent treatment. Many patients, however, are found to have thyroid disorders on the basis of laboratory tests alone, particularly when an investigation is initiated from specialty, such as neurology or psychiatry. Because the spectrum of

thyroid disease merge so gradually with that of euthyroid state, it has become quite common to classify the thyroid status of an individual almost exclusively on the basis of the thyroid biochemical profile (Malan 1982).

c. Main Thyroid Function Tests:

T3/T4 levels free and bound calculated indirectly by resin binding assays. True values can be obtained but tests are difficult and involve dialysis of the sample.

T4- (l-5 to 13 ug/dL). Free T4 (l 0.8-2.3 ng/dL)

T3- (80-200 ng/mL)

Thyroxine binding globulin (2-4.8 mg/Dl)

Radio-iodine uptake (l 5-35% in 24 hrs)

TSH levels, serum.

Radioiodine scan or technetium scan (Cold or Hot nodules).

d. Hormone estimation:

The introduction of radio-imunologic assay (RIA) methods by Yalow and Berson in 1959 provides superior measurement for serum T3, T4 and TSH. Normal values for T3 ranges from 5 to 20 nm/l and 5 .5 to 12.5 microgram/d for T4. Normal values are generally higher for women, but they fall below 10 micro IU/ml for both.

The measurement of T4 by RIA has been particularly useful in differentiating hypothyroidism and hyperthyroidism from the euthyroid. Measurement of TSH is the most reliable method available for validating the adequacy of thyroid hormone replacement in hypothyroidism. Accurate measurement of TSH also allows the detection of more subtle forms of thyroid diseases. The ability to assay the alpha and beta subunits has permitted identification of pituitary or TSH – induced hyperthyroidism.

Direct measurement of very small quantities of free T3 and T4 is not possible without the use of radioactive tracers.

e. I-131 Uptake: The thyroid uptake is the percentage of an administered radio-pharmaceutical incorporated by the thyroid gland in a defined period of time. If the radio-pharmaceutical is administered orally, the measured uptake increases progressively reaching a plateau between 18 to 24 hours after intake.

The normal thyroid uptake values vary with the environment, so each laboratory must establish a normal range for its particular patient population. Higher levels of iodine in processed foods have substantially lowered the normal range in most regions. The normal 24 hours I-131 thyroid uptake is 6 to 30%. The wide variations produce some overlap between euthyroid and hyperthyroid ranges and serves to emphasize that the uptake alone does not permit a definition of functional thyroid state (Herbert 1996).

f. Sonography of the Thyroid Gland:

The normal thyroid parenchyma has a characteristic sonographic appearance of homogenous medium to high-level echoes, with little identifiable internal architecture. This uniform background makes detection of focal thyroid lesion relatively easy in most cases. The strap muscles (Sternohyoid and sternothyroid) of the neck are seen as thin sonolucent band along the anterior surface of thyroid gland. The larger sternocleidomastoid muscles are located further anterio-laterally. Each thyroid lobe is bounded laterally by the common carotid artery and jugular vein, which serve as useful anatomical landmarks. Posterior and slightly lateral to each thyroid lobe is the longus coli muscle, which is seen as a wedge shaped sonolucent structure anterior to the cervical vertebrae.

The air filled trachea in the midline gives a characteristics curvi- linear reflecting surface with associated reverberation artifact. Often the esophagus may swing laterally, usually towards the left where it may lie adjacent to postero-medial surface of the thyroid. However usually the esophagus is hidden from sonographic visualization by the tracheal air shadow. Each lobe has a smooth globular-shaped contour and is no more than 3-4 cm in height, 1-1.5 cm in width, and 1 cm in depth. The isthmus is identified, anterior to the trachea as a uniform structure that is approximately 0.5 cm in height and 2-3 mm in depth. The pyramidal lobe is not identified unless it is significantly enlarged. In females the upper pole of the each lobe may be seen at the level of the thyroid cartilage, lower in male. The parathyroid glands are observed only when they are enlarged and are less dense ultrasonologically than thyroid tissue because of the absence of iodine.

Thyroid sonography is not cost effective in evaluating the average patient with thyroid enlargement. Since thyroid goiters are common and rarely associated with malignancy, there is little useful purpose to sonographically document the size, shape, or uniformity of a goiter. However, sonography may be used effectively to answer a specific clinical

question about a patient with a goiter. At times, it will be useful to know the ultrasonic appearance of a dominant nodule in a goiter, a tender spot, a region of focal hardness because it might give a clue about pathology. For example, sonography can identify one region in a goiter whose echo pattern is distinct from the rest of the goiter suggesting a second type of pathology, especially if the region is surrounded by a sonoleucent rim. Among the lesions that have been demonstrated in goiters using sonography are neoplasm and lymphoma. Other uses of sonography in goitrous patients include: differentiation of thyroid enlargement from adipose tissues or muscle, identifying a large unilateral mass in distinction to an asymmetric goiter, confirming substernal extension, providing the correct interpretation to varying clinical impressions among several examiners, and objectively documenting volume changes in response to suppressive therapy with thyroid hormone which may be particularly useful when patients change physicians.

An interesting public health use of sonography in underdeveloped countries has been to objectively identify goiter as a screen for iodine deprivation.

3.2. LIPIDS

3.2.1. Physio-Bio Chemistry of Lipid

The lipids are heterogenous group of compound related actually or potentially to fatty acids. They have common property of being relatively insoluble in water and soluble in non-polar solvents such as ether, chloroform and benzene (Mayes, 1998) lipids constitute over 10% of the body weight of normal adult individual. Fatty percent of total caloric requirement per day are derived from lipid. Fat absorbed from the diet and lipids synthesized by the liver and adipose tissue are transported between tissue and organs for utilization and storage. Since lipids are insoluble in an aqueous medium, so their transport in the plasma presents a special problem. This problem is solved by associating non-polar lipids (triglycerol and cholesteryl ester) with amphipathic lipids and proteins to makes water soluble lipoproteins are called apo-proteins. Cholesterol and triglycerides are the two main lipids in the blood.

Classification of total cholesterol –

Total cholesterol mg/dl

<200 – desirable

200-239: Borderline high

>240 : High

Lipoproteins

Lipoproteins are water soluble complex of high molecular weight composed of lipids (cholesterol, triglycerides, phospholipids) and one or more specific proteins, called apo-proteins. Lipoproteins represented the functional unit of for water soluble lipids in the blood (Assman 1982). The lipoproteins are divided into various categories according density, as determined by ultracentrifugation (Mayes et al, 2007)

Chylomicrons: Chylomicrons formed in the intestine, transport exogenous triglycerides, composed of 98.00-99.50%lipid and 1.2% protein. It floats to form a superficial layer on serum when allowed to stand overnight (Assman 1982). Partially degraded chylomicrons, called chylomicron remnants, carry some atherogenic potential.

Functions of cholesterol:

Cholesterol is essential to life, as it performs a number of important functions. It is a structural component of cell membrane.

1. Cholesterol is a precursor for the synthesis of all other steroids in the body. These include steroid hormones, vitamin D and bile acids.
2. It is an essential ingredient in the structure of lipoproteins in which form the lipids of the body are transported.
3. Fatty acids are transported to liver as cholesteryl ester for oxidation.

Very low density lipoproteins (VLDL) or Pre-β lipoproteins :

Very low density lipoproteins formed in the liver, transport the bulk of endogenous triglycerides and consist of 90-93% lipid and 7-10%protein. These are the precursor for CDC. Some forms of VLDL remnants are atherogenic similar to CDL, VLDL remnants consists particularly of degraded VLDL and are relatively rich in cholesteryl ester. Strictly speaking, IDL (Intermediate density lipoproteins) belong to VLDL remnants, although in clinical practice IDL included in the LDL fraction. A normal VLDL cholesterol can be defined as that present when triglycerides are <150mg/dl; this value typically <30mg/dl (Heiss *et al.* 1980) conversely when triglycerides level >150mg/dl, VLDL value is usually >30mg/dl.

Intermediate density lipoproteins (IDL):

Intermediate density lipoproteins are formed in significant amount the presence of metabolic disease only. It is present at low concentrations are through to be metabolic products of VLDL and precursor particles of LDL (Assman 1982).

Low density lipoproteins (LDL)

Low density lipoprotein transports the bulk of the total cholesterol (60-70%) in the blood. LDL arises as metabolic products of VLDL and contains approximately 79% lipid and 21% proteins. LDL is the major atherogenic lipoproteins and has long been identified by NCEP as the primary target of cholesterol lowering therapy.

ATP classification of LDL cholesterol:

LDL mg/dl	**Classification**
<100	Optimal
100-129	Near optimal /above normal
130-159	Borderline high
160-189	High
≥190	Very high

LDL cholesterol level as low 25-60mg/dl is physiologically sufficient (Brown and Goldstein 1986). Animal species that can not develop atherosclerosis generally have LDL cholesterol level below 80mg/dl. LDL concentrations approximately 30mg/dl, indicating that such low levels are safe. Moreover, persons who have extremely low levels of LDL throughout life due to familial hypobetalipoproteinaemia have documented longevity.

HDL (alpha-lipoproteins):

High density lipoproteins contain approximately 50% lipid and 50% protein. It is produced by the intestine and liver in precursor form, they then fully developed in plasma capable of taking up cholesterol from cells and transporting it back to liver. HDL subfractions such as HDL1, HDL2 and HDL3 are differentiated on the basis of varying composition as well as structural and functional properties (Assman *et al.* 1982). There are several factors that contribute to low HDL levels that need to be identified in clinical practice. (Stone 1994, Chait and Brunzell 1990, Krauss 1982).

Classifications:

<40mg/dl	Low HDL cholesterol
≥60mg/dl	High HDL cholesterol

Triglycerides:

Lipoprotein metabolism is integrally linked, and elevations of serum triglycerides can be confounded by significant correlation with total LDL and HDL cholesterol levels.

Classification of TG:

TG categories	Levels
Normal	<150mg/dl
Borderline high	150-199mg/dl
High	200-499mg/dl
Very high	>500mg/dl

Triglyceride rich lipoproteins are (TGRLP) remnant lipoproteins; these lipoproteins include small very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL). They are cholesterol enriched particles and have many properties of LDL.

Apolipoproteins (Mays PA et al, 2007)

One or more apolipoproteins are present in each lipoprotein. They have amino acid sequences and functional properties. According to ABC nomenclature, the main apo-proteins of HDL (α-lipoprotein) are designated as A (A-I, A-II). The main apo-protein of LDL (β-lipoprotein) is apo-β. Apo-β (β-100) is found also in VLDL. Chylomicron containing a truncated apo-β (β-48) that is synthesized in the intestine, which β-100 in the liver. Besides these, other apo-proteins i.e. C-I, C-II, CIII, D,E are also in various lipoproteins. They serve as cofactors for enzyme involved in lipoprotein metabolism (Champe and Harvey 1987).

Functions of apolipoproteins:

(1) They can form part of the structure of lipoprotein e.g. apo-B.

(2) They are enzyme cofactor e.g. C-II for lipoprotein lipase, A-I for LCAT.

(3) They act as ligands for interaction with lipoprotein receptors in tissues e.g. apo B-100 and apo E for LDL receptor; apo E for LDL receptor related protein (LRP), which has been identified as the remnant receptor and apo A-I for HDL receptor. The function of apo A-IV and apo D; however are not yet clearly defined, although apo D is believed to be important factor in human neurodegeerative disorders.

3.2.2 Dyslipidemia:

Dyslipidemia is a disorder of lipoprotein metabolism including lipoprotein over production or deficiency. It may be manifested by elevation of the total cholesterol, the low density lipoprotein (LDL) and triglyceride (TG) concentrations and a decrease in the high density lipoproteins (HDL) in the blood. It is also called dyslipidemia.

Dyslipidemia is a major risk factor for atherosclerotic cardiovascular diseases, the most common cause of death in the USA and the most European countries. In future it will be the number one killer of developing countries like Bangladesh also. More than 50% patients with unstable angina are dyslipidemic (Siddique *et al.* 2005).

Types of dyslipidemia:

(1) Primary

(2) Secondary

Primary: Several monogenic disorders have been defined that lead to different type of dyslipidemias but for many cases, the etiology is polygenic. These disorders affect plasma lipoprotein levels, by overproduction of lipoprotein and/or by decreased clearance e.g, for ↑LDL.

(1)Heterozygous familial hypercholesterolemia

(2)Homozygous familial hypercholesterolemia

(3)Familial defective apo-lipoprotein B-100

(4)Polygenic hypercholesterolemia

For elevated triglycerides:

(1)Familial combined hyperlipidemia

(2)Familial hypertriglyceridemia

(3)Polygenic hypertriglyceridemia

(4)Familial dysbetalipoproteinemia

For low HDL:

As those for elevated triglycerides, since elevated triglycerides are usually associated with low HDL. Genetic factors undoubtedly play an important role as well as in many persons (Cohen *et al.* 1994). In rare cases, genetic defects in metabolism of HDL alone can cause isolated low HDL.

Secondary dyslipidemias: Many medical conditions are associated with mild or even severe dyslipidemia even in the absence underlying genetic factors.

Major causes of secondary dyslipidemia:-Diabetes mellitus, Nephrotic syndrome, Obstructive liver disease, Chronic renal failure, Drugs like β-blockers, Diuretics, steroids, progestins, etc.

Family, drug and diet history may reveal clues to secondary causes of dyslipidemia. If secondary dyslipidemia is suspected, urianalysis (for protein), serum creatinine, alkaline phosphatase, blood sugar should be done. Glycated hemoglobin is a standard method of glucose control.

Atherogenic dyslipidemia

A common form of dyslipidemia is characterized by three lipid abnormalities: elevated triglycerides, elevated LDL, and reduced HDL cholesterol (Austin *et al.* 1998, Grundy 1998, Krauss 1998). Although there is evidence that each component of lipid triad is individually atherogenic, the relative quantitative contribution of each can not be determined. For this reason it is reasonable to view the lipid triad as a whole as a "risk factor".

3.2.3 Atherosclerosis

It is a generalized macrovascular disease. It has been defined (by a World Health Organization study group) as a variable combination of change of intima of arteries (as distinguished from arterioles) consisting of a focal accumulation of lipids, complex carbohydrates, blood and blood products, fibrous tissues and calcium deposits and associated with medial changes. It is synonymous with atheroma but not with arterisclerosis which is less specific term used to describe hardening of arteries and

arterioles (Julian et al. 1998). Atherosclerosis is a chronic, multifocal immuno-inflammatory, fibroproliferative disease of medium sized and large arteries mainly driven by lipid accumulation.

Components of atherosclerosis:

Atherosis- the accumulation of cholesterol-rich gruel (i.e, atheroma)

Sclerosis- the expansion of fibrous tissue

Inflammation- which involves monocytes/macrophages, T lymphocytes and mast cells (Newby 2006).

The pathogenesis of atherosclerosis:

Atherosclerosis results from interaction between arterial wall blood constituents. A number of elements play important roles:

The endothelium;

Monocytes/Macrophages;

Smooth muscle cells;

Platelets;

Blood lipids (Julian *et al.* 1998).

Atherogenesis is a multi-step process beginning relatively early in life. The first stage is the fatty streak, which consists largely of cholesterol-filled macrophages; most of cholesterol in the fatty streak is deriving from LDL cholesterol. The second stage consists of fibrous plaques in which a layer of scar tissue overlies a lipid rich core. Other risk factors contribute to plaque growth at this phase. The third stage is represented by the development of unstable plaques that are prone to rupture and formation of luminal thrombosis. Plaque rupture (or erosion) is responsible for the most acute coronary syndrome (myocardial infarction, unstable angina, and coronary death) (Libby *et al.* 1998, Fuster *et al.* 1999; Théroux and Fuster 1998) Atherosclerosis is not a continuous linear process but rather a disease with alternate phases of stability and instability.

3.2.4 Clinical atherosclerotic diseases

(a) Coronary Artery Disease (CAD)

Cardiovascular diseases are presently the leading cause of death in industrialized countries and expected to become so in emerging countries by 2020 (Murray *et al.* 1996). Among these, coronary artery disease (CAD) is the prevalent manifestation and is associated with high mortality and morbidity. Clinical presentations of ischaemic heart disease include silent ischaemia, stable angina pectoris, unstable angina, myocardial infarction (MI), heart failure, and sudden cardiac death. This is life threatening state atherothrombotic disease. In spite of modern treatment, the rates of death, MI, and re-admission of patients with ACS (acute coronary syndromes) remain high.

(b) Non-coronary artery diseases

Clinical forms of non-coronary atherosclerotic disease carry a risk for clinical CHD approximately equal to that established CHD and hence contribute a CHD equivalent. These conditions include -

1. Carotid artery disease.

(a)Transient Ischaemic Attack(TIA)

(b) Stroke of carotid origin

(c) >50% stenosis of carotid artery on angiography or ultrasound (Ferguson *et al.*1999, Barnett *et al.* 1998, Norris *et al.*1991).

2. Peripheral artery diseases (PAD) which is indicative of CHD risk equivalent (Chambless *et al.*1997, Hodis *et al.*1998, Salonen and Salonen 1991).

(a) Reno-vascular disease leading to CRF

(b) Intermittent claudications and ischaemic ulcers

PAD is diagnosed by the ankle/brachial blood pressure Index (ABI), by lower limb blood flows and or clinical symptoms. Different studies (Leng *et al.* 1996, Vogt *et al.*1993, McKenna *et al.*1991, Poulias *et al.*1992) support the concept that PAD where diagnosed by ABI, lower limb blood flow studies, or clinical symptom, is a CHD risk equivalent.

3.2.5 Atherosclerosis and Lipids

a. Atherosclerosis and Total cholesterol

Atherosclerosis generally can first be identified by gross pathological examination of coronary arteries in adolescence or early adulthood (McGill *et al.* 1997, 1998, 2000). The

subsequent rate of atherogenesis is proportional the severity of ambient risk factors including serum cholesterol levels. Moreover, the cholesterol level in young adulthood predicts development of CHD later in life. In three prospective studies with long term follow up (Anderson *et al.*1987, Klag *et al.*1993, Stamler *et al.* 2000), detection of elevated serum cholesterol in early adulthood predicted an increased incidence of CHD in middle age.

High total cholesterol is a marker for atherogenic lipoproteins. In population studies, the serum total cholesterol is a good surrogate for LDL cholesterol levels. The Framinham Heart Studies (Wilson *et al.* 1998). The Multiple Risk Factors Intervention Trial (MRFIT) (Stamler *et al.*1986), and the Lipid Research Clinic (LRC) trial (Lipid Research Clinic Program 1984) found a direct relationship between levels of LDL cholesterol (or total cholesterol) and new onset CHD in men and women who were initially free of CHD. The same relation holds for recurrent coronary events in people with established CHD (Rossouw *et al.* 1990, Pekkanem *et al.* 1990).

Studies across different populations reveal that those with higher cholesterol levels have more atherosclerosis and CHD than do those having lower levels (McGill 1968, Keys *et al.* 1980, 1984). People who migrate from regions where average serum cholesterol in general populations is low to areas with high cholesterol levels show increases in their cholesterol levels as they acculturate. These higher levels are accompanied by more CHD (Law *et al.* 1994). Only in populations that maintain very low levels of serum cholesterol, e.g., total cholesterol <150mg/dl, or LDL cholesterol<100/dl throughout life do we find a near absence of clinical CHD (Keys *et al.*1980, Law *et al.*1994, 1999, Grundy *et al.*1990)

Three ranges of total cholesterol are compared : <200,200-239mg/dl and ≥240mg/dl; these ranges approximately corresponds to LDL cholesterol ranges of <130, 130-159mg/dl, and ≥160mg/dl.Increased life time risks associated with high total cholesterol levels (>240mg/dl) which corresponds to categorically high LDL cholesterol (≥160mg/dl) are clearly evident and justifies clinical therapies to reduce long term risk. But even borderline high total cholesterol 200-239mg/dl carries significant long term risk and deserves clinical intervention.

b. Atherosclerosis and LDL cholesterol

Elevated LDL cholesterol is the primary driving force for coronary atherogenesis. Since LDL cholesterol levels <100mg/dl throughout life are associated with a very low risk for CHD in populations, they can be called optimal. Even when LDL cholesterol concentrations are near optimal (100-129mg/dl) atherogenesis occurs; hence, such levels must be also called above optimal. At levels that are borderline high(130-159mg/dl) atherogenesis progresses at a significant rate. These relationships are confirmed by the Log-Linear relationship between serum cholesterol levels and CHD risk observed in many populations (Law *et al.* 1994, 1999). The relationship of elevated LDL cholesterol to the development of CHD must be viewed as multi-step process beginning early in life (Stary *et al.* 1992, 1994, 1995). Also elevated LDL cholesterol plays a role in the development of mature coronary plaque, which is the substrate for the unstable plaque. Recent evidence also indicates elevated LDL cholesterol contributes to the plaque instability as well; conversely, LDL cholesterol lowering stabilizes plaque and reduces likelihood of acute coronary syndromes. Clinical intervention with LDL lowering therapy in patients with advanced coronary atherosclerosis thus aims to stabilize plaque and to prevent acute coronary syndromes (Brown *et al.*1995, 2000).

c. Atherosclerosis and Triglycerides

Many prospective epidemiological studies have reported a positive relationship between serum triglycerides and evidence of CHD (Austin *et al.* 1998; Assman *et al.*1998). Non lipid risk factors of obesity, hypertension, diabetes and cigarette smoking are also interrelated with triglycerides (Grundy 1998). Raised triglycerides are in fact an independent risk factor for CHD. This independence suggests that some triglycerides-rich lipoproteins are atherogenic. The most likely candidate for atherogenic TGRLP are remnant lipoproteins which include very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL). They are cholesterol enriched particles and have many properties of LDL. Their elevations emerged as strong predictors of coronary atherosclerosis or CHD (Tatami *et al.*1981, Steiner *et al.* 1987, Krauss *et al.*1987, Phillips *et al.*1993, Tornvall *et al.*1993, Hodis *et al.*1994, Koren *et al.*1996, Karpe *et al.*2001, Takaichi *et al.*1999, Thompson 1998, Sacks *et al.* 2000).

d. Atherosclerosis and HDL cholesterol

Strong epidemiological evidence links low levels serum HDL cholesterol to increased CHD morbidity and mortality (Wilson *et al.* 1998, Gordon *et al.* 1989, Abbott *et al.*1988). High HDL cholesterol levels conversely convey reduces risk. In fact, in prospective studies (Wilson *et al.* 1980, Assman *et al.* 1996) low HDL cholesterol usually proves to be the lipid risk factors mostly correlated with CHD. High levels of HDL appear to protect against atherogenesis (Rubin *et al.*1991, Plump *et al.*1994, Tangirala *et al.*1999). In vitro, HDL promotes efflux of cholesterol from foam cells in atherosclerotic lesions (reverse cholesterol transport).Antioxidant and anti-inflammatory properties of HDL also inhibit atherogenesis (Van Lenten *et al.* 1995, Navab *et al.* 2000). A low level of HDL correlates with the presence of other atherogenic factors (Vega and Grundy 1996). In many persons, a low HDL level correlates with elevation of serum triglycerides and remnant lipoproteins (Schaefer e*t al.*1994, Phillips *et al.* 1981). In addition, low HDL commonly shows linkage with small, dense LDL particles (Austin *et al.* 1990, Luck *et al.*1997, Rainwater 2000, Austin *et al.*2000).

e. Atherosclerosis and Non-HDL cholesterol

Since VLDL cholesterol is highly correlated with atherogenic remnant lipoproteins, it can reasonably be combined with LDL cholesterol to enhance risk prediction when serum triglycerides are high. The sum of VLD Land LDL cholesterol is called non-HDL cholesterol. It is calculated routinely as total cholesterol minus HDL cholesterol. Non-HDL cholesterol includes all lipoproteins that contain apo-B which has been shown to be strong predictive power for severity of coronary atherosclerosis and CHD events (Tornvall *et al.*1993, Sniderman 1988, Marcovina *et al.* 1988, Reinhart *et al.*1990, Sniderman *et al.* 1991, Levinson *et al.* 1992; Kwiterovich *et al.* 1992, Westerveld *et al.* 1998, Gotto *et al.* 2000, Lamarche *et al.* 1996, Lemieux *et al.* 2000).

f. Atherosclerosis and Emerging lipid risk factors

1. Lipoprotein remnants

Lipoproteins called beta-VLDL, which are apolipoprotein E-enriched remnants and typical of dysbetalipoproteinemia are almost certainly atherogenic because dysbetalipoproteinemia is accompanied by increased risk for CHD. High serum levels of lipoproteins enriched in apolipoprotein C-III, another form of VLDL remnants appear to

be atherogenic as well (Koren *et al.*1996, Sacks *et al.*2000, Alaupovic *et al.* 1997). Even so, prospective studies relating various remnant measures to CHD risk are limited and measurement with specific assays can not be recommended for routine practice.

2. Lipoprotein (a)

Several studies (Moliterno *et al.*1993, Stubbs *et al.*1998, Budde *et al.*1994, Seman *et al.*1999) report a strong association between Lp(a) levels and CHD risk. Indeed, a recent meta-analysis of reported prospective studies support an independent predictive power for elevated Lp(a). (Danesh *et al.* 2000).Issues related to measurement of Lp(a) in clinical practice have not been fully resolved (Marcovina *et al.* 1998, Marcovina *et al.* 1999).

3. Small LDL particles

They are formed in large part, although not exclusively, as a response to elevations of triglycerides. Their presence is associated with an increased risk for CHD (Austin *et al.* 1990, Miller *et al.* 1993), however, the extent to which they predict CHD independently of other risk factors is un resolved (Mykkänen *et al.*1999). Moreover, standard and inexpensive methods are not available for their measurement.

4. HDL subspecies

HDL comprises several components and sub-fractions that also have been related to CHD risk. While low HDL cholesterol is the risk indicator most often used, HDL sub-fractions (Lp AI and Lp AI/AII and or HDL3 and HDL2) have been used for risk prediction. Although small studies suggests greater predictive power of one or another HDL component, their superiority over HDL cholesterol has not been demonstrated in large, prospective studies. Moreover, measures of HDL subspecies are not readily available in clinical practice.

5. Apolipoproteins

a. Apolipoprotein-B:Apolipoprotein-B is a potential marker for all atherogenic lipoproteins. Although LDL cholesterol and apolipoprotein-B are highly correlated in persons with normal triglyceride levels, the apolipoprotein B level typically is disproportionately higher in persons with hypertriglyceridemia.ATP III takes this difference into account and sets a secondary target, non HDL cholesterol, in persons with hypertriglyceridemia. Non HDL cholesterol is significantly correlated with apolipoprotein

B and can serve as a surrogate for it. Standardized apolipoprotein B measures are not readily available, and in any case, would add expense beyond routine lipoprotein analysis.

b. Apolipoprotein A-I:Apolipoprotein A-I is carried in HDL and it is usually low when HDL is reduced. A low apolipoprotein A-I is associated increased risk for CHD ,but not independently of low HDL. Where as independent predictive power beyond HDL cholesterol is uncertain. In any case, standardized methodology for estimating this is not available.

6. Total cholesterol/HDL cholesterol ratio

Many studies show that the total cholesterol /HDL cholesterol ratio is a powerful indicator of CHD risk. Some investigators (Hong *et al.* 1991, Castelli *et al.* 1992, Criqui *et al.* 1998) propose that "cholesterol ratio" is a simple approach for lipid risk assessment. This ratio reflects two powerful components of risk. High total cholesterol is a marker for atherogenic lipoproteins, whereas low HDL cholesterol correlates with multiple risk factors of metabolic syndromes probably imparts some independent risk. Regardless, ATP III does not define total cholesterol /HDL cholesterol ratio as a specified lipid target of therapy.

TC/HDL

Values	Risk stratification
<4.5	Low risk
4.5 - 8.5	Risk
> 8.5	High risk

3.2.6 Thyroid and Lipids

Physiology of lipid metabolism and thyroid

Cholesterol is endogenously produced from acetyl-CoA throughout the action of 3-hydroxyl-3-methylglutaryl-CoA (HMG-CoA) reductase. The activity of this enzyme is mainly regulated by the intracellular concentration of cholesterol.An increase of intracellular cholesterol reduces the production of HMG-CoA reductase, where-as reduction of intracellular cholesterol leads to an increase of HMG-CoA reductase activity.

Thus the maintenance of homeostasis of intracellular cholesterol is dependent on the enzymatic activity of HMG-CoA.

A high intake of carbohydrates increases the production of free fatty acids (FFA), which are used in the liver as substrate for the production of triglycerides and lipoproteins. The FFA represent the main energy source of the organism as their oxidation in the cell produces the necessary quantity of energy, in the form of ATP molecules, used for the metabolism of the cell. Endogenous triglycerides are rich in VLDL particles which are physiologically synthesized from glucose or substrate provided by FFA and degraded in the liver into the main transporters of cholesterol, LDL. The delipidation of VLDL occurs by means of lipoprotein lipase (LPL) resulting in inter low density lipoproteins (IDL) and release of phospholipids and free cholesterol into HDL.

Free cholesterol is esterified by lecithin cholesterol acyl transferase (LCAT) and is accumulated slowly into HDL. Thus, HDL are subdivided into HDL2 and HDL3 depending on their content in cholesteryl esters. HDL2 are strong antiatherogenic particles and their levels are another key enzyme, hepatic lipase (Valemarsson *et al.*1987). The partition of lipids among lipoproteins is based on cholesteryl ester transfer protein (CETP) which transfers cholesteryl esters from HDL2 to VLDL, IDL and inversely triglycerides to HDL2 (Lagrost 1994).

The catabolism of lipoproteins occurs through the LDL receptors or apo B/E, the LDL receptor related protein (LRP), the VLDL receptor, the apo B48 receptor and the scavenger receptors (SR). The SR is composed of five classes. The SR-AI and AII are expressed on the macrophages and on the endothelial cell, they are regulated by intracellular concentration of cholesterol and they can accumulate large amounts of oxidized-LDL in the macrophages. The SR-BI plays a key role in the reverse transport of cholesterol and is regulated, as is also the LDL receptor, by the intracellular concentration of cholesterol.

Both enzymes, HL and CETP are regulated by thyroid hormone and it appears that their activity is inversely dependent on the status of thyroid function.

Thyroid hormones act on lipid metabolism affecting synthesis and mainly degradation of lipids. These effects include enhanced utilization of lipid substrates, mobilization of triglycerides stored in adipose tissue and increased activity of HL and of CETP (Pucci *et*

al. 2000, Dredecjus *et al.* 2003). HL is a glycoprotein secreted by the liver that contributes to the remodelling of the different lipoproteins. CETP is a plasma protein that mediates the exchange of cholesteryl ester between lipoproteins and is a key factor in HDL metabolism and in the reverse cholesterol transport pathway. Plasma CETP activity was found increased in hyperthyroidism and decreased in hypothyroidism. (Tan *et al.* 1998). The LDL receptor is negatively regulated by cellular cholesterol through the sterol regulatory element-binding protein-2 (SREBP-2). Recently, it was reported that SREBP-2 is regulated by thyroid hormones. (Shin *et al.* 2003).

3.2.7 Thyroid Dysfunction and Lipid Abnormalities (Dyslipidemia)

a. Hypothyroidism and dyslipidemia

The depletion of thyroid hormones leads to a reduced number of LDL receptors in the liver and, consequently, to decreased biliary excretion of cholesterol resulting in elevated serum LDL and VLDL which are rich in apo B lipoproteins (Walton *et al.* 1965).

Chronic hypothyroidism provokes structural changes in HDL and apo A-I, a strong co-activator of CETP, by increasing phospholipids and apo-E content and lowering their fractional catabolic rate (Dredecjus *et al.* 2003) It also reduces the activity of CETP and HL enzymes, the driving force for the reverse cholesterol transport, by up to 30% (Franco *et al.* 2003). Therefore, decreased activity of HL and CETP in hypothyroidism may lead to a diminished conversion of VLDL remnants into IDL, cessation of IDL- to- LDL conversion and consequent remodelling of LDL and HDL (Zambon *et al.* 2003).

Lack of thyroid hormones leads to a decreased metabolism of serum remnants-like particles (RLPs).

Finally, another important component in the development of the dyslipidemia in hypothyroidism is renal glomeruler dysfunction, characterized by high intraglobular pressure (low glomerular filtration rate-GFR, and renal functional reserve-RFR). (Nikolaeva *et al.* 2002).

In sub-clinical hypothyroidism or mild thyroid failure (MTF) changes in lipid abnormalities similar to those (raised total cholesterol, LDL cholesterol, and triglycerides) with overt hypothyroidism (Alvarez *et al.* 1993).

Hypothyroid also cause other cardiovascular abnormalities that include diastolic and systolic dysfunction (Vora *et al.* 1985) with reduced exercise capacity, and diastolic

hypertension (Saito *et al.* 1994) resulting from increased vascular resistance and arterial stiffness (Obuobie *et al.* 2002). In long standing hypothyroidism these alterations become clinically significant, leading to pericardial and pleural effusions and, rarely, to congestive heart failure (Klein *et al.* 2001).

b. Dyslipidemia and hyperthyroidism

Hyperthyroidism is usually accompanied with decreased serum concentration of total cholesterol and LDL and normal or decreased HDL. The lipid profile is usually normal in sub-clinical hyperthyroidism (Duntas 2002).

CHAPTER-4

MATERIALS AND METHODS

4.0. MATERIALS AND METHODS

4.1. Type, place and period of study:

This prospective study was carried out in the Institute of Biological Sciences, University of Rajshahi, Bangladesh during the period of July 2004 to June 2009, availing the laboratory facilities of the Centre for Nuclear Medicine and Ultrasound, Mymensingh and Rangpur, and the Department of Biochemistry, BSMMU (Bangabandhu Sheikh Mujib Medical University), Dhaka, Bangladesh.

4.2. Study population

Five hundred thirty three (533) patients with clinically suspected thyroid disorder were examined for T3, T4 and TSH. Out of them 162 were hyperthyroid, 164 were hypothyroid and 207 were euthyroid *i.e.* with normal thyroid activity. All individuals were taken into consideration for measuring lipid profile after overnight fasting.

Selection of the study population was done on the basis of history taking and clinical examination of the patients and on some inclusion and exclusion criteria.

4.3. History and clinical examination:

Clinical history including chief complaints, present history, past history, family history, socio-economic condition, drug history, etc. were taken and general physical examination including neck and systemic examination were done.

4.4. Inclusion and exclusion criteria:

Inclusion criteria:

Patients suspected clinically with thyroid dysfunction.

Exclusion criteria:

a. Diabetes mellitus

b. Nephrotic syndrome

c. Renal failure

d. Obstructive liver disease

e. Drugs that raise cholesterol or cause dyslipidemia-

- β blocker
- Diuretics
- Steroids

f. Family history of hypercholesterolemia if any

Before examination a detailed briefing about the purpose of the study was given to the subjects and written consents were taken for all of the study population.

The ethical committee of the institute approved the protocol.

4.5 Collection of blood and sample processing:

a. Thyroid hormones estimation (T3, T4 and TSH)

Estimation of T3 and T4 were done by Radioimmunoassay (RIA) and estimation of TSH was done by Radioimmuno radiometric assay (IRMA) in laboratory of the Centre for Nuclear Medicine and Ultrasound, Mymensingh and Rangpur, Bangladesh.

The basic principle of RIA and IRMA is antigen-antibody reaction. In RIA, antigen is labelled and specific antibody used. In IRMA, antibody was labelled (Heel 1985).

With all aseptic precautions, 5cc of venous blood was drawn from each subject. Blood was kept for 30 minutes at room temperature for clotting and then centrifuged for 10 minutes, supernatant serum was separated and stored at -20°C. Room temperature of the sample was attained prior to assay. Tests were carried out as early as possible.

Values of T3, T4 and TSH:

Age adjusted range of T3, T4 and TSH in different age groups (Sills *et al.* 1992).

Age Groups (In year)	**Normal range**
T3 (nmol/L)	
1-15 years	1.57 to 3.30
>15 years	1.23 to 3.10
T4 (nmol/L)	
1-12 years	70.80 to 160.90
>12 years	58.00 to 154.40
TSH (mIU/L) for all ages	0.60 to 4.80

Ultra sonogram of thyroid and uptake scan was done whenever needed.

b. Estimation of lipid profile:

Sample collection:

After selection, all subjects in study population were asked to fast overnight (12 hours). 5cc of blood was collected from each subject with the help of disposable syringe by antecubital puncture, taking full aseptic precautions. After removal of the needle from the syringe blood was taken into plain test tube with gentle push to avoid haemolysis.

Separation and storage of serum:

Serum was separated by centrifugation at 2000 rpm for 10 minutes as soon as clot retraction had been completed. The clean serum obtained was preserved in a screw-capped test tube for subsequent analysis.

Estimation of lipid profile was carried out as early as possible, whenever there was delay in experiment; samples were stored in refrigerator of -2^0C to -4^0C.

Laboratory investigations:

Blood and serum were used for biochemical analysis of:

a. Serum triglycerides (TG) by GPO-PAP method.
b. Serum total cholesterol (TC) by CHOD-PAP method.
c. Serum high density lipoprotein cholesterol (HDL-cholesterol) by precipitation.
d. Low density lipoprotein cholesterol (LDL-cholesterol) by calculation.

   LDL-cholesterol (LDL-C) = Total cholesterol (TC) - HDL-cholesterol (HDL-C) - {Triglycerides (TG) ÷ 5}.

ATP III Classification of Lipid Profiles:

TG (mg/dl):

TG categories	Levels
Normal	<150mg/dl
Borderline high	150-199mg/dl
High	200-499mg/dl
Very high	>500mg/dl

Total Cholesterol (mg/dl):

Categories	Levels
Desirable	<200mg/dl
Borderline high	200-239mg/dl
High	>240mg/dl

LDL-C (mg/dl):

Categories	Levels
Optimal	<100mg/dl
Near optimal or above normal	100-129mg/dl
Borderline high	>130-159mg/dl
High	160-189mg/dl
Very high	≥190mg/dl

HDL-C (mg/dl):

Categories	Levels
Low HDL-C	<40mg/dl
High HDL-C	≥60mg/dl

Fasting blood sugar, blood urea, serum creatinine and urine for routine examination were done, whenever needed.

Statistical analysis:

Data were well recorded and statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 16 for windows. Chi-square tests, Pearson's correlations and simple linear regressions were done with p value <.05 taken as statistically significant.

CHAPTER-5

OBERVATIONS AND RESULTS

5.0. OBSERVATIONS AND RESULTS

This prospective study was carried out in the Institute of Biological Sciences University of Rajshahi, Bangladesh, during the period of July 2004 to June 2009, availing the laboratory facilities of the Centre For Nuclear Medicine And Ultrasound, Rangpur and Mymensingh, Bangladesh and Department of Biochemistry, BSMMU (Banga Bandhu Sheikh Mujib Medical University), Dhaka Bangladesh .

Five hundred thirty three patients with clinically suspected thyroid disorders were randomly selected and examined for serum T3, T4 and TSH. Fasting lipid profile of all the patients was measured after overnight fasting. Data were analyzed using SPSS (Statistical Package for Social Science) Version 16 for Windows and presented in graphs and charts.

5.1. Age distribution of the study population:

Minimum age in the study population was 3 months and maximum age was 82 years (37.58 ± 16.17). In the study population there was 132(24.76%) patients in the age group of 30-39 years, next prevailing group was the age group of 20-29 years consisting of 126(23.64%) patients. Age group 40-49 years consists of 76(14.24%) patients. Sixty to sixty nine years age groups consists of 63(11.82%) patients, age group 50-59 years consists of 56(10.51%), age group 10-19 years consists of 54(10.13%) and age group 70 years and above consists of 21(3.94%) patients. Least numbers of patients were in the age group of 0-9 years (0.94%).

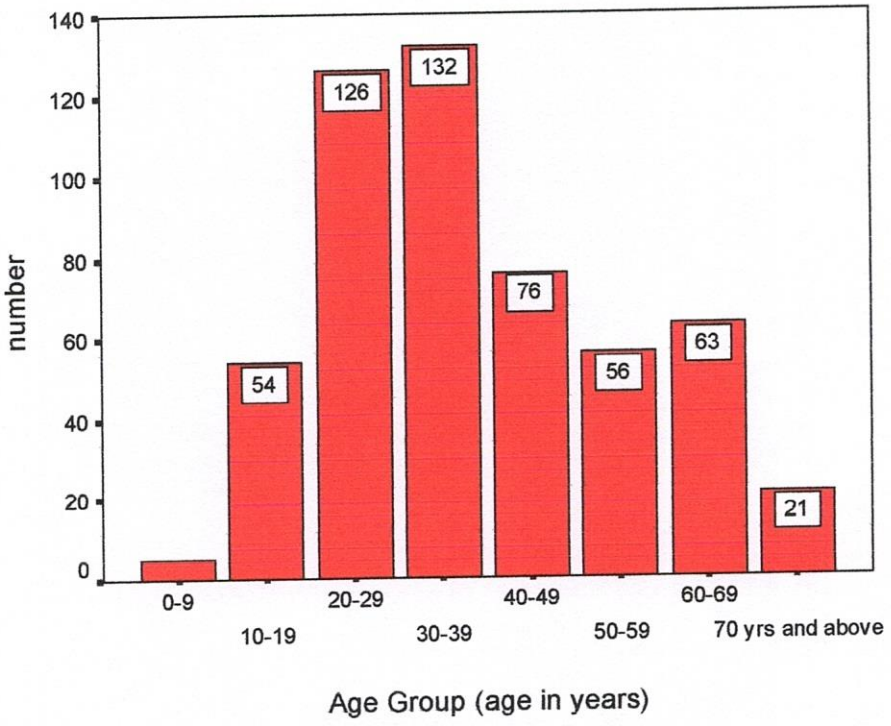

Fig.2. Showing age distribution of the study population.

5.2. Sex distribution of the study population:

In the study group there was 168 (31.5%) patients were male and 365(68.5%) patients were female.

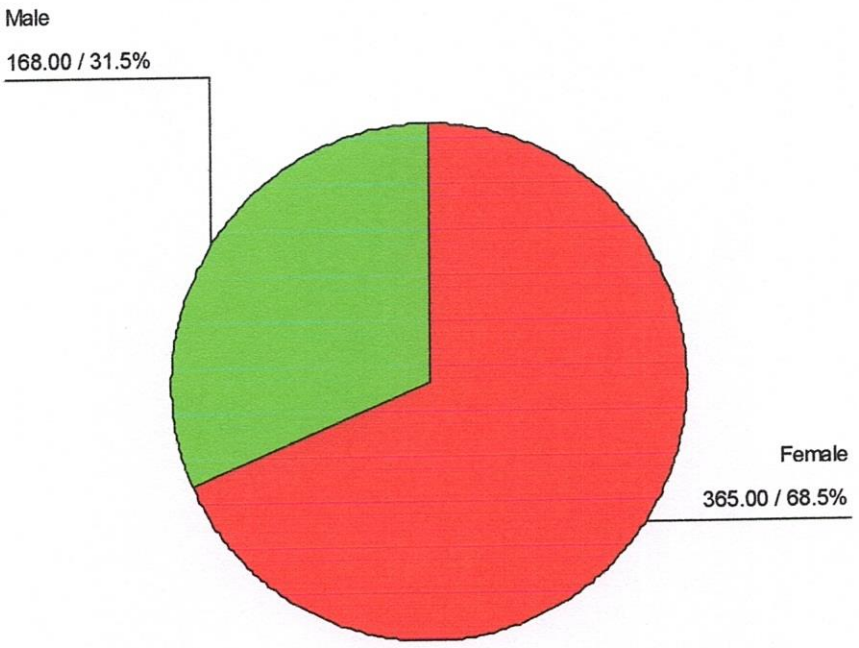

Fig.3. Sex distribution of the study population.

5.3. Distribution of study population by age and sex:

In the age group 30-39 years there were 88(16.51%) female patients and 44(8.25%) patients were male. In the age group 40-49 years there was 54(10.13%) female patients, in the age group 20-29 years 98(18.39%) patients were female, in the age group 50-59 years 37(6.94%) patients was female, in the age group 60-69 years 36(6.75%) patients were female, , in the age group 70 years and above 12(2.25%) patients was female. In the age group 0-9 years 2(0.37%) patients was female. In the present study, in all the age groups, the number of female patients exceeded the male.

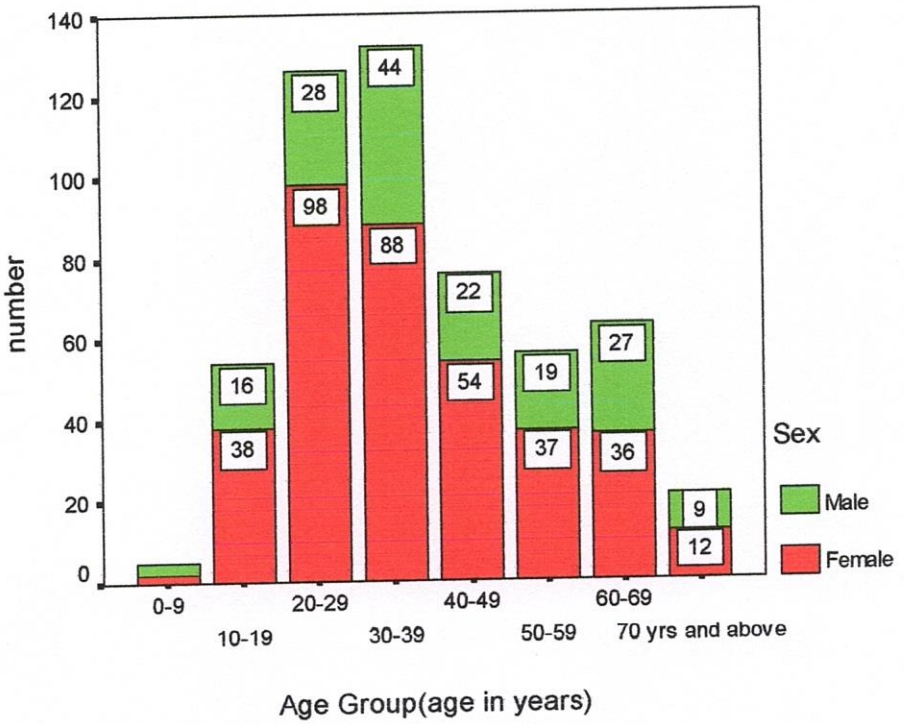

Fig.4. Age and sex distribution of the study population.

5.4. Level of thyroid activity:

In the study population there were 207 (38.84%) patients having normal thyroid activity. Three hundred and twenty six (61.16%) patients were having abnormal thyroid activity. Of them 162 (49.69%) patients were hyperthyroid and 164(50.31%) patients were suffering from hypothyroidism.

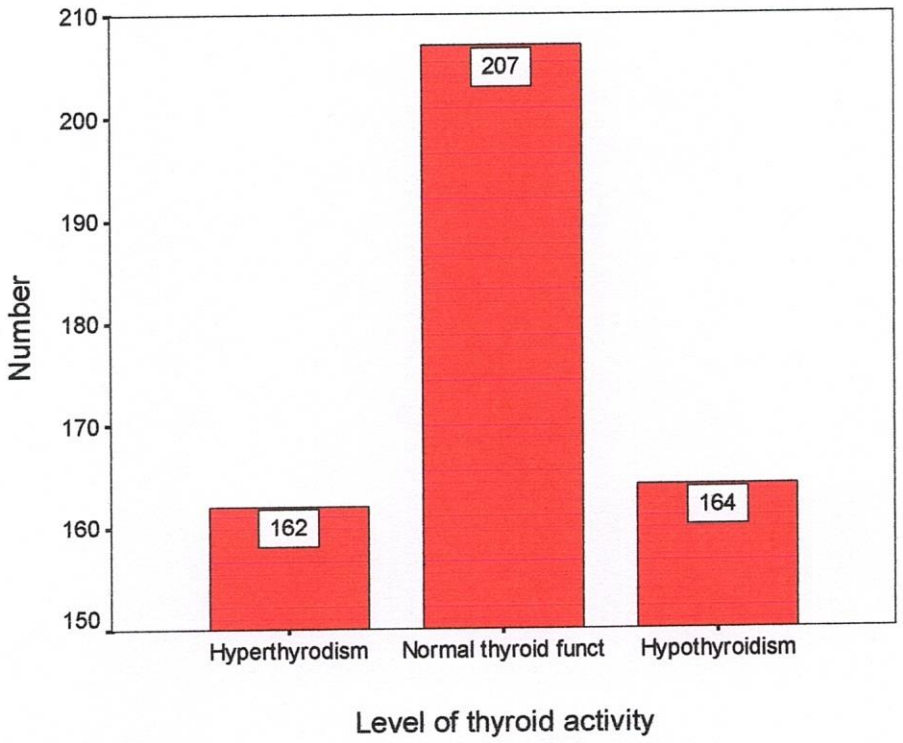

Fig.5. Bar diagram showing the distribution of level of thyroid activity.

5.5. Level of thyroid activity according to age group:

In the current study it has been observed that the level of thyroid activity abnormality increases with the advancement of age of the patients and such change in thyroid activity is found to be statistically highly significant as the calculated p value was <0.001.

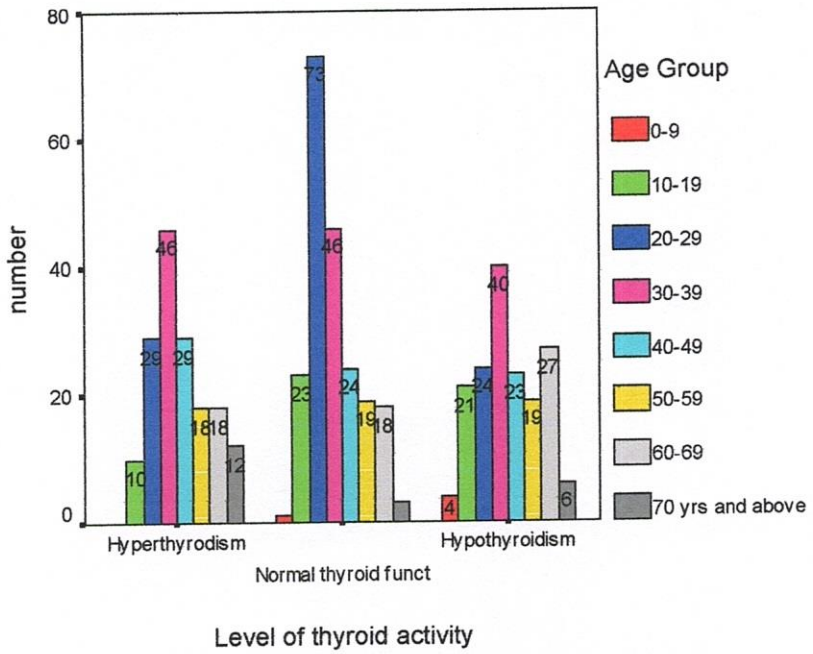

Fig. 6. Bar diagram showing the level of thyroid activity according to age group.

Table 3. Level of thyroid activity according to age group.

Age group	0-9 years	10-19 years	20-29 years	30-39 years	40-49 years	50-59 years	60-69 years	70 years & above	p value
hyperthyroidism	00	10	29	46	29	18	18	12	<0.001[s]
Normal thyroid function	01	23	73	46	24	19	18	03	
hypothyroidism	04	21	24	40	23	19	27	06	

Chi-Square = 47.039, df = 14, p-value <0.001

5.6. Sex distribution and thyroid abnormality:

Among the study population, 124 (23.26%) female and 83(15.57%) male patients had normal thyroid function. Hyperthyroidism was detected in 123(23.07%) female and 39(7.01%) male patients. Hypothyroidism was recorded in 118(22.13%) female and 46(8.63%) male patients. So thyroid abnormality varied between sexes and this finding was statistically significant (p value = 0.002).

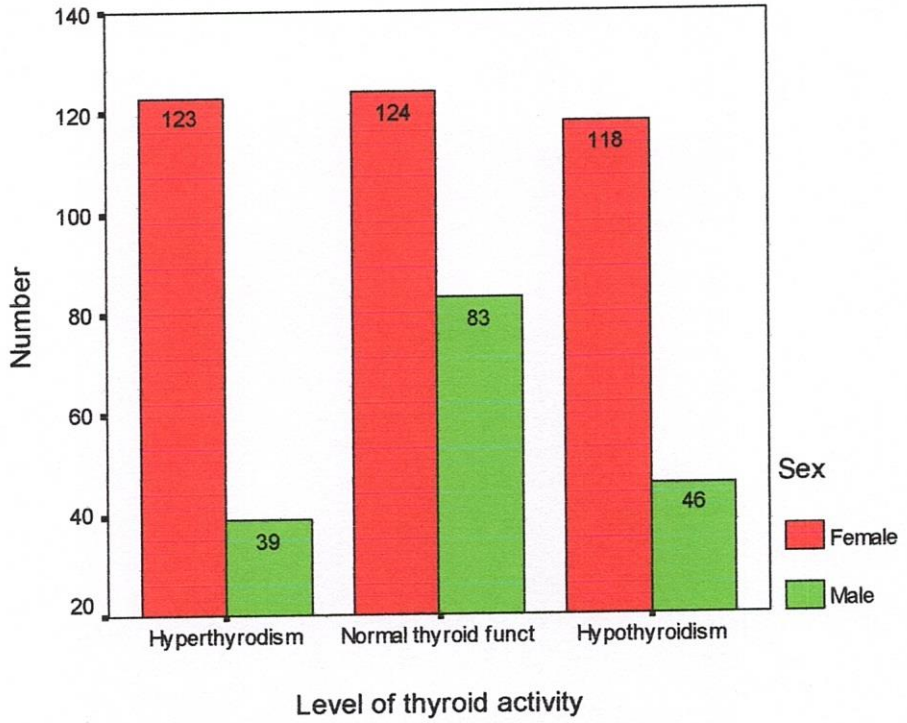

Fig.7. Bar diagram showing distribution of thyroid activity according to sex.

Table 4. Distribution of thyroid activity according to sex.

Gender	Hyperthyroidism	Normal thyroid function	hypothyroidism	p value
Male	39	83	46	0.002[s]
Female	123	124	118	

Chi-Square = 12.131, df = 2, p-value = 0.002

5.7. Clinical presentation of thyroid activity (Thyroid enlargement):

In the study population, among 207 patients having normal thyroid activity, none had severe thyroid enlargement. Of them 152 (72.42%) patients thyroid gland was not enlarged. Mild to moderate enlargement was observed in 55 (26.57%) patients. In patients with hyperthyroidism, 5(3.09%) patients had severe thyroid enlargement. No thyroid enlargement was noticed in 38 (23.45%) patients, moderate to severe enlargement was in 118 (72.84%) patients. In patients with hypothyroidism no thyroid enlargement was in 94(57.32%) patients. Severe thyroid enlargement was in 9(5.48%) patients. Moderate to severe enlargement was in 68 (41.46%) patients. p value was <0.001, which is statistically significant.

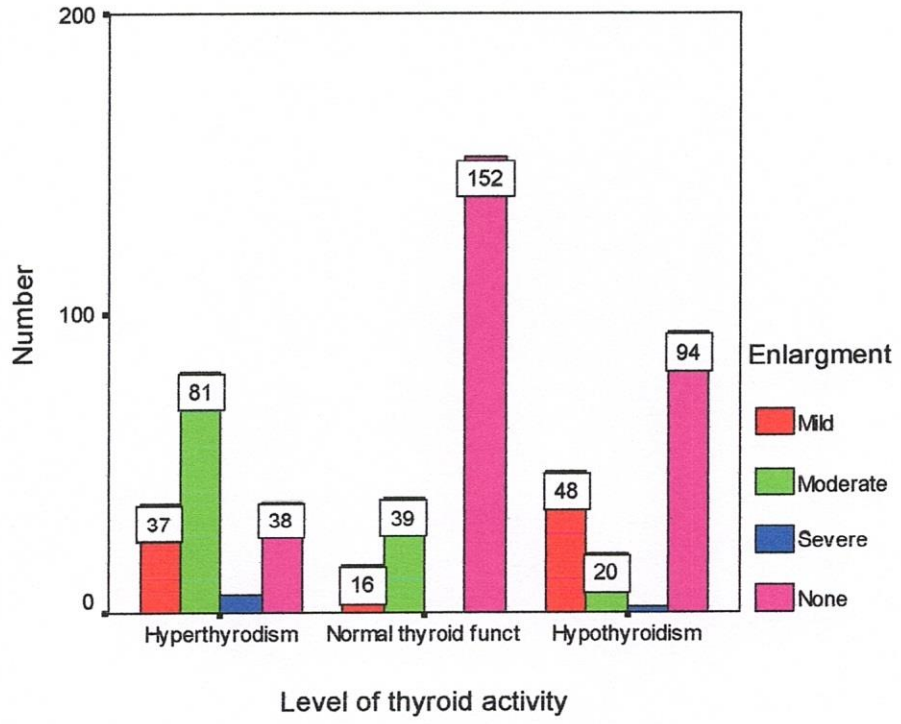

Fig.8. Showing thyroid enlargement and level of thyroid activity.

Table 5. Thyroid enlargement and level of thyroid activity.

	Hyperthyroidism	**Normal thyroid function**	**Hypothyroidism**	**p value**
Mild enlargement	37	16	48	<0.001[s]
Moderate enlargement	81	39	20	
Sever enlargement	06	00	02	
No enlargement	38	152	94	

Chi-Square = 127.457, df = 6, p-value <0.001

5.8. Hot or cold intolerance:

Among the study population, 136(25%) patient had no intolerance to hot or cold. In patients with normal thyroid function cold intolerance was 67(32.36%), in patients with hyperthyroidism cold intolerance was 70(43.20), in patients with hypothyroidism cold intolerance was 131 (79.87%).

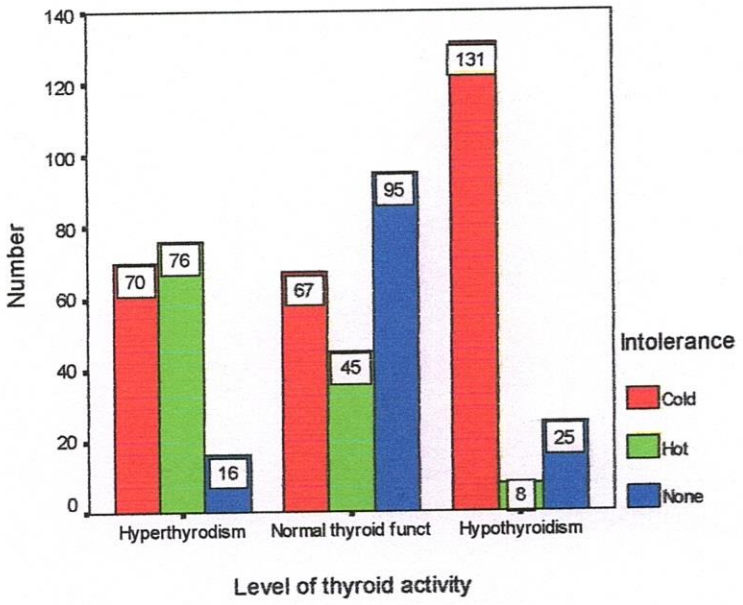

Fig. 9. Bar diagram of hot or cold intolerance in the study population.

Table 6. Level of thyroid activity and intolerance to hot or cold

		Intolerance			Total	p value
		Cold	Hot	None		
Level of thyroid activity	Hyperthyroidism	70	76	16	162	<0.001
	Normal thyroid function	67	45	95	207	
	Hypothyroidism	131	08	25	164	
Total		**268**	**129**	**136**	**533**	

Chi-Square = 159.74, df = 4, p-value <0.001

5.9 Appetite:

In the study population, appetite was normal in 247(46.3%) patients. Appetite was decreased in 145(27.2%) patients and was increased in 141(26.5%) patients.

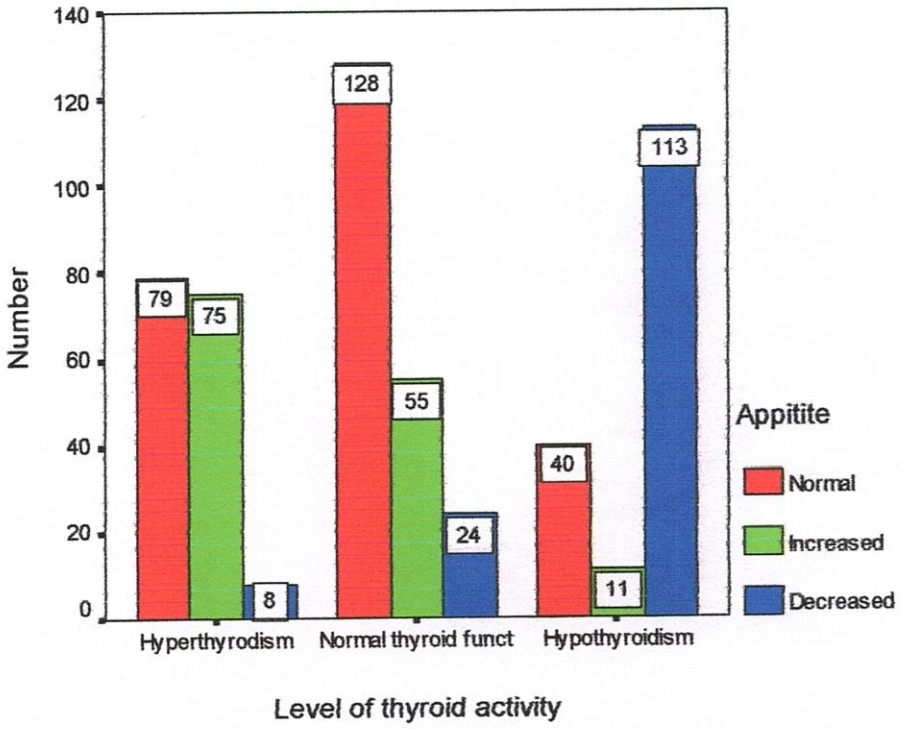

Fig.10. Bar diagram of changes in appetite of study population.

Table 7. Level of thyroid activity and appetite.

		Appetite			Total	p value
		Normal	Increased	Decreased		
Level of thyroid activity	Hyperthyroidism	79	75	08	162	<0.001
	Normal thyroid function	128	55	24	207	
	Hypothyroidism	40	11	113	164	
Total		**247**	**141**	**145**	**533**	

Chi-Square = 229.149, df = 4, p-value <0.001

5.10. Sleep changes:

Among the study population, 213(43.3%) patients had no changes in sleep pattern. Sleep was decreased in 80(49.38%) patients with hyperthyroidism and was increased in 121(73.78%) patients with hypothyroidism.

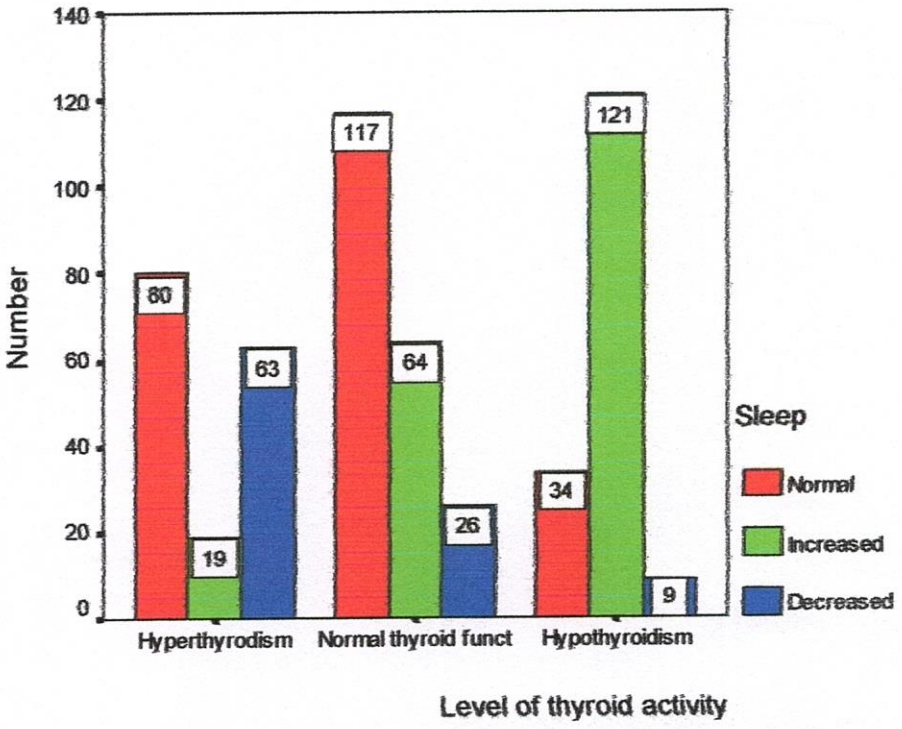

Fig.11. Distribution of sleep pattern in the study population.

Table 8. Showing level of thyroid activity and sleep.

		Sleep			Total	p value
		Normal	Increased	Decreased		
Level of thyroid activity	Hyperthyroidism	80	19	63	162	<0.001
	Normal thyroid function	117	64	26	207	
	Hypothyroidism	34	121	9	164	
Total		**231**	**204**	**98**	**533**	

Chi-Square = 171.476, df = 4, p-value <0.001

5.11. Body Weight:

In the study population, no change of body weight occurred in 205(38.5%) patients, weight was lost in 76(46.91%) patients with hyperthyroidism and weight was gained in 125(76.61%) patients having hypothyroidism.

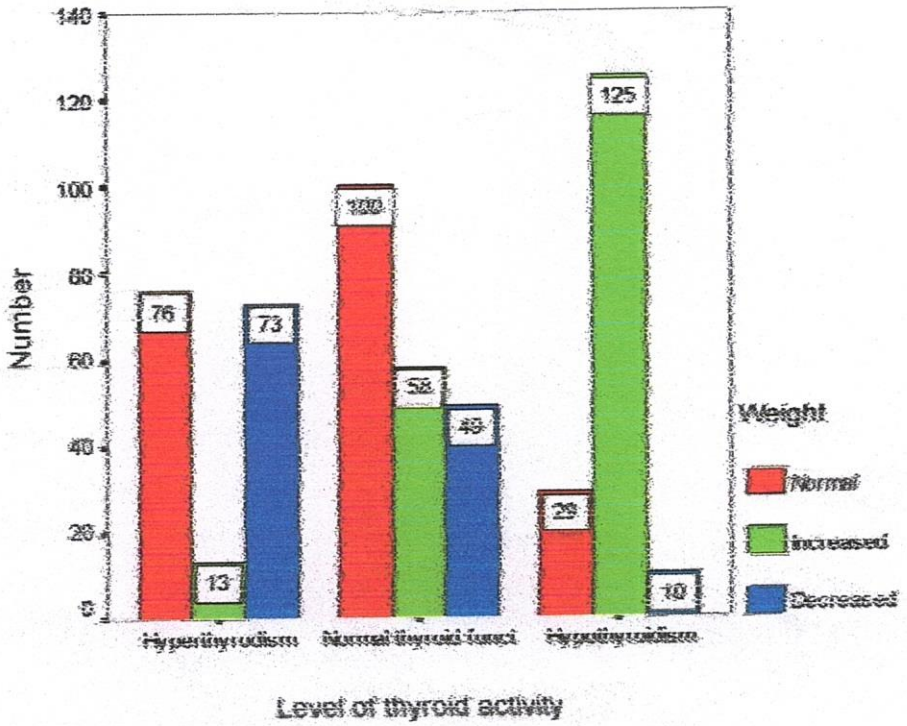

Fig.12 Showing distribution of weight changes in the study population

Table 9. Showing level of thyroid activity and weight.

		Weight			Total	p value
		Normal (No change)	**Increased**	**Decreased**		
Level of thyroid activity	Hyperthyroidism	76	13	73	162	<0.001
	Normal thyroid function	100	58	49	207	
	Hypothyroidism	29	125	10	164	
Total		**205**	**196**	**132**	**533**	

Chi-Square = 186.880, df = 4, p-value <0.001

Table 9. Showing level of thyroid activity and weight.

		Weight			Total	p value
		Normal (No change)	**Increased**	**Decreased**		
Level of thyroid activity	Hyperthyroidism	76	13	73	162	<0.001
	Normal thyroid function	100	58	49	207	
	Hypothyroidism	29	125	10	164	
Total		**205**	**196**	**132**	**533**	

Chi-Square = 186.880, df = 4, p-value <0.001

5.12. Palpitation:

Among the study population, 393 (73.7%) patients had normal heart rate. Palpitation was found in 85(52.46%) patients with hyperthyroidism and no palpitation was found in 148 (90.20) patients with hypothyroidism.

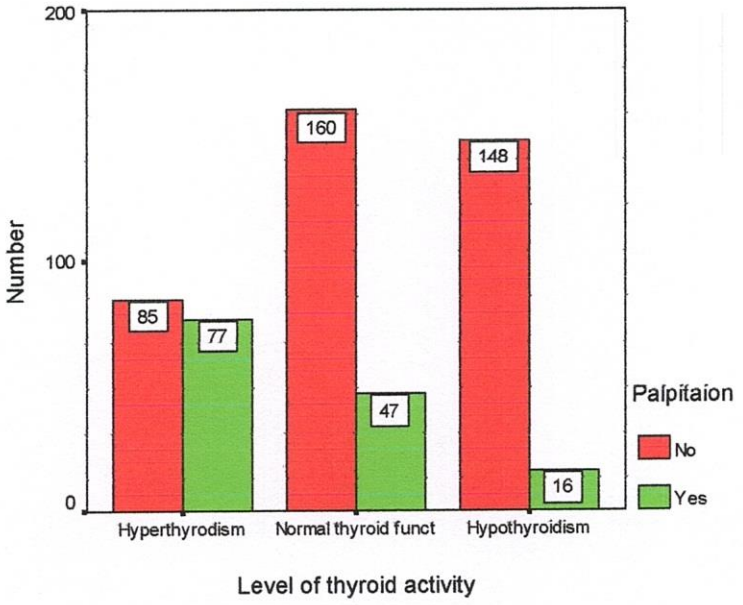

Fig. 13. Showing distribution of palpitation in the study population.

Table 10. Showing level of thyroid activity and Palpitition

		Palpitition		Total	p value
		No	Yes		
Level of thyroid activity	Hyperthyroidism	85	77	162	<0.001
	Normal thyroid function	160	47	207	
	Hypothyroidism	148	16	164	
Total		393	140	533	

Chi-Square = 62.261, df = 2, p-value <0.001

5.13. Tremor:

In the study population, tremor was found in 85 (52.46%) in patients having hyperthyroidism and no palpitation was found in 150(91.46%) patients with hypothyroidism.

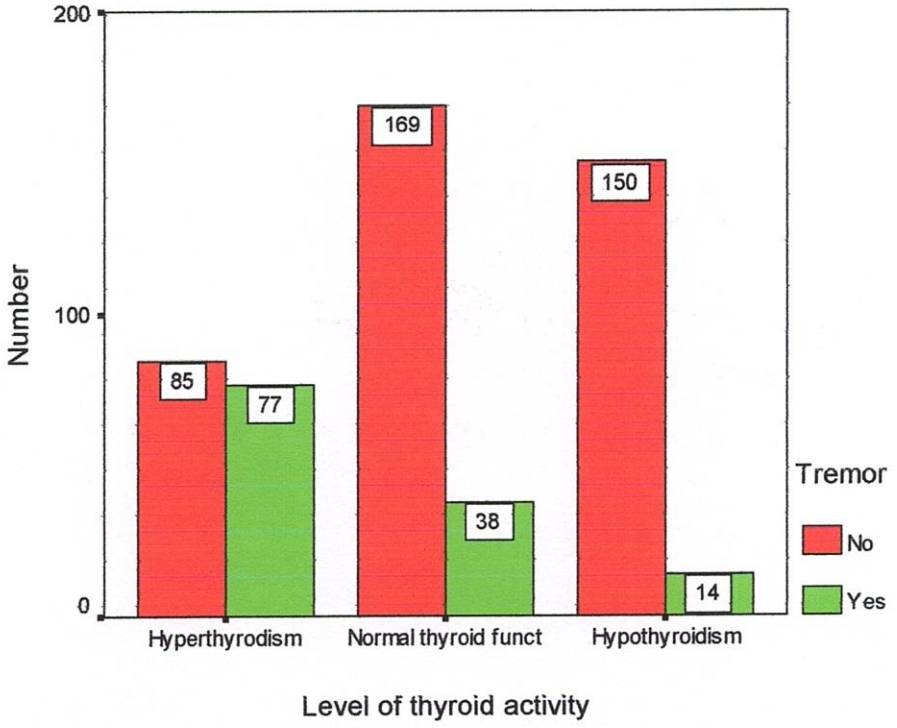

Fig.14. Showing distribution of tremor in the study population.

Table 11. Showing level of thyroid activity and Tremor

		Tremor		Total	p value
		No	Yes		
Level of thyroid activity	Hyperthyrodism	85	77	162	<0.001
	Normal thyroid function	169	38	207	
	Hypothyroidism	150	14	164	
Total		**404**	**129**	**533**	

Chi-Square = 73.853, df = 2, p value <0.001

5.14. Weakness:

Among the study population, weakness was experienced in 141(85.97%) patients with hypothyroidism and in 88(54.32%) patients with hyperthyroidism.

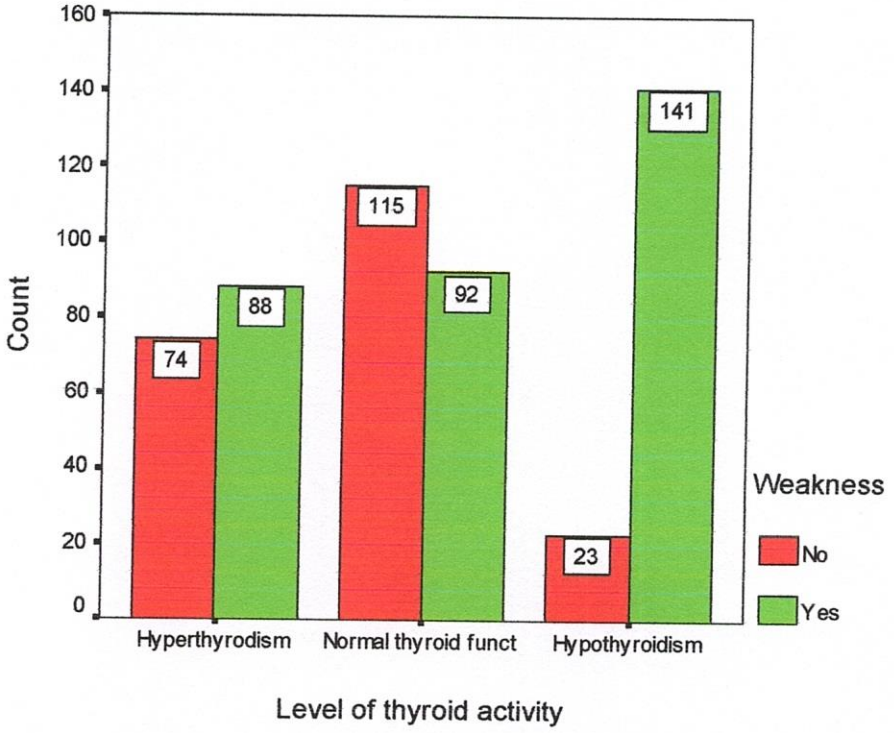

Fig. 15. Showing distribution of weakness in the study population.

Table 12. Showing level of thyroid activity and Weakness.

		Weakness		Total	p value
		No	Yes		
Level of thyroid activity	Hyperthyroidism	74	88	162	<0.001
	Normal thyroid function	115	92	207	
	Hypothyroidism	23	141	164	
Total		**212**	**321**	**533**	

Chi-Square = 69.274, df = 2, p value <0.001

5.15. Heart failure:

In the study population, had heart failure was detected among 16 (3.0%) patients. However, this was statistically insignificant as the p value was 0.059.

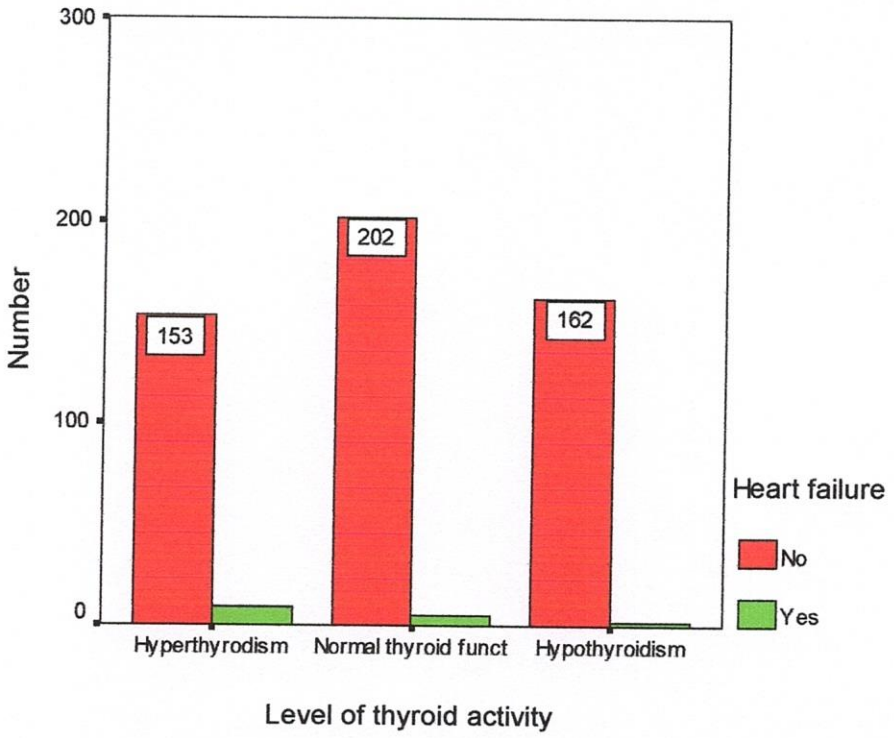

Fig.16. Showing distribution of heart failure in the study population.

Table 13. Distribution of heart failure in the study population.

Heart failure	Hyperthyroidism	Normal thyroid function	hypothyroidism	p value
Yes	09	05	02	0.059^{ns}
No	153	202	162	

Chi-Square = 5.662, df = 2, p value = 0.059

5.16. Changes in voice:

In the study population, changes in voice was found in 19 (11.72%) cases of hyperthyroidism and in 126 (76.82%) in patients with hypothyroidism.

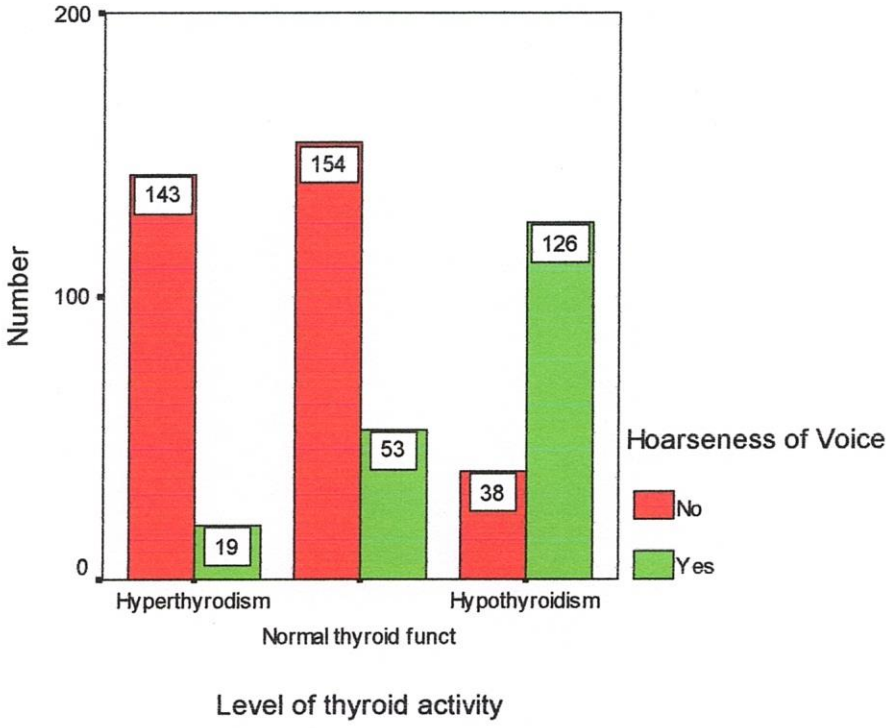

Fig.17. Showing changes of voice in the study population.

Table14. Showing distribution of change of voice in the study population.

		Hoarseness of Voice		Total	p value
		No	Yes		
Level of thyroid activity	Hyperthyroidism	143	19	162	<0.001
	Normal thyroid function	154	53	207	
	Hypothyroidism	38	126	164	
Total		**335**	**198**	**533**	

Chi-Square = 167.249, df = 2, p-value <0.001

5.17. Myxedema:

In the study population myxedema was found in 27(16.67%) cases of hyperthyroidism and 38 (23.17%) cases of hypothyroidism. p value was 0.05 which is statistically just significant.

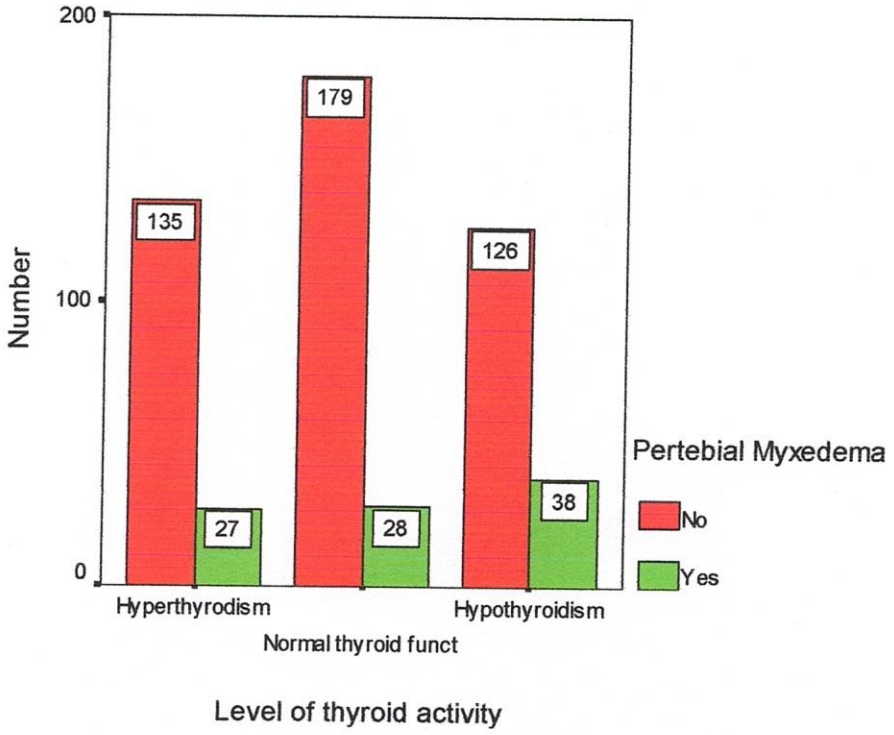

Fig.18. Showing distribution of myxedema in the study population.

Table 15. Showing distribution of myxedema in the study population.

Pretibial Myxedema	Hyperthyroidism	Normal thyroid function	Hypothyroidism	p value
Yes	27	28	38	0.050[s]
No	135	179	126	

Chi-Square = 6.007, df = 2, p-value = 0.050

5.18. Thyroid activity and level of triglyceride:

In the study population, normal TG level was found in 67(32.67%) cases in the normal thyroid activity. In hyperthyroidism, normal TG level was fond in 48(29.63%) cases. In hypothyroidism normal TG level was found in 47(27.67%). p value was <0.001, which is highly significant.

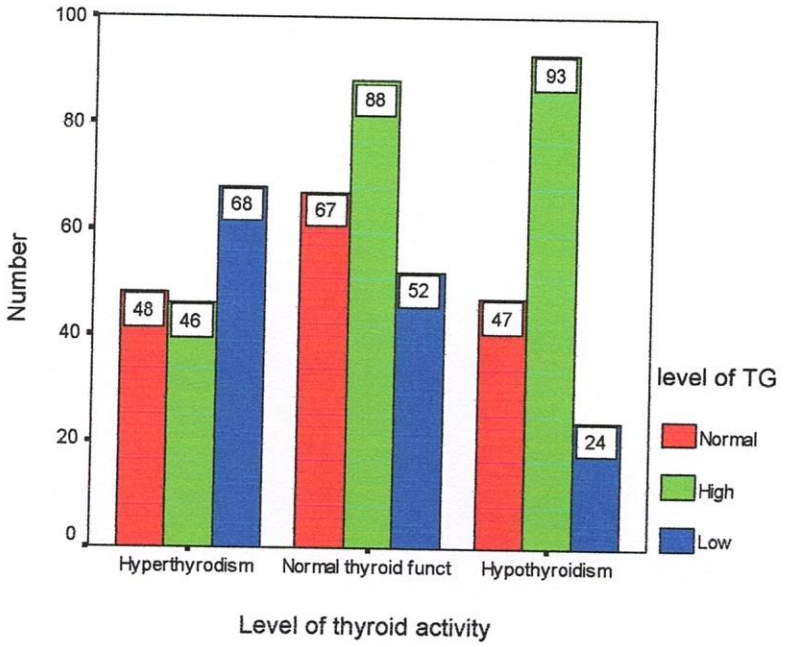

Fig. 19. Showing relation between level of thyroid activity and level of Triglyceride.

Table 16. Showing level of thyroid activity and level of Triglyceride

		Level of TG			Total	p value
		Normal	**High**	**Low**		
Level of thyroid activity	Hyperthyroidism	48	46	68	162	<0.001[s]
	Normal thyroid function	67	88	52	207	
	Hypothyroidism	47	93	24	164	
Total		**162**	**227**	**144**	**533**	

Chi-Square = 38.798, df = 4, p value <0.001

5.19. Thyroid activity and level of HDL:

Among the study population, level of HDL was high in 115(55.55%) individuals having normal thyroid activity. And level was high in 102 (62.96%) cases. p value was 0.077, which is statistically not significant.

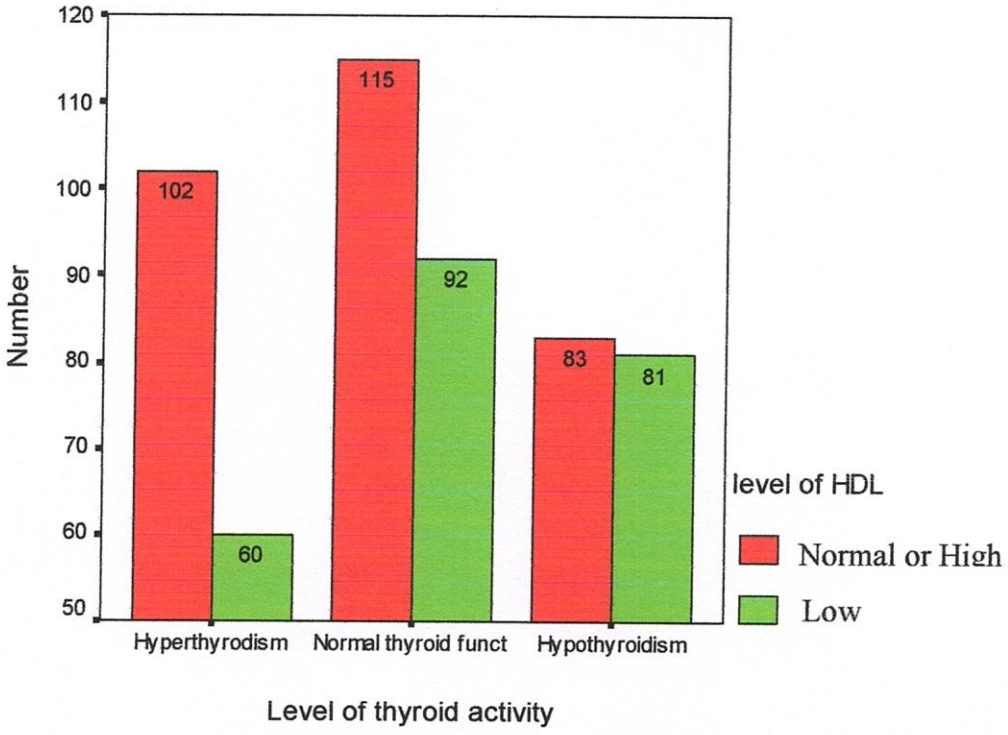

Fig. 20. Showing relation between level of thyroid activity and HDL level.

Table 17. Showing relation of HDL and level of thyroid activity.

		Level of HDL		Total	p value
		Normal or High	Low		
Level of thyroid activity	Hyperthyroidism	102	60	162	0.077^{ns}
	Normal thyroid function	115	92	207	
	Hypothyroidism	83	81	164	
Total		**300**	**233**	**533**	

Chi-Square = 5.128, df = 2, p value = 0.077

5.20 Level of low density lipoprotein and level of thyroid activity:

In the study population 85(41.06%) patients had high level of LDL with normal thyroid activity. In 148 (45.40%) cases had elevated LDL with abnormal thyroid activity. p value was <0.001, which is statistically significant.

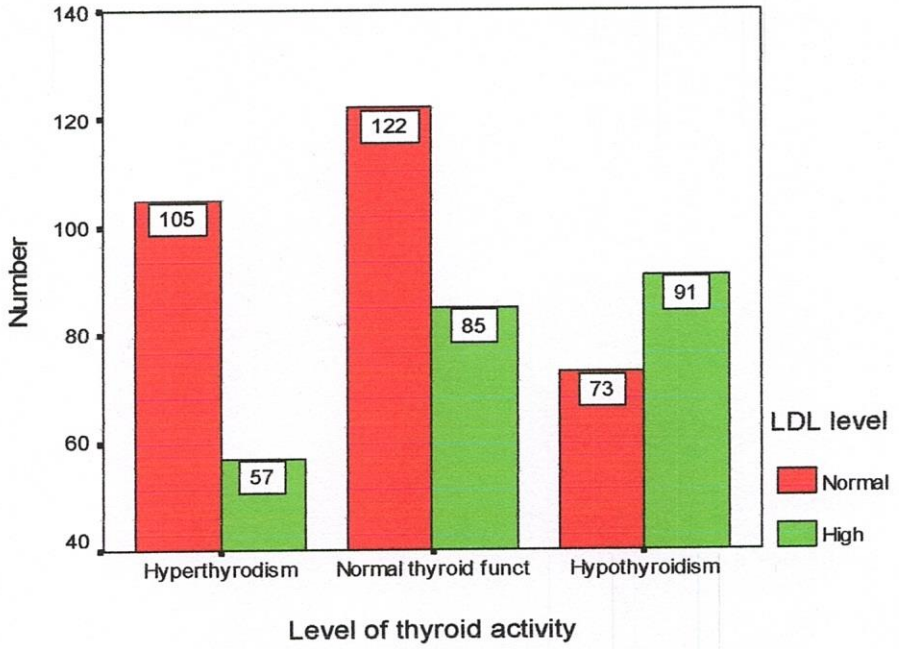

Fig. 21. Showing relation between LDL level and level of thyroid activity.

Table 18. Showing Level of thyroid activity and LDL level:

		LDL level		Total	p value
		High	Low		
Level of thyroid activity	Hyperthyroidism	105	57	162	<0.001
	Normal thyroid function	122	85	207	
	Hypothyroidism	73	91	164	
Total		**300**	**233**	**533**	

Chi-Square = 14.620, df = 2, p-value <0.001

5.21 Level of total cholesterol and level of thyroid activity:

In the study population 88 (42.51%) with normal thyroid activity, 48 (29.63%) with hyperthyroidism and 93 (56.71%) with hypothyroidism patients had high level of total cholesterol.

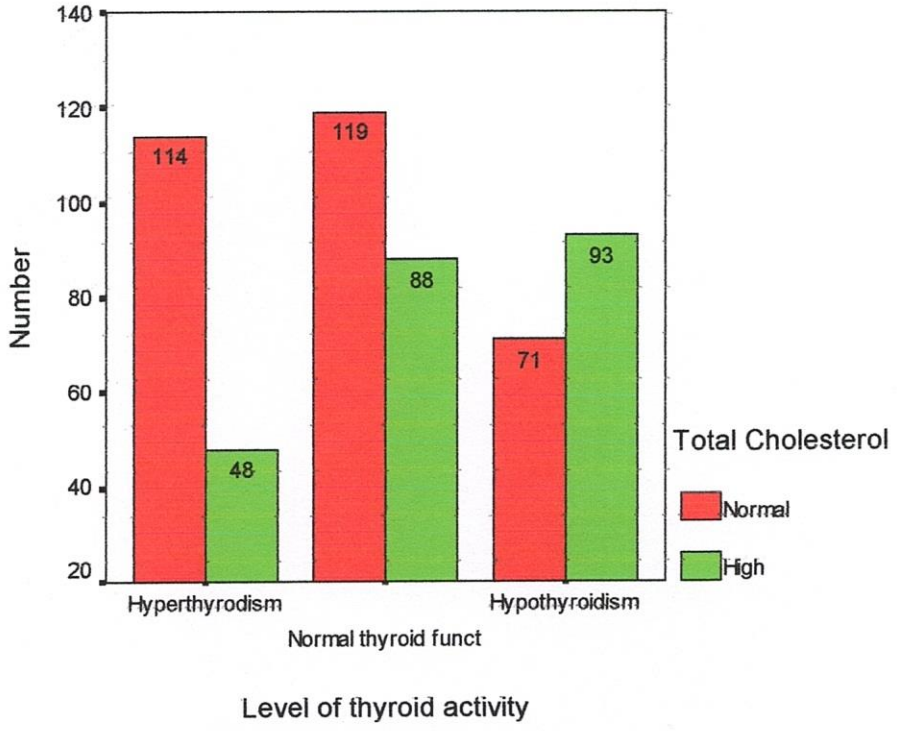

Fig. 22. Showing relation between Total cholesterol and level of thyroid activity.

Table 19. Showing relation of Total cholesterol and level of thyroid activity:

		Total Cholesterol		Total	p value
		Normal	**High**		
Level of thyroid activity	Hyperthyroidism	114	48	162	0.001[s]
	Normal thyroid function	119	88	207	
	Hypothyroidism	71	93	164	
Total		**304**	**229**	**533**	

Chi-Square = 24.413, df = 2, p value <0.001

Table 20. Showing Pearson's correlation between thyroid status and the lipid profile.

	TSH		T4		T3	
	r	p	r	p	r	p
TC	0.288	<0.01	-0.214	<0.01	-0.053	ns
HDL	-0.153	<0.01	0.145	<0.01	-0.026	ns
LDL	0.238	<0.01	-0.183	<0.01	-0.040	ns
TG	0.147	<0.01	-0.120	<0.01	0.103	ns

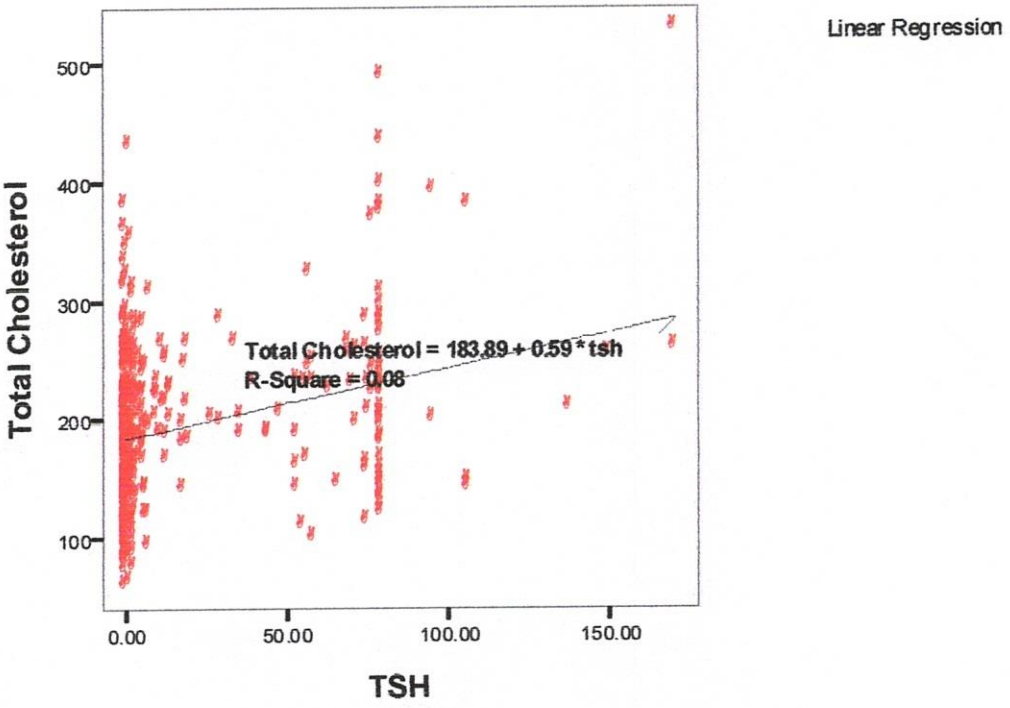

Fig. 23. Positive and linear association of TSH and TC

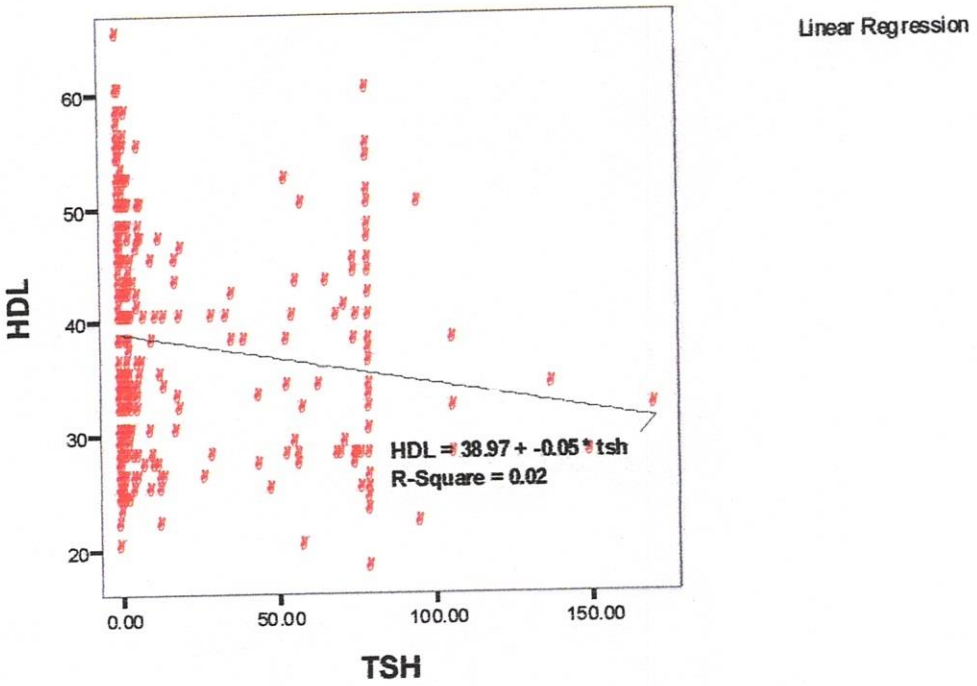

Fig.24. Negative and linear association of TSH and HDL

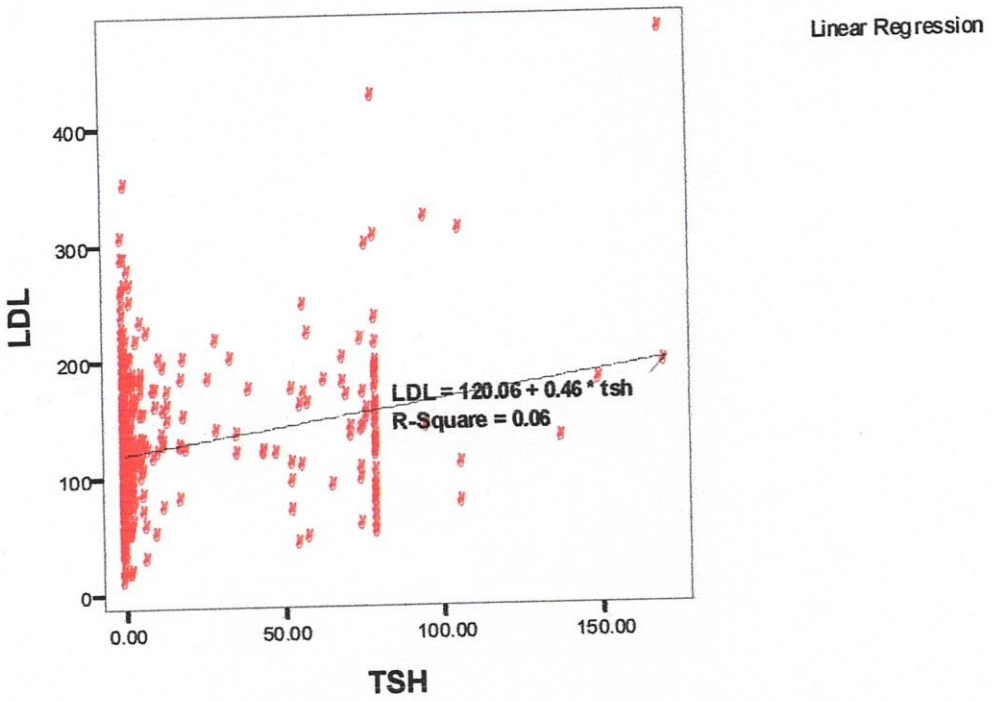

Fig. 25. Positive and linear association of TSH and LDL

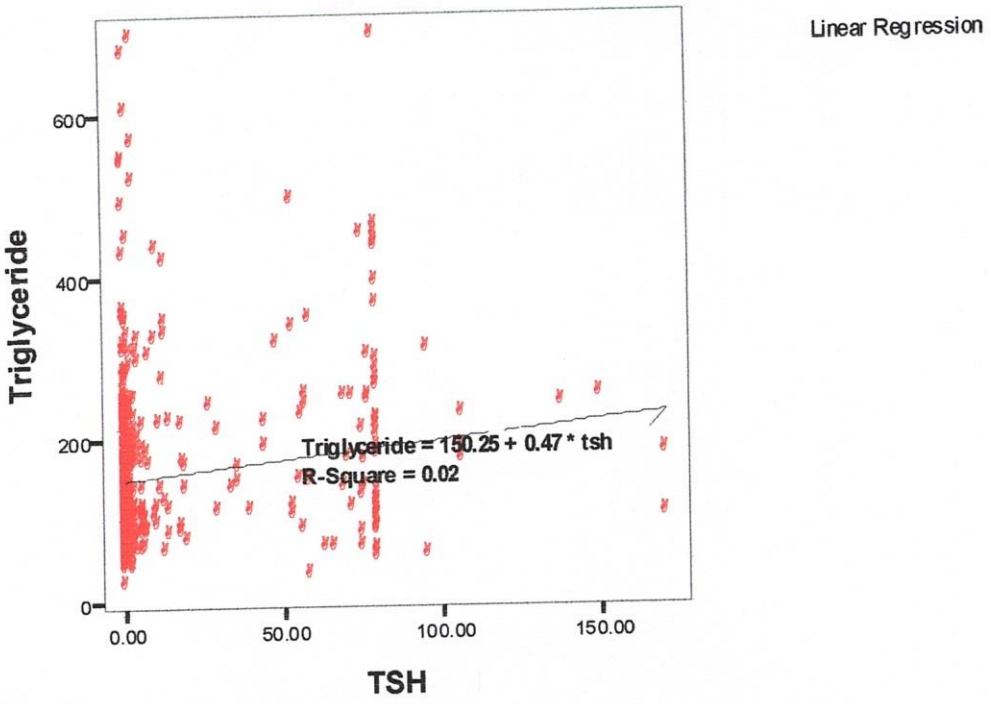

Fig. 26. Positive and linear association of TSH and TG

CHAPTER-6

DISCUSSION

6.0. DISCUSSION

Coronary heart disease is the leading cause of death in the middle aged people all over the world. It is the most common cause of death in the USA and in most European countries. In Bangladesh the number of coronary heart disease cases is also increasing. In future it will be the number one killer disease in Bangladesh and other developing countries. Dyslipidemia is a major risk factor for atherosclerotic cardiovascular diseases. It is a disorder of lipoprotein metabolism including lipoprotein over production or deficiency. It may be manifested by elevation of total cholesterol, LDL cholesterol, triglycerides and a decrease in high density lipoprotein (HDL) in the blood.

Thyroid disease, one of the major health hazards in Bangladesh, is mainly seen in the northern part along the belt of the river Brahmaputra and the river Jamuna. Thyroid disorders are the second endocrine problem, next to Diabetes Mellitus (DM) in Bangladesh. Thyroid glands through its secreted hormones maintain the level of metabolism in the tissue that is optimal for their normal function. No tissue in the body is spared from its action. Thyroid hormone influences all major metabolic pathways with specific regard to lipid metabolism affecting synthesis, mobilization and degradation of lipids.

In the present study, patients clinically suspected with thyroid disorders were taken into consideration. A Total of 533 patients were studied. Minimum age of the study population was 3 months and maximum age was 82 years with mean age 37.58 $\pm$16.17 years and maximum patients were in the middle age group of 30-39 years (Fig. 2, in Chapter 5- Results).

Out of the total 533 patients, 365 (68.5%) were females and 168 (31.5%) were male (Fig. 3). Therefore, a female predominance was noticed in the current study (Fig.4, in Chapter 5- Results).

In the present study, there were 207 (38.84%) patients with normal thyroid activity (euthyroid) and 326 (61.16%) were having abnormal thyroid activity. Of them, 162 (30.39%) had hyperthyroidism and 164 (30.77%) were with hypothyroidism (Fig. 5, in Chapter 5- Results).

Studies on the prevalence of abnormal thyroid activity have been done earlier at different places of Bangladesh earlier. Alam *et al.* (1995) in a hospital based study in the Institute of Nuclear Medicine (INM), BSMMU, Dhaka, Bangladesh reported 10.12% hypothyroid and 23.57% hyperthyroid cases.

Siddique *et al.* (1992) in a similar type of study, also done in INM, Dhaka found 5.80% hypothyroid and 5.09% hyperthyroid cases. Khalilullah *et al.* (1996) in another community based study in greater Dinajpur district, Bangladesh found 17.39% hypothyroid and 17.88% hyperthyroid cases.

In a study conducted on the thyroid disorders in greater Rajshahi district, Bangladesh Faisal Kabir *et al.* (1992) reported 8.72% hypothyroid and 12% hyperthyroid cases. Paul *et al.* (1998) carried out a study in greater Khulna district, Bangladesh and found 34.11% hypothyroid and 10.85% hyperthyroid. In 2000, a study of INM, Dhaka, Bangladesh showed 22.30% hyperthyroid and 16.62% hypothyroid cases.

In our study, 164 i.e (30.77%) cases of hypothyroidism were found, which is very close to the prevalence of 34.11% hypothyroidsim as reported in Khulna, Bangladesh by Paul *et al.* (1998). But the prevalence of both hypo and hyperthyroid patients are more in the Northern part of Bangladesh (Rangpur and Mymensingh) than those found in other studies done elsewhere in Bangladesh. Hypothyroidism is a common disorder with a prevalence rate up to 20% (Sawin *et al.* 1985).

In the present study it was observed that the level of thyroid activity i.e. function according to age group, thyroid abnormality increases as the age advanced; more prevalent in middle age group (Fig. 6, Table 3, in Chapter 5- Results). The association between age and goiter prevalence or thyroid volume with or without thyroid dysfunction seems to be dependent on iodine status.

This finding of ours agree very well with the findings of Konno *et al.* (1993) who found that the level of thyroid activity increased up to an age around 40 years, but after that age no further increase was seen. Tunbridge *et al.* (1977) in their study also found that thyroid dysfunction increases with age, especially in women.

The results of sex distribution and thyroid function abnormality of our study (Fig. 7 and Table 4, in Chapter 5- Results) showed 123 (23.07%) female patients and only 39 (7.01%) male patients in hyperthyroid group; 118 (22.13%) female and 46 (8.63%) male

patients in hypothyroid group. There was a significant predominance of female patients ($p<.002$).

Our finding is in good agreement with the study of Konno *et al.* (1993) who also found that thyroid disorders are more prevalent among women particularly in iodine-insufficient populations. Our results also show a good harmony with that reported by Helfand and Redfern (1998) who reported that women are more likely to have thyroid dysfunction than men.

Elderly women have special concerns about treatment of serum cholesterol as it relates to sub-clinical hypothyroidism. The prevalence of hypothyroidism is much higher among women than men, and among women over 60, the prevalence of subclinical hypothyroidism is as high as 20% (Wood *et al.* 1995). Although cardiac disease in women is much less frequent than in men in the younger population, incidence increases dramatically with age.

Among women over 70, cardiac disease affects 50% of women and is a leading cause of death (Miller 1993) with hyperlipidemia being an important independent risk factor for myocardial infarction (Sacks et al. 1996). As TSH levels and dyslipidemia increase with age, it can be assumed that thyroid disease becomes increasingly important as a factor in cardiac disease. In fact, a study in Scotland found an increased prevalence of overt and subclinical hypothyroidism in patients with cholesterol levels >310 mg/dl, and this association was strongest in older women. Other studies note that the degree of coronary artery narrowing was greater in patients with hypothyroidism (Hak *et al.* 2000).

Clinical presentation regarding thyroid enlargement of the present study was shown in Fig. 8 and Table 5 (in Chapter 5- Results) where mild to moderate enlargement of thyroid was with high prevalence among all the patients ($p<0.001$).

According to the results among the study population of the present study regarding clinical aspects, patients with hyperthyroidism, were associated predominantly with hot intolerance, increased appetite, decreased sleep and weight loss, palpitation and tremor. Patients with hypothyroidism were associated with predominantly with cold intolerance, decreased appetite, increased sleep, weight gain, hoarseness of voice and pretibial myxoedema. Both groups showed weakness but more marked in patients with hypothyroidism. Heart failure with shortness of breath in clinical presentation in both

groups showed no significant difference between these groups (p-value = 0.059, as shown in Table 13, in Chapter 5- Results).

All of the above findings of the present study co-relate very well with the the general features of hyper and hypothyroidism as reported by Strachan and Walker (2010).

Triglyceride (TG) level of our study population has been shown in Fig. 19 and Table 16 (in Chapter 5- Results). The results showed that there was a high level of TG in hypothyroidism with p value highly significant ($p<0.001$). Fig. 20 and Table 17 (in Chapter 5- Results) showed the relation between level of thyroid function and HDL-cholesterol level and the results indicated no significant difference ($p>.077$).

The relation between level of thyroid function and LDL cholesterol of the preset study was shown in Fig. 21 and Table 18 (in Chapter 5- Results). The results indicated that there was a significant elevation of LDL level in patients with hypothyroidism ($p<.001$). Also there was significant elevation of total cholesterol in patients with hypothyroidism ($p <0.001$) as shown in Fig.22 and Table 19 (in Chapter 5- Results).

Therefore, this prospective study shows an elevation of total cholesterol, LDL cholesterol and triglycerides in hypothyroidism; with no significant abnormalities in lipid profile in hyperthyroidism.

According to the results of simple correlation (Table-20, in Chapter 5 – Results) , T3 was not correlated with lipid profiles in these subjects and T4 was correlated negatively with TC, LDL and TG, and positively with HDL ($r= -0.21$, $p <0.01$; $r = -0.18$, $p<0.01$; $r = -0.12$, $p <0.01$ and $r = 0.14$, $p <0.01$). As expected T4 was closely and negatively correlated with TSH ($r = -0.53$, $P<0.01$) and T3 was positively correlated with T4 ($r = 0.195$, $p <0.01$). Here increase in serum T4 levels has got potential beneficial effect on lipid profile that means hyperthyroidism is not associated with risk of development of dyslipidemia.

Moreover, as shown in Table 20, (in Chapter 5- Results) there were positive associations between serum TSH levels and TC, LDL-C and TG and negative correlation with HDL-C ($r = 0.28$, $p<0.01$; $r = 0.23$, $p<0.01$; $r = 0.14$, $p <0.01$ and $r = -0.15$, $p< 0.01$ respectively).

Also according to linear regression as shown in the Figures 23 – 26 (in Chapter 5-Results), TSH level was also positively and linearly associated with T, LDL-C and TG

levels, and negatively associated with HDL-C. As T3 and T4 levels were negatively correlated with TSH level, these were not taken for linear regression.

The more increase in the TSH values, the more was the degree of hypothyroidism. Therefore, hypothyroidism is associated with elevated TC, LDL-C and TG, and thereby with risk of dyslipidemia.

This study correlated with the study of Xing Wanjia *et al.* (2012) which showed TSH levels were correlated in a positive linear manner with the TC, non-HDL-C i.e. LDL-C and VLDL-C, and TG respectively as a risk factor in the context of CHD; with Nivedita Nanda *et al.* (2007) where there was a significant increase in TC and TG levels among the hypothyroid patients, LDL-C and VLDL-C were significantly increased, where as there was no significant changes in HDL-C level.

Elizabeth *et al.* (2008) also showed that there was a statistically significant gradual increase in total cholesterol, triglycerides and low density lipoprotein cholesterol (LDL-C) concentrations as thyroid function declined, even including among those with serum TSH values 5-10mIU/L.

Pazos *et al.* (1995) reported that even in sub-clinical hypothyroidism or mild thyroid failure changes in lipid abnormalities (raised total cholesterol, LDL cholesterol and triglycerides) similar to those with overt hypothyroidism.

The lipid profile is usually normal in sub-clinical/overt hyperthyroidism (Duntas 2002). Hypothyroid patients have high serum concentrations of total cholesterol (TC) and low density lipoprotein (LDL-C) cholesterol (Lithel *et al.* 1981) and some have in addition high serum concentrations of triglycerides (TG) (Nikkila and Ketti 1973).

With respect to serum high density lipoprotein (HDL-C) cholesterol concentrations, high, normal or low values have been reported in different series (Muls *et al.* 1984, Agdeppa *et al.* 1979).

In the study from the Mayo clinic, the lipid profiles of 268 consecutive patients with hypothyroidism were reviewed and it was found that 91.4% of these patients had abnormal lipid values. In frank hypothyroidism an atherogenic lipid profile is observed with elevated total serum cholesterol and low density lipoprotein (LDL) (O'Brien *et al.* 1993).

Increased levels of total and LDL cholesterol are a common finding in hypothyroidism and may represent an increased risk factor for coronary heart disease. In this context it is also remarkable that thyroid antibodies are much more common in subjects with coronary heart disease than in general population. Several investigators found a deleterious effect of mild TSH elevation on serum lipid concentrations (Canaris *et al.* 2000, Walsh *et al.* 2005a, Gussekloo *et al.* 2004, Walsh *et al.* 2005b)

Thyroid hormone and lipids share a long history because the link between thyroid disease and lipid disorders, in terms of hypercholesterolaemia, was described more than 70 years ago (Mason *et al.* 1930). Since then the association between thyroid disease and lipid disorders has been well established (Kutty *et al.* 1978). However, as Hippocrates once said "... the medical art is long lived, the moment deceptive, the experience illusory, and right judgment difficult" (Hippocrates), a re-evaluation of the connections between thyroid disease and lipid disorders seems justified in the light of a pleiad of new data presented in recent years. In hypothyroidism, hypercholesterolemia with increased concentration of very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and low high density lipoprotein (HDL) has been recognized as a major feature (Benvenga *et al.* 1996, Loeb 1996).

It is well recognized that changes in the composition of lipoproteins are present in hypothyroidism and hyperthyroidism (Muls *et al.* 1984; Friis *et al.* 1987). Because thyroid hormone regulates the activity of some key enzymes in lipoprotein transport, it is not surprising that disturbances in lipoprotein transport are usually encountered in thyroid disease. Intracellular free cholesterol suppresses endogenous cholesterol production by inhibiting the rate-limiting enzyme of cholesterol synthesis (hydroxymethyl glutaryl coenzyme A [HMG CoA] reduc-tase), and therefore enabling the cell to regulate its own cholesterol content by a local feedback control system (Ness *et al.* 1973).

Thyroid hormone stimulates the hepatic *de novo* cholesterol synthesis by inducing the enzyme HMG CoA reductase, which catalyzes the conversion of HMG CoA to mevalonate. This results in an enhanced synthesis of cholesterol in hyperthyroidism and a decreased one in hypothyroidism. However, serum cholesterol levels are conversely decreased in hyperthyroidism, or increased in hypothyroidism because thyroid hormone may simultaneously affect the synthesis and degradation of LDL cholesterol (Friis *et al.* 1987). Additionally, approximately 3% of thyroxine is bound to lipoproteins, mainly to

HDL (92%) and less to LDL (6.7%) (Benvenga *et al.* 1988). The T4-LDL complex is recognized by the LDL receptor, and constitutes a credible mechanism of T_4 entry into the cells (Benvenga *et al.* 1990). Finally, thyroid hormone activates the LDL receptor leading to an increased fractional catabolic rate of apoB without influencing its synthetic rate (Statels *et al.* 1990).

Plasma HDL concentrations have been reported as normal or decreased in hyperthyroidism, and normal or even elevated in severe hypothyroidism (Loeb 1996; Statels *et al.* 1990; Scottolini *et al.* 1980). These conflicting results are partly because of the recently reported regulation of CETP and HL activity by thyroid hormone (Tan et al. 1998). CETP transports cholesteryl esters from HDL2 to VLDL, IDL and remnants, and inversely triglyceride to HDL2 (Lagrost 1994). The latter are consequently hydrolyzed and converted to HDL_3 by HL. Thus, HL is the main responsible lipolytic enzyme for the conversion of IDL to LDL and HDL2 to HDL_3. CEPT and more specifically HL seem to be dependent on the status of thyroid function, and they are low in severe thyroid failure and increased in hyperthyroidism (Kussi *et al.* 1980).

Hyperthyroidism exhibits an augmented excretion of cholesterol by the bile together with unchanged or increased en-terohepatic circulation of bile acids (Loeb 1996; Müller *et al.* 1984). Thyroid hormones, as stated before, may stimulate HMG CoA, the key enzyme of cholesterol biosynthesis, and induce an increased synthesis of cholesterol (Ness et al. 1973). However, the serum cholesterol levels are decreased mainly because of simultaneous enhancement of the turnover of LDL. This leads to a further decrease of total and LDL cholesterol in hyperthyroidism. The promoter of the LDL receptor gene contains a thyroid hormone responsive element (TRE), which could allow triiodothyronine (T3) to modulate gene expression of the LDL_receptor resulting in an increase, LDL clearance (Bakker *et al.* 1998).

In recent years, more and more evidence has demonstrated that hypothyroidism is associated with the increased prevalence of CHD (Cappola *et al.* 2003, Neves *et al.* 2008) This association is partly due to decreased levels of thyroid hormones, which lead to an atherogenic lipid profile characterized by increased levels of total cholesterol (TC) and low density lipoprotein cholesterol ILDL-C) (Duntas 2002).

High total cholesterol is a marker for atherogenic lipoproteins. In population studies, the serum total cholesterol is a good surrogate for LDL cholesterol levels. The Framinham

Heart Studies (Wilson *et al.* 1998). The Multiple Risk Factors Intervention Trial (MRFIT) (Stamler *et al.* 1986), and the Lipid Research Clinic(LRC) trial (Lipid Research Clinic Program,1984) found a direct relationship between levels of LDL cholesterol (or total cholesterol) and new onset CHD in men and women who were initially free of CHD. The same relation holds for recurrent coronary events in people with established CHD (Rossouw *et al.* 1990; Pekkanem *et al.* 1990).

Studies across different populations reveal that those with higher cholesterol levels have more atherosclerosis and CHD than do those having lower levels (McGill 1968, *Keys et al.*1980, 1984). The relationship of elevated LDL cholesterol to the development of CHD must be viewed as multi-step process beginning early in life (Stary 1992, 1994, 1995).

Many prospective epidemiological studies have reported a positive relationship between serum triglycerides and evidence of CHD (Austin *et al.*1998, Assman *et al.* 1998). Non lipid risk factors of obesity, hypertension, diabetes and cigarette smoking are also interrelated with triglycerides (Grundy 1998). Raised triglycerides are in fact an independent risk factor for CHD.

In conclusion, the results of lipid profile analysis in patients with thyroid disorders of the present study shows that, there were significant elevation of triglycerides (TG), LDL cholesterol (LDL-C) and total cholesterol with no significant change of HDL cholesterol (HDL-C) in hypothyroidism. Hyperthyroidism shows no significant change in lipid profile relating to dyslipidemia. Also TSH levels were positively and linearly associated with TC, LDL, TG and negatively with HDL. The more increase in the TSH values, the more was the degree of hypothyroidism. Therefore, hypothyroidism is associated with elevated TC, LDL-C and TG, and thereby with risk of dyslipidemia.

Finally, it may be concluded that hypothyroidism relates to dyslipidemia - a major risk factor for development of Coronary Heart Disease (CHD) in particular and proper replacement of thyroid hormones in hypothyroid patients (overt/sub-clinical) can change total scenario of atherosclerotic disease with coronary heart disease in particular, cerebrovascular disease, peripheral vascular disease, etc.

The limitation of the present study is its relatively small size. Furthermore, the thyroid status was classified in all patients based on one blood test. Thus, some individuals with transient TSH elevations might have been misclassified. Further study should involve

repeated thyroid hormones testing, especially TSH. Also study is not community based, rather higher centre/hospital based. Therefore, the study population does not represent the whole population. In the statistical analysis, relatively simple models we used explained only a limited amount of variations in the serum lipid profiles. Free peripheral thyroid hormone levels were not measured, making to some extent difficult to assign definite thyroid diagnoses.

CHAPTER-7

SUMMARY AND CONCLUSION

7.0. SUMMARY AND CONCLUSION

Thyroid disorders have no age barrier but most prevalent with manifestation among the middle aged people. In our country thyroid disorders are mainly seen in Northern part, more along the belt of the river Brahmaputra and the Jamuna where most peoples are poor, instead there is increasing number of Ischemic Heart Disease (IHD).

This prospective study was aimed to find out relation between thyroid function and dyslipidemia in patients with thyroid disorders. This study was carried out among the patients clinically suspected with thyroid disorders during the period of July 2004 to June 2009, availing the laboratory facilities of the Centre for Nuclear Medicine and Ultrasound, Mymensingh and Rangpur, Department of Bio-chemistry, BSMMU (Banga Bandhu Sheikh Mujib Medical University), Dhaka and the Institute of Biological Sciences, the University of Rajshahi, Rajshahi, Bangladesh.

Five hundred thirty three (533) patients with clinically suspected thyroid disorders were selected and examined for T3, T4 and TSH. Lipid profiles of all the patients were measured after overnight fasting. Out of the total 533 study population, 207 were euthyroid, 162 were hyperthyroid and 164 were hypothyroid.

There was female preponderance with no exemption of age with maximum patients in the middle age group. Patients with hyperthyroidism were associated clinically with hot intolerance, increased appetite, decreased sleep, weight loss, palpitation and tremor. where as patients with hypothyroidism were associated predominantly with cold intolerance, decreased appetite, increased sleep, weight gain, hoarseness of voice and pretibial myxoedema.

With thyroid enlargement in consideration, mild to moderate enlargement was with high prevalence.

Regarding lipid profile in patients with thyroid disorders, there were significant elevation of triglycerides (TG), LDL cholesterol (LDL-C) and total cholesterol with no significant change of HDL cholesterol (HDL-C) in hypothyroidism. Also TSH levels were positively and linearly associated with TC, LDL, TG and negatively with HDL. The more increase in the TSH values, the more was the degree of hypothyroidism. Therefore, hypothyroidism is associated with elevated TC, LDL-C and TG, and thereby with risk of dyslipidemia.

Hyperthyroidism shows no change significant change in lipid profile relating to dyslipidemia.

Finally, it may be concluded that hypothyroidism relates to dyslipidemia; major risk factor for development of Coronary Heart Disease (CHD) in particular.

Recommendations

Screening of dwellers particularly middle aged group in Northern part of Bangladesh along the belt of river Brahmaputra and the river Jamuna for thyroid function and thereafter, proper replacement of thyroid hormones in hypothyroid patients (overt/sub-clinical) can change total scenario of atherosclerotic disease with coronary heart disease in particular, cerebrovascular disease, peripheral vascular disease, etc. Also it may change the treatment strategy in prophylaxis at risk group from using antilipid and antiplatelets and thereby reducing cost effectiveness. In the treatment of CHD and dyslipidemia thyroid function, especially the serum TSH level should be monitored and maintained in the relatively low normal range. Further large prospective studies are needed to clarify the above relationship and to confirm its clinical implications.

CHAPTER-8

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8. REFERENCES

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CHAPTER-9

APPENDICES

9. APPENDICES

APPENDIX-I

PATIENT RECORD SHEET

Date: .....

Reg. No. .....

Name of the Patient: ..... Age: ..... Sex: .....

Address:

Monthly income (if possible): ..... Tk.

Name of the Institute: ----------

CLINICAL HISTORY

Appetite: Normal/Increased/Decreased

Bowel Habit: Constipation/Loose motion

Sleep: Normal/Increased/Decreased

Palpitation: Yes/No

Tremor: Yes/No

Sweating: Normal/Increased/Decreased

Intolerance to hot/cold.

Weakness:

Shortness of breath

Oedema

Family History:

Weight: Unchanged/Loss/Gain

Menstrual History: Regular/Irregular.

Neck Swelling:

Drug History: Thyroid drugs, Beta blockers, Diuretic, Iodinated drugs

History of any severe illness: Diabetes Mellitus, Nephritic syndrome, Renal failure, Obstructive liver disease

GENERAL PHYSICAL EXAMINATION:

Anaemia:

Jaundice:

Pulse:

Tremor:

Eye Sign

Pretibial myxoedema

Height:

Weight:

Hoarseness of voice

LOCAL EXAMINATION (NECK) FOR THYROID

Size of thyroid gland: Not Palpable / Palpable

Consistency:

Tenderness:

Mobility:

Nodule: Present/Absent: Number of nodule: Single/multiple

Location: Rt. Lobe /Lt. Lobe /Isthmus /both lobes.

Consistency of nodule: Firm/Hard

Thyroid hormones: T3 level: ---------- T4 level: ---------- TSH level: ----------

Uptake & scan (whenever needed):

Ultra Sonogram of Thyroid (whenever needed):

Lipid Profile: Total Cholesterol……., HDL-C ……..

TG …… LDL-C ……

Diagnosis: Hyper Thyroid / Hypo Thyroid / Euthyroid (Normal thyroid function)

Signature of the investigator

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APPENDIX-II

CONSENT FORM

Study for evaluation of relationship between thyroid function in patients with thyroid disorders and dyslipidemia which is the main and major risk factors for development of atherosclerosis causing Coronary Heart Disease (CHD) in particular.

We shall take history and then examine you physically. Later blood samples will be taken for estimation of T3, T4 and TSH. Then you will be asked to come next day morning after overnight fasting for another blood sample for estimation of lipid profile. We shall record the illness that you may suffer during the study period and we shall treat you effectively.

This research work may help us to understand the role of thyroid function abnormality in lipid profile and thus may enable us to find a new dimension of management strategy in patients with thyroid disorders who are at high risk for development of Ischemic Heart Disease (IHD).

You can withdraw yourself from the study at any time. If you are willing to participate in this study, then please put your signature/left thumb impression below.

Signature of the Principal Investigator

Signature/left thumb impression of the participants

APPENDIX-III (A)

THYROID HORMONES ESTIMATION

Blood level of thyroid hormones:

Basic principles of radioimmunoassay (RIA) (Heel 1985)

This method depends on the competition between biologically important substances and tracer label (usually ^{125}I) of these substances for a limited number of binding sites on a specific antibody. The proportion of the tracer labelled substances bound to the antibody is inversely related to the concentration of the substance present in the serum.

T_3/T_4 ASSAYS

Estimation of T_3 and T_4 was done by RIA method.

Principle:

^{125}I-labelled ^{T3}, T_4 contained in standard or patients sample competes for a fixed amount of T_3, T_4 antibody. Amount of tracer-bound antibody is inversely related to the amount of T_3, T_4 in the sample. by measuring the count of the tracer-bound antibody, concentration of T_3, T_4 in the unknown is interpolated from the standard curve.

Contents of the test kit:

Tracer: ^{125}I-labelled T_3, T_4

Standard: 6 vials of different concentrations.

For T_3: 0, 0.8, 1.5, 3.1, 6.2, and 12.3 nmol/L, For T_4: 0, 26, 52, 103, 206 and 309 nmol/L

T_3, T_4 antibody

Precipitant

Equipments:

1. Test tube with rack
2. Micropipette (50, 200 and 500 μl) with disposable tips
3. Vortex mixer
4. Centrifuge machine

5. Gamma counter

Assay protocol

Test tubes: Labelling and arrangement done in tube rack

Tube No.:

--> Total

--> Standard

--> Quality control (QC)

Rest --> Patients

(two tubes for each)

50 μl pipetted from standard, QC and patients serum in respective test tube.

200μl pipetted from tracer (^{125}I-labelled T_3/T_4) in all test tubes. Tube 1-2 separated.

200 μl antibody added to all test tubes except 1-2.

Vortexed for 10 seconds.

Kept in water bath at 37°C – 60 minutes for T_3 45 minutes for T_4.

Vortex mixing done and kept at room temperature for one hour.

Centrifuged at 3500 rpm for 20 minutes.

Decantation of all test tubes done except 1-2.

Counting was performed by RIA gamma counter.

The PC (personal computer) based RIA counter calculated the counts of each sample (unknown, standard and quality control) and also gave different quality control parameters. From the computer reading, values (expressed in nmol/L) of T_3 and T_4 were recorded.

Thyroid stimulating Hormone (TSH) assay by Immuno-radiometric assay (IRMA) (Heel 1985).

Principle:

TSH antigen of the standard/QC/sample makes non-competitive binding with monoclonal antibody (McAb) as well as polyclonal antibody (PcAb). Here, McAb is the tracer which is tagged with ^{125}I PcAb is coupled with magnetic iron oxide particle PcAb<M>. The complex ^{125}I-McAb-TSH-PcAb<M> (sandwich) is separated from free tracer by magnetic separator and

decantation. Activity of the complex is directly proportional to the TSH concentration in the sample.

Contents of TSH kit:

Tracer: ^{125}I-labelled McAb.

PcAb<M>: Polyclonal antibody coupled with magnetic iron oxide.

TSH standard:

Lyophilized:

Labelled

A, B, C, D, E, F, G

Concentration

0, 0.35, 1.0, 3.3, 10, 22, 75 mIU/L.

Concentrated wash buffer.

Equipments:

1. Test tube with rack
2. Micropipette (50, 200, 500μl and 1 ml) with disposable tips
3. Vortex mixer
4. Magnetic separator
5. Absorbent blotting paper
6. Gamma counter

Preparation for assay:

Standard: Reconstituted each vial with 1 ml of distilled water to get exact concentration.

Wash buffer (concentrated): Diluted with distilled water at ratio of 1:10.

Magnetic TSH-Ab suspension: resuspended by gentle mixing.

The kit and sample were allowed to reach temperature before use.

Assay protocol:

Labelling of the test tubes and arrangement in the tube rack.

Tube No.:

--> Total

--> Standard

--> Quality control (QC)

Rest --> Patients (samples)

(Two tubes for each)

Standard, QC, sample: 200 μl pipetted in respective test tubes.

Tracer (^{125}I-McAb): 50 μl pipetted in all test tubes.

Tube 1-2 was separated. Then vortex mixing was done.

Incubated in water bath at 37°C for one hour.

Second antibody [PcAb<M>]: 500 μl pipetted in all test tubes except 1-2.

Again vortex mixing done.

Incubated at room temperature for one hour.

Test tube rack was placed on a magnetic separator for 10 minutes -> decantation was done and rack was removed from separator.

One ml wash buffer was added in all test tubes except 1-2.

Again vortex mixing done.

Again, test tube rack was placed on a magnetic separator for 10 minutes -> decantation was done and rack was removed from separator.

Again, wash buffer was added.

Again, vortex mixing done.

Again, test tube rack was placed on a magnetic separator for 10 minutes -> decantation was done and rack was removed from separator.

Counting was performed by PC based RIA gamma counter.

The computer of RIA counter calculated the counts of each sample (Unknown, standard and quality control) and also gave different quality control parameters. From the computer reading, values (mIU/L) of TSH were recorded.

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APPENDIX-III (B)

ESTIMATION OF LIPID PROFILE

CHOLESTEROL (TOTAL)

Enzymatic colorimetric test (CHOD-PAP)

PRINCIPLE

Cholesterol and its esters are relesed from lipoproteins by detergents. Cholesterol esterase hidrolizes the esters the esters and H202 is formed in the subsequent enzymatic oxidation of cholesterol by cholesterol-oxidase according to the following equation.

$$\text{Esters cholesterol} + H_2O \xrightarrow{CHE} \text{Cholesterol} + \text{fatty acids.}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CHOD} \text{Cholest-4-en-one} + H_2O_2$$

$$H_2O_2 + \text{4-AP} + \text{phenol} \xrightarrow{POD} \text{Quinonimine} + H_2O$$

The quantity of this red dye quinonimine formed is proportional to the cholesterol concentration .

REAGENTS

Reagents 1	Pipes pH 6.9	90 mmol/1.
	Phenol	26 mmol/1.
Reagents 2	Peroxidase	1250U/L
Vial of enzymes	Cholesterol esterase	300 U/L
	Cholesterol oxidase	300 U/L
	4- Aminophenazone	0.4 mmol/l

PREPARATION AND STABILITY

Dissolve the contents of one bottle R.2 to the contents of one bottle Buffer Reagent R.1

This working reagent is stable 4 months at 2-8 C. or 15 days at + 15-25 C

PROCEDURE

	Blank	Standard	Sample
Standard	--	20 ul	--
Sample	--	--	20 ul
Working reagent	2 ml.	2 ml	2 ml

Mix, incubate 5 min. at + 37 C . or min. at + 15-25 C.
Measure the extintion (E) of standard and sample against
Blank reagent at 505 nm. (500-550) or Hg. 546 nm.
The colour is stable for 30 min.
Cholesterol (total)

Calculation

$$\text{Cholesterol conc.} = \frac{\text{E. sample}}{\text{E. stanadrad}} \times \text{conc.standard}$$

Mg/dl x 0.0258 = mmol/l.

Linearity

This method is linear up to 600 mg/dl. (15.4 mmol/l.)

If the cholesterol concentration is greater than 600 mg/dl. In th serum or plasma, dilute the sample 1:2 with saline solution and repeat the determination and multiply the result by 2.

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QUALITY CONTROL

SPINTROL. Normal and pathological.

HDL

Enzymatic colorimetric (CHOD-PAP)

PRINCIPLE

Low density lipoproteins (LDL and VLDL) are specifically precipitated by phosphostungtic acid and magnesium ions and can then be removed by centrifugation. High density lipoproteins (HDL) remain in the supernatant. Determination of HDL cholesterol is performed using the clear supernatant.

REAGENTS

Reagents A **Prep. Reag**	Phosphotungstic Acid 14 mmol/1. Magnessium Chloride 2 mmol/1.
Reagents B	Cholesterol Reagent Cod. : 1001092-1001093

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PREPARATION AND STABILITY

R.A is ready for used. Stored at +2 to +8 C up to the date of expiration as specified.

Rf. S-2012.

Dissolve the contents of one bottle R.2 to the contents of one bottle Buffer Reagent R.1

This working-reagent is stable 4 months at +2 to +8 C. or 15 days at + 15 to +25 C

PROCEDURE

Pipette into a centrifuge tube:

Serum	1 ml.	0.5 ml.
R.A (Reagent)	0.1 ml.	0.05 ml.
Mix well, allow to stand for 10 min. at room temperature and centrifuge at 4000 rpm. For 20 min. or at 12000 rpm. For 2 min.		

Pipette into reaction vessel :

	Macro-test		Semimacro test	
	Blank	Sample	Blank	Sample
Clear Supernatant	--	40 ul.	--	20 ul.
Working Reagent	2 ml.	2 ml.	1 ml.	1 ml.
Mix and incubate at 37 C. for 5 min. or at room temperature for 10 min. Measure at 505 nm (500-550) or Hg. 546 nm. The extinction (E) of sample against Blank reagent.				

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Calculation

HDL – Cholesterol (mg/dl) = E.Sample×320 at 505 nm.

HDL – Cholesterol (mg/dl) = E.Sample×475 at 546 nm.

Linearity

This method id linear up to 275 mg/dl. Of HDL. Chol.

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TRIGLYCERIDES

Enzymatic-colorimetric test (GPO-PAP).

PRINCIPLE

The triglycerides are enzimatically hindrolized to glycerol and free fatty acids. The glycerol liberated reacts with Glycerol Kinaseand Glycerol

-3-Phpsphate Oxidase yielding H2O2. The H2O2 concentration is determined throught the Trinder's reaction.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol+fatty acids.}$$

$$\text{Glycerol} + ATP \xrightarrow{\text{GK}} \text{Glycerol -3-Phosphate} + ADP$$

$$\text{Glycerol -3-P} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone-P} + H_2O_2$$

$$H_2O_2 + \text{4-AP} + \text{p-chlorophenol} \xrightarrow{\text{POD}} \text{quinoneimine} + H_2O\ .$$

Chapter -9

REAGENTS

Reagent 1	GOOD Buffer pH 7.5	50 mmol/l.
	p-chlorophenol	2
	mmol/l.	
Reagent 2	Lipoproteinlipase	150000 U/L.
	Glycerol Kinase	500 U/L
	Glycerol-P-oxidase	2500 U/L.
	Peroxidase	440 U/L.
	4-Aminophenazone	0.1 mmol/l.
	ATP	0.1 mmol/l.

PREPARATION AND STABILITY

Dissolve the contents of one bottle R.2 to the contents of one bottle Buffer reagent R.1.

This working reagent is stable 4 weeks at +2+8 C. or 1 week at room temperature .

PROCEDURE

	Blank	Standard	Sample
Standard	--	20 ul	--
Sample	--	--	20 ul
Working Reagent	2 ml	2 ml	2 ml

Mix, incubate 4 min. at 37 C. or 10 min. at room temperature .

Measure the extinction at 505 nm (490-550) against Blank.

The colour is stable for 30 min.

Calculation

$$\frac{\text{E. sample}}{\text{E. standard}} \text{ X Standard conc. = sample conc.}$$

Mg/dl X 0.0113 = mmol/l

Linearity

This method is linear up to 1000 mg/dl
If the triglycerides concentration is greater than 1000 mg/dl. dilute the sample 1:2 with saline solution and repeat the determination and multiply the result by 2.

Bibliography

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Calculation of LDL-Cholesterol

According to the Friedewald Formula (1972):

$$\text{LDL-Chol} = \text{Total Cholesterol} - \frac{\text{Triglycerides}}{5} - \text{(HDL-Chol)}$$

APPENDIX-IV

ABBREVIATIONS

ABI	Ankle/Brachial Blood Pressure Index
ACS	Acute Coronary Syndromes
ATP	Adenosine Tri Phosphate
ATP Classification III	Adult Treatment Panel III
BRAC	Bangladesh Rural Advancement Committee
BSMMU	Bangabandhu Sheikh Mujib Medical University
CAD	Coronary Artery Disease
CETP	Cholesteryl Ester Transfer Protein
CHD	Coronary Heart Disease
DIT	Di-iodotyrosine
EID	Environmental Iodine Deficiency
FSH	Follicular Stimulating Hormone
GH	Growth Hormone
HDL-C	High Density Lipoprotein - Cholesterol
HL	Hepatic Lipase
ICCIDD	International Council for Control of Iodine Deficiency Disorders
IDD	Iodine Deficiency Disorders
INM	Institute of Nuclear Medicine
IRMA	Immuno-radiometric Assay
LCAT	Lecithin Cholesterol Acyltransferase
LDL-C	Low Density Lipoprotein - Cholesterol
LH	Luteinizing Hormone
MIT	Mono-iodotyrosine
MTF	Mild Thyroid Failure
RIA	Radio Immunoassay

T3	Triiodothyronine
T4	Tetraiodothyronine
TBG	Thyroxine Binding Globulin
TBPA	Triiodothyronine Binding Protein
TC	Total Cholesterol
TG	Triglyceride
TGRLP	Triglycerides Rich Lipoproteins
TIA	Transient Ischaemic Attack
TRH	Thyroid Releasing Hormone
TSH	Thyroid Stimulating Hormone
TSIS	Thyroid-Stimulating Immunoglobulins
UNICEF	United Nations International Children's Emergency Fund
VLDL-C	Very Low Density Lipoprotein - Cholesterol
WHO	World Health Organization