ROLE OF ANTI-TG AND ANTI–TPO IN HYPO– AND HYPERTHYROIDISM

BY

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DEPARTMENT OF BIOLOGICAL SCIENCES
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CERTIFICATE

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ABBREVIATIONS

T3	Tri-iodothyronine
T4	Thyroxine
TSH	Thyroid stimulating hormone
TRH	Thyrotropin releasing hormone
TG	Thyroglobulin
TPO	Thyroid peroxidase
RF	Rheumatoid Factor
FPIA	Fluorescence Polarization Immunoassay
MEIA	Microparticle Enzyme Immunoassay
TBP	Thyroxine Binding Proteins
TBG	Thyroxine Binding Globulin
TBA	Thyroxine Binding Albumin
TBPA	Thyroxine Binding Pre Albumin
MIT	Mono-iodotyrosine
DIT	Di-iodotyrosine
Ig	Immunoglobulin
µg	Micro gram
IU	International Units
ml	Milliliter
dl	Deciliter

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INTRODUCTION

INTRODUCTION

Thyroid Gland Structure & Function

Thyroid Gland, endocrine gland found in almost all vertebrate animals and so called because it is located in front of and on each side of the thyroid cartilage of the larynx Like man, thyroid is also present in lower animals, where it has well defined role in regulating metabolic processes. Although present in all vertebrates its shape and position varies in different vertebrate groups. In lower vertebrates there is no compact, encapsulated thyroid gland but the gland is in the form of follicles. In cyclostomes such as lamprey the thyroid follicles are spread along the ventral aorta and frequently found near the branchial arches of the gills. In teleosteans, thyroid follicles are present from pharyngeal region to the distal region of the body, and again, no compact gland but only the follicles are present (Bentley, 1998).

The fully developed thyroid gland in man is a brownish-red organ composed of two lobes, connected by a thin band of tissue, the Isthmus, which gives the gland the appearance of a butterfly. The gland is closely attached to trachea in the anterior aspect of neck. Each lobe measures about 2.0- 2.5 cm in both thickness and width and about 4.0 cm in length. The isthmus measures about 2.0 cm in both length and width and about 0.5 cm in thickness. The gland normally weighs about 28 g (about 1 oz), (Hole,1993).

The secretory units of thyroid gland are follicles that consist of an outer layer of epithelial cells. These cells rest on basement membrane and enclose an amorphous material called colloid which is mainly composed of thyroglobulin (an iodinated glycoprotein) and small quantities of iodinated thyroalbumin. The follicles are embedded in stromal tissue, which contain blood vessels and autonomic nerve fibers. Increased activity of gland is characterized by a decrease in the quantity of colloid with subsequent reduction of follicular volume. Accordingly the lining cells become columnar and may even proliferate into colloid. On the other hand, during decreased glandular activity the follicles enlarge because of accumulation of colloid, and follicular cells become flattened. The cytoplasm of the follicular cells has a micro-tubular network, and microvilli that extend from apices of cells into the colloid. Important reactions of the thyroid hormone synthesis, such as iodination and the initial phase of hormone

secretion (colloid resorption), are believed to take place at or near apical surface of the cell. Thyroid follicular cells, like cells of other endocrine glands, have a prominent endoplasmic reticulum (Refetoff, 1979).

Thyroid hormones

The thyroid gland secretes two hormones, triiodothyronine and tetraiodothyronine (thyroxin), which are commonly known as T3 and T4, respectively. T4 is the primary secretory product of the gland. In addition thyroid gland secretes small amounts of biologically inactive reverse T3 as well as minute quantities of monoiodotyrosine (MIT) and di-iodotyrosine (DIT), which are precursors of T3 and T4. Approximately 40% of secreted T4 is deiodinated by liver and other peripheral tissues to yield T3 and about 45% is deiodinated to yield r T3. From the estimated daily production rates for T3 and rT3, it is evident that at least 85 % of normal T3 production and essentially all of the rT3 production can be accounted for by peripheral deiodination of T4 rather than by direct secretion by thyroid gland. T3 is 4-5 times more potent in biological system than T4 (Teitz, 1996).

Thyroid hormones have many actions and these hormones are indispensable for growth, development, and sexual maturation in mammals. One is calorigenic effect (increased oxygen consumption) on many tissues that is produced by effects on membrane transport and mitochondrial metabolism (stimulation of mitochondrial respiration and oxidative phosphorylation). However some tissues for example those of the brain, retina, lung, spleen and testes do not appear to be affected by this action of hormones (Shambaugh, 1978). They cause stimulation of heart rate and heart contraction, enhancement of sensitivity of beta-adrenergic receptors to catecholamines, stimulation of protein synthesis and carbohydrate

metabolism, increase in synthesis and degradation of cholesterol and triglycerides, and increase in vitamin requirement. These effects are usually magnified in patients with hyperthyroidism and minimized in patients with hypothyroidism (Sterling, 1979).

Biosynthesis, Secretion and Metabolism

The biosynthesis of thyroid hormones involves thyroid trapping of serum iodide, incorporation of iodine into tyrosine, coupling of iodinated tyrosyl residues of thyroglobulin, and proteolytic cleavage of follicular thyroglobulin to release iodothyronines (Berger and Quinn, 1976).

The important element involved in the synthesis of thyroid hormones is iodine, which is normally ingested in the form of iodides. Iodide transport to the follicles is the first and rate-limiting step in the synthetic process. The follicular cells concentrate iodide some 30-40 times the normal plasma levels by means of an energy-dependant Na-I co transport pump mechanism (Larsen et al., 1987). The iodide is oxidized in the thyroid gland to more reactive iodine, possibly as the free radical of iodine, in a matter of seconds; it gets bound to tyrosine molecules attached to a thyroidal protein called thyroglobulin (TG). TG is also synthesized within follicular cells before being secreted into follicular lumen by exocytosis. The enzyme responsible for the oxidation and binding of iodide is thyroid peroxidase (TPO). In this process hydrogen peroxide accepts the electron and MIT and DIT are formed. Two DIT molecules undergo an oxidative condensation with the release of an aniline residue for the formation of T4, which is still bound by peptide linkage to TG. Similarly T3 is yielded from coupling of MIT and DIT. A small amount of rT3 is also formed by condensation of DIT and MIT. The condensation reaction is an aerobic, energy-requiring reaction, and like oxidation and binding, is considered to be catalyzed by TPO (Larsen et al., 1987).

Synthesis of T3, T4, DIT and MIT in TG molecules occurs mainly at the follicular cell-colloid interface but also within the colloid. TG is present in highest concentration within the colloid, where it is stored. The follicular cells engulf colloid globules by endocytosis; these globules then merge with the lysosomes in the follicular cell. Lysosomal proteases break the peptide bonds between iodinated residues and TG, and T4, T3, DIT and MIT are released into cytoplasm of the follicular cell. From here T4 and T3 diffuse into the systemic circulation. DIT and MIT are deiodinated by an intracellular microsomal iodotyrosine dehalogenase and the freed iodide is re-utilized. Each step involved in the synthesis of thyroid hormones is regulated by TSH from anterior pituitary. This hormone stimulates the "iodide pump", TG synthesis and colloid uptake by follicular cells. The rate of proteolysis of TG for the liberation of T4 and T3 is also regulated by TSH. In addition, TSH induces an increase in size and number of thyroidal follicular cells. Prolonged TSH stimulation leads to increased vascularity and eventual hypertrophic enlargement of thyroid gland (Besser and Thorner, 2002).

Clinical Significance of Thyroid Hormone Measurement

Thyroid Physiology

A tightly coordinated feedback relationship exists among thyroid gland, the hypothalamus, and the pituitary gland (Larson, 1982). The systems of these glands are closely interrelated and integrated, the net result being maintenance of thyroid hormone levels in blood within appropriate range under different physiological conditions (Brown et al., 1974).

Thyrotropin releasing hormone (TRH) secreted by the hypothalamus, acts on pituitary thyrotropes to cause synthesis and release of TSH (Jackson, 1982). A rise in thyroid hormone level inhibits the pituitary response to TRH via negative feedback loop system. A fall in

thyroid hormone level causes an increase in TRH and TSH secretion. T4 undergoes peripheral deiodination of the outer ring to yield T3; this occurs primarily in the liver. Reverse T3, produced by removal of one iodine from inner ring of T4, is metabolically inactive and is the end product of T4 metabolism Peripheral deiodination is a rapidly responsive mechanism of control for thyroid hormone balance. Acute or chronic stress or illness causes a shift in the direction of this deiodination favouring formation of rT3 rather than T3, whereas the T4 level remains essentially unchanged. Various medications also shift peripheral deiodination towards the inactive product rT3 (Ermans, AM. 1986).

T4 and T3 in circulation are reversibly and almost completely bound to carrier proteins. These proteins are T4-binding globulin (TBG), T4-binding prealbumin (TBPA) and T4-binding albumin (TBA). About 99.7% of T4 and T3 exist in bound form and only a considerably small fraction of each of these hormones is unbound and free for biological activity. Because there is a wide variation in the concentration of T4-binding proteins, even under normal circumstances, there is a wide variation in total T4 levels among euthyroid individuals (i.e., those having normal thyroid functions). Total T3 concentrations also vary with alteration in binding proteins, although to a lesser degree than T4 levels (Glinoer et al., 1978).

Clinical significance of T3

Triiodothyronine (T3) was first identified in human serum by Gross and Pitt-Rivers., (1952). Since then the physiological effects of T3 have been widely investigated (Braverman et al., 1970), and appreciation of its clinical significance has greatly increased. T3 and its associate thyroid hormone (T4), are responsible for regulating diverse biochemical processes throughout the body which are essential for normal development, metabolic and neural activities. T3 has a molecular weight of 651 dalton and contains 58% iodine. Although T3 has a considerably lower concentration in human serum than T4, T3 is less avidly bound to serum

proteins and has a greater metabolic activity by weight (Felig et al., 1987). There is evidence that T3 is metabolically active hormone, with T4 serving as a "prohormone" for T3 just as thyroglobulin is for T4 (Schwartz et al., 1971). T3 has a half life in serum of only 1.5 days (Larsen, 1972). It has become apparent in recent years that T3 plays an important role in the maintenance of the euthyroid state. Serum T3 measurement can be a valuable component of a thyroid-screening panel in diagnosis of certain disorders of thyroid function as well as conditions caused by iodide deficiency (Marsden and McKerron., 1975). Under conditions of strong thyroid stimulation, the T3 measurement provides a good estimation of thyroid reserve (Larsen, 1972). Recognition of a thyroid dysfunction called T3-thyrotoxiosis is associated with an increased serum T3 level but T4 and free T4 remain normal and *in vitro* uptake results have further highlighted the importance of serum total T3 measurement (Ivy, 1971; Larsen, 1972; Hollander, 1972).

Clinical significance of T4

It is an iodine-containing hormone, which has a molecular weight of 777 dalton. T4 and its associate thyroid hormone T3 are responsible for regulating diverse biochemical process throughout the body as mentioned earlier. Although T3 has greater biologic potency, T4 is normally present in human serum in approximately 50–fold excess of circulating T3 and account for more than 90% of the circulating protein bound iodine (Lerman, 1953). T4 is 99.9% bound to serum thyroxin binding proteins (TBP). Less than 0.05% of the total circulating T4 is unbound and therefore biologically active (Robbins and Rall., 1967; Ekins, 1979).

Clinically T4 measurements have long been recognized as an aid in the assessment and diagnosis of thyroid status. Elevated T4 values are characteristically seen in patients with overt hyperthyroidism while T4 levels are generally depressed in patients with overt hypothyroidism. T4 levels are altered by physiological or pathological changes in thyroxine binding protein capacity (Robbins and Rall., 1967) , which has a pronounced effect on the concentration of thyroid hormones. Consequently, T4 levels may be elevated with increased concentrations of TBG, such as in pregnancy, administration of oral contraceptives or estrogen, infectious and

chronic active hepatitis, biliary cirrhosis or congenital increase in TBG levels. Conversely, when TBG levels are decreased, such as in nephritic syndrome, androgen therapy, glucocorticoid therapy, major systemic illness or congenital decrease of TBG, T4 may be reduced. In most of the cases T4 values give good indications of thyroid status (Oppenheimer, 1968).

Clinical significance of TSH

Human thyroid stimulating hormone or thyrotropin is a glycoprotein with a molecular weight of approximately 28,000 KDa. It is synthesized by basophilic cells (thyrotropes) of anterior pituitary. Human TSH is composed of two non covalently linked α and β subunits. Although α subunit is common to all glycoprotein hormones, the β subunits are hormone specific and confer biological as well as immunological specificity. Both α and β subunits are required for biological activity (Pierce, 1971). TSH stimulates the production and secretion of metabolically active thyroid hormones, by interacting with a specific receptor on the thyroid cell surface (Reese-Smith et al., 1977). The synthesis and secretion of TSH is stimulated by thyrotropin releasing hormone (TRH), the hypothalamic tripeptide, in response to low levels of circulating thyroid hormones (Sterling and Lazarus., 1977; Patel et al., 1972). Elevated levels of T3 and T4 suppress the production of TSH via a classic negative feedback mechanism.

Diagnosis of Thyroid hormone Excess and deficiency states

Several different kinds of laboratory tests are commonly employed to evaluate the patients with thyroid hormone dysfunction. These include.

i. Estimation of hormone concentration
ii. Estimation of free hormone fraction
iii. Estimation of free hormone concentration
iv. Estimation of serum binding proteins such as Thyroxin binding globulin (TBG), Thyroxin binding prealbumin (TBPA) and Thyroxin binding albumin (TBA).

v. Measurement of other hormones and thyroid related proteins such as thyrotropin releasing hormone, thyroglobulin and calcitonin.

When the immune system is active against thyroid tissue, antibodies are formed against various antigenic components of the gland. The most commonly encountered antibodies are directed against thyroglobulin, microsomes, thyroid peroxidase, and TSH receptors. The level of these antibodies can be measured in the serum of patients with suspected auto-immune thyroid disease.

Hypothyroidism It is mainly of three kinds.

Primary Hypothyroidism

It is a common disease that affects 2-3% of population. It results when the thyroid gland is damaged and unable to produce an adequate amount of T4 and T3, such as that happens in case of chronic lymphocytic thyroiditis. It also occurs in inherited conditions in which thyroid hormone synthesis is inefficient such as with enzyme abnormalities associated with dyshormonogenesis. Ablation or removal of thyroid tissue with radioactive iodine or surgery can also result in primary hypothyroidism. Thyroid enlargement may or may not be present, depending on the underlying cause. The decrease in T3 and T4 concentration consequently leads to an increase in TSH level and it has been found that a two-fold change in FT4 produces a 160 fold change in TSH level. This relationship makes TSH measurement a powerful tool in early detection of thyroid failure (Spencer et al., 1989).

Congenital hypothyroidism

This may be due to absence of thyroid gland (athyreosis) or may occur secondarily due to defects of thyroid hormone synthesis. Screening programs for congenital hypothyroidism are

helpful for an early diagnosis of the condition (Walfish, 1984; Fisher, 1985). This disorder occur once in every 3500 to 4000 live births, and an early treatment is critical if mental retardation is to be prevented (Fisher and Folly., 1989).

Secondary hypothyroidism

It results from pituitary or hypothalamic diseases that produce a deficiency of TSH, TRH or both. Isolated TSH deficiency is however rare, and most patients with secondary hypothyroidism also have other pituitary hormone deficiencies. In secondary hypothyroidism serum thyroid hormone concentrations are low, but TSH level is either low or within the reference intervals (Inappropriately low for the low T4 level), or only slightly elevated. When both T4 and TSH levels are low, a TRH test proves helpful (Watts and Keffer,1982).

Hyperthyroidism

It occurs due to increased thyroid hormone in circulation. It may be TSH independent as in Grave's disease, toxic goiter, thyroiditis and iatrogenic, or it may be TSH dependent as in TSH secreting pituitary tumor and excessive placental hormonal secretion. Serum TSH is low in all forms of hyperthyroidism except in rare cases in which hyperthyroidism is mediated by TSH itself (Watts and Keffer, 1989). Finding a low TSH level and an elevated T4 level is usually sufficient to establish the diagnosis of hyperthyroidism. Occasionally, increase in serum T4 and T3 occur owing to release of thyroid hormones as a result of damage to the thyroid parenchyma associated with subacute thyroiditis or chronic lymphocytic thyroiditis. The increase in T4 and T3 levels may be associated with clinical findings suggestive of hyperthyroidism. Clinically, measurement of serum T3 concentration are especially valuable in

diagnosis of hyperthyroidism and in following the course of therapy for this disorder (Wahner and Gorman, 1971, Larson, 1972, Marsden and McKerron, 1975).

Thyroglobulin

Thyroglobulin is the major component of thyroid follicular colloid. It is a 670 KDa glycoprotein, produced by the thyroid epithelial cells, the thyrocytes. It is synthesized in the initial stages of the thyroid hormones production. Carbohydrates constitute about 10% of the total structure and 2% of amino acids in thyroglobulin are tyrosines; which from the backbone of thyroid hormone molecules. Since the hormones are synthesized on this protein before being released into circulation, thyroglobulin may be considered a pro-hormone. Thyroglobulin is comprised of two identical subunits and represents the major protein found in the thyroid. This protein provides 40 tyrosine residues, of the 140 in the molecule, for iodination during the biosynthesis of T4 and T3 and therefore is responsible for accumulation of iodine by the thyroid gland (DeGroot et al., 1996).

The degradation of thyroglobulin occurs in lysosomes. Whole of this process is precisely regulated. TG uptake occurs by micropinocytosis, which can result from both, fluid phase pinocytosis and receptor mediated endocytosis. Because TG is highly concentrated in the colloid, fluid phase pinocytosis or low-affinity receptors also provide sufficient TG uptake for hormone release; high affinity receptors may also serve to target TG away from lysosomes, through recycling into colloid or by transcytosis into blood stream. Several apical receptors have been suggested to play a role in TG uptake and intracellular trafficking (Shimojo et al., 1988).

A thyroid asialoglycoprotein receptor may internalize and recycle immature forms of TG back to colloid. This function is attributed to an as yet unidentified N-acetylglucosamine receptor that mediates TG uptake by thyrocytes especially under intense TSH stimulation. This results in transcytosis of TG from colloid to the blood stream, thus preventing excessive hormone release (Maclagan et al., 1957).

Thyroid peroxidase

The enzyme thyroid specific peroxidase (TPO) is present on the microsomes of thyrocytes and is expressed at its apical cell surface. It is a membrane-bound glycoprotein with an approximate mass of 107 kD. In synergy with thyroglobulin it has an essential function in the iodination of L-tyrosine and the chemical coupling of the resulting mono and di-iodination to form the thyroid hormone T4, T3 and rT3 (DeGroot and Neipomniszcze, 1977). TPO is a potential auto-antigen and is recognized as major microsomal component. It is believed to be polyclonal and heterogeneous in nature with a minimum of six antigenic determinants being recognized. (Doble et al., 1988).

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Concept of autoimmunity

An effective immune response leads to harmonious appearance of normal immune reactivity expressed appropriately with self/non-self discrimination. Autoimmunity represents a breakdown of such self / non-self discrimination that may or may not result in adverse effects in the host (Theofilopoulos and Dixon, 1982). It is also considered as a termination of natural unresponsive (tolerant) state. Such tolerance is induced by two mechanisms, involving contact between antigen and immunocompetent cells: (1) elimination of small clone of cells "programmed" to react with the antigen (Burnet's Clonal selection theory), (Burnet, 1972), and (2) induction of unresponsiveness in the immunocompetent cells through excessive antigen binding to them and / or through triggering of a suppressor mechanism. The normal immune response is modulated by both antigen-specific and non-specific suppressor cell activity as part of an immunologic network (Miller and Schwartz, 1982).

There is increasing evidence that much of the tolerance to tissue antigen is an active process and involves T cells. In normal circulation B cells with surface receptors for DNA and other tissue components are present in small number. Tolerance in T cell population is achieved much more readily than in B cell with low concentration of antigen that would be released from tissues in normal catabolism (Allison, 1974; Howard and Mitchison, 1975). Therefore immune response to such substances develops weakly in normal individuals.

In T-cell tolerance (1), the "helper" T cells function to concentrate antigen more effectively for presentation to B cells may be depressed; (2) suppressor T cells may reduce the helper-T cell function that does persist; and (3) there is lack of conversion of IgM to IgG antibody synthesis

by those B cells that may be activated directly by certain antigens. It is also apparent that B cell tolerance is terminated more readily as well as induced with more difficulty (Theofilopoulos, 1982). Therefore, in many cases, B cells are ready to produce autoantibodies at any time, and frequently do this weakly in normal individuals, especially with increasing age. However, such autoimmune reactivity increases markedly and prematurely when helper-T cells are made responsive (or lose tolerance to auto antigens). Conditions that may lead to such T-cell responsiveness include:

i. Exogenous alteration of normal host component by agents such as type-C viruses incorporating membrane components as they bud from the infected cell.

ii. Haptens complexing to tissue proteins acting as a carrier.

iii. Tolerance to tissue components may also be lost when immune responses are induced to a foreign antigen and these immune responses cross react with normal tissue components.

iv. Non-specific stimulation of helper-T cells by adjuvant or depression of the regulating suppressor-T cell activity (Miller and Schwartz, 1982).

The lack of normal regulation of immune responses may play a role in the increased production of autoantibodies by mutant lymphoid clones in lymphoproliferative and immune deficiency states (Stiller, 1975). In this regard, clinical autoimmune disease may commonly reflect a form of immunologic deficiency.

It is of note that in selective IgA deficiency, a particularly high prevalence of autoimmune disorders is seen (Wells, 1975). This may relate to a defective barrier to entry of foreign substances at mucosal surfaces associated with IgA deficiency. It is evident that the tendency

for autoimmune reactivity is constantly present in healthy individuals. Several factors modulate the autoimmune response.

(1) Sex: Autoimmune responses are more common in females; and certain hormones affect autoantibody formation.

(2) Genetics: There is an increasing evidence for presence or formation of autoimmune antibodies in sera of near relatives of those with certain autoimmune diseases (De Horatius and Messener, 1975).

(3) Age: Many auto antibodies (e.g. anti-nuclear antibodies, rheumatoid factor, antithyroglobulin antibodies) are found more commonly in aged individuals (Bernard JH, 1991).

(4) Thymic Control : The evidence that thymus plays a role in autoimmunity includes the observations that (a) the onset in life of naturally occurring autoimmune disease and immunodeficiency in NZB/W mice is accelerated by neonatal thymectomy, (b) Thymic hyperplasia is seen in at least some clinical autoimmune states (Levinson, 1987).

(5) Exogenous factors : Exogenous factors may also modulate autoimmune response, and sunlight, drugs or certain virus infection may "trigger" a prominent autoimmune response by T cell activation or some other mechanisms. (Phillips, 1975).

Organ-Directed Autoimmune States

Thyroid gland appears to be one of the best examples of how a postulated autoimmune pathogenesis might cause organ directed disease. The current theories suggest that normal thyroglobulin (released from the thyroid) circulates systemically in very low amount (Doniach and Roitt, 1988). This may be sufficient to induce a "low zone" (low dose) T lymphocyte

tolerance in normal subjects, with weak production of anti-thyroglobulin by those B cells, which have receptors for thyroglobulin. This tolerance increases gradually with age, particularly in females. Likewise immune response to one or more thyroid components is induced in predisposed individuals because of alteration of thyroid function by infection or chemicals. These immune responses may or may not cause tissue destruction but are frequently valuable as diagnostic markers (Tanner, 1982). Possibly, there are defects in specific or non-specific suppressor cell activity in those with presumed autoimmune thyroid disease (Volpe, 1986).

An account of immunologic studies of assistance in major thyroid disorders is given below:

(i) Hashimoto's thyroiditis

It is an inflammatory condition occurring in about 1-2 % of population mainly in middle-aged women, and characterized by gland enlargement as a result of marked lymphocytic inflammatory changes (Volpe, 1986). These changes may consist of lymphoid follicles with active germinal centers, in which much of the anti thyroglobulin antibody appears to be synthesized. Normal thyroid glandular structures are adversely altered and progressive disease may lead to thyroid atrophy and myxedema (Scherbaum, 1987).

(ii) Thyrotoxicosis or Graves' disease

Grave's disease is a multisystemic disorder, occurring particularly in young to middle-aged females consisting of (1) hyperthyroidism with diffuse hyperplasia of the thyroid, (2) myopathy; and (3) an infiltrative ophthalmopathy, frequently leading to exophthalmos. The thyroid may contain small areas of lymphoid infiltration as well as typical glandular hyperactivity.

Immune reactivity may not be the primary pathogenic event but, once present, causes further tissue damage. Evidence against a primary pathogenic role for thyroid autoantibodies in Hashimoto's Thyroiditis and Graves' disease is the lack of correlation between the level of autoantibody and severity of disease in individual cases, and the lack of development of thyroid disease in infants with high levels of antithyroid antibodies because of placental transfer (Bernard JH, 1991).

Anti-Thyroglobulin Antibodies (Anti-TG)

Anti thyroglobulin are directed against TG, which is a major constituent of thyroid colloid. Historically, anti-TG antibody determinations were used in tandem with antimicrosomal antibody determination to maximize the probability of a positive result in patients with autoimmune disease. Although the prevalence of anti TG antibodies in thyroid autoimmune disease is significant, 85% and 30% in Hashimoto thyroiditis and Graves' disease respectively, it is much lower than the prevalence of the anti-TPO antibodies. Although Anti-TG are found in conjunction with anti-TPO in majority of cases of Hashimoto's thyroiditis, Graves' disease, and primary Myxedema (Rosenbaum and Davies., 1992 ; Burek and Rose., 1996) upto 1% cases of hypothyroidism are associated with anti-TG alone (Nordyke et al., 1993).

Anti-Thyroid peroxidase Antibody (Anti-TPO)

Thyroid peroxidase is a potential auto antigen, and the anti-TPO antibody was historically referred to as the antimicrosomal antibody. The thyroid peroxidase enzyme was subsequently identified as major microsomal component recognized by these autoantibodies. Autoantibodies against TPO have emerged as the most useful marker for diagnosis and management of autoimmune thyroid disease (Khoury et al., 1981).

Anti-TPO antibodies mediate antibody-dependent thyroid cell destruction; levels correlate with the active phase of disease. Measurement of this autoantibody is useful for resolving the diagnostic dilemma presented by apparent inconsistency between elevated TSH and normal free T4 results. Given abnormally elevated TSH and euthyroid T4 results, a positive anti-TPO antibody test provides strong evidence for early, sub clinical autoimmune disease. This assay is also used to monitor to immunotherapy, to identify at risk individuals (with family history of

thyroid disease) and as a predictor of postpartum thyroiditis. Elevated levels are found in virtually all cases of Hashimoto's thyroiditis and in approximately 85% cases of Graves' disease (Banga, 1985).

Aims and Objectives

The study was carried out taking into consideration the following objectives:

i. To asses the element of autoimmunity in cases of thyroid disease.

ii. To ascertain the prevalence of autoimmunity in this region, since northern areas of Pakistan is a region of endemic thyroid disease.

iii. To asses any differences in results, which may arise due to racial, climatic and socio-economic factors etc.

MATERIALS AND METHODS

MATERIAL AND METHODS

Since the patients from various socio economic groups visit the hospitals daily, many of these patients are investigated for thyroid disease. The most common reason being the symptoms pointing to the abnormal thyroid function. These symptoms are either indicative of hypo-function or hyper-function of the thyroid gland. The symptoms pointing to hypo functions are those attributed to low basal metabolic rate. These patients also complain cold intolerance, decreased sweating, weight gain without increased caloric intake, bradycardia, slowness of movements, speech and thought, lethargy and sleepiness. The symptoms which indicate the hyper function of thyroid gland include (1) increased metabolic rate, (2) heat intolerance and sweating, (3) increased appetite but weight loss, (4) palpitations and tachycardia, (5) nervousness and emotional lability, (6) muscle weakness, and (7) tiredness but inability to sleep. Patients with above complaints were selected from Holy family Hospital Rawalpindi and Attok Hospital (Pvt) Ltd. Rawalpindi and sent to laboratories of respective hospital for evaluation of hypo- and hyperthyroidism. The most commonly asked investigations by clinicians to evaluate thyroid function are the measurement of T3, T4 and TSH levels in serum.

After taking histories, 300 patients were selected for thyroid hormone analysis to ascertain abnormal thyroid function. Blood samples were taken by standard aseptic sampling technique. Blood samples were taken from anti cubital veins, after applying tourniquet to the upper arm and sterilizing the venipuncture area. About 10 ml blood was taken from each patient and put into plain centrifuge tubes. Blood samples were allowed to remain undisturbed until clotting of the blood and clot retraction. These samples were centrifuged at 10,000 rpm

(Hetich company, Japan) and the clear serum samples thus obtained were transferred to torpedo tubes, in aliquots. These tubes were numbered, labeled and then stored at –20°C until further analysis. The serum samples were aliquoted to avoid repeated thawing and freezing of these samples because different investigations on these samples had to be carried out at different times. Twenty apparently healthy persons including equal numbers of males and females were also evaluated for T3, T4, and TSH levels, to be included in the study as healthy controls. Ten samples out of these 20 persons, having their hormonal levels well within the reference range were isolated, to be included as control subjects in the study.

The study involved in the first stage, measurement of T3, T4 and TSH and, in the second stage the evaluation of the samples with abnormal hormone levels for antibodies against thyroglobulin (anti-TG) and thyroid peroxidase (anti-TPO). The next step was to evaluate these samples for presence of Rheumatoid factor (RF).

For analysis of T3, T4, and TSH levels, these samples were processed on the automated immunoassay analyzer, IMX (Abbott Laboratories, USA). This system is designed to run assays using MEIA (Microparticle Enzyme Immuno Assay) and FPIA (Fluorescent Polarization Immuno Assay) technologies.

The samples having abnormal values of T3, T4 and TSH i.e. values consistent with either hypo- or hyper-function of thyroid gland were segregated. 87 specimens having clearly abnormal hormonal levels were selected for evaluation of element of autoimmunity. The tests for anti TG and anti TPO were performed on AxSYM analyzer (Abbott laboratories, USA). The technique employed for the antibody detection was microparticle enzyme immunoassay (MEIA), which quantitatively measures the IgG class of antibodies against thyroglobulin and

thyroid peroxidase. The aliquots of samples were also processed for detection of Rheumatoid factor by rapid agglutination test, employing the latex serology test AVITEX RF (Omega Diagnostics Limited, UK).

Estimation of serum T3 (Triiodothyronine)

Clinically, measurement of serum T3 concentration is especially valuable in diagnosis of hyperthyroidism and in following the course of therapy for this order (Larsen, 1972). Serum T3 was measured using IMX T3 reagent kit manufactured by Abbott Laboratories. All reagents used in the method were provided in the kit.

Reagents

1. Anti-T3 (Goat) coated microparticles in buffer with protein stabilizers
2. Alkaline phosphate conjugate in buffer with protein stabilizers.

   Minimum concentration 0.4 ng/ml.
3. 4-Methylumbelliferyl Phosphate 1.2mM in AMP buffer.
4. Calibrators: 6 calibrators with following values were used to calibrate the analyzer and to make a calibration curve against which the individual samples were compared.

A	=	0.0	ng/ml
B	=	0.5	ng/ml
C	=	1.0	ng/ml
D	=	2.0	ng/ml
E	=	4.0	ng/ml
F	=	8.0	ng/ml

During a run of assay, commercially prepared controls supplied along with the reagents were also processed at the same time to confirm the validity of the assay. These controls were designated low, medium and high controls according to the hormonal level contained therein. These had the following values.

Low	=	0.40 – 1.0	ng/ml
Medium	=	1.10 – 1.9	ng/ml
High	=	2.90 – 4.5	ng/ml

Samples

Aliquots of the samples already stored and frozen were thawed at room temperature for analysis of T3 levels. The samples were mixed thoroughly after thawing and those, showing any turbidity were again centrifuged at 10,000 rpm for 15 minutes to remove any particulate matter which could hinder the analysis.

Test Procedure Following test protocol was adopted.

(i) 150 µl serum was pipetted to incubation wells and anti-T3 coated microparticles were added to these wells for the formation of antigen-antibody complex.

(ii) An aliquot of reaction mixture, containing the antibody-antigen complex bound to the microparticles, was transferred to the glass fiber matrix.

(iii) The T3-alkaline phosphatase conjugate was then dispensed into the matrix. This conjugate binds to the available sites on the anti-T3 coated microparticles.

(iv) The matrix was washed to remove unbound material.

(v) The substrate 4-Methyumbelliferyl phosphate was added to the matrix and fluorescent product was measured by the MEIA optical assembly of IMX.

Reference Range = 0.51— 1.65 ng/ml.

Estimation of serum T4 (Thyroxine)

Serum T4 was measured using IMX T4 reagent kit manufactured by Abbott Laboratories. All reagents used in the method were provided in the kit.

Sample

Aliquots of serum samples already stored and frozen were thawed at room temperature for analysis of T4 levels. The samples were mixed thoroughly after thawing and those, showing any turbidity were again centrifuged at 10,000 rpm for 15 minutes to remove any particulate matter which could hinder the analysis.

Reagents

1. Mouse monoclonal T4 antiserum in buffer with protein stabilizers.
2. T4-Flourescein tracer in buffer containing surfactant..
3. T4-Pretreatment solution. Surfactant in buffer.
4. Calibrators: 6 calibrators containing thyroxine prepared in processed human serum, were used to prepare the calibration curve against which the unknown samples were compared to evaluate the level of T4. These calibrators had the following values.

A	=	0.0	µg /dl
B	=	3.0	µg /dl
C	=	6.0	µg /dl
D	=	12.0	µg /dl
E	=	18.0	µg /dl
F	=	24.0	µg /dl

During a run of assay commercially prepared controls supplied along with the reagents were also processed at the same time to confirm the validity of the assay. These controls were designated low, medium and high controls according to the hormonal level contained therein. These had the following values.

Low	=	3.3 – 5.7	µg /dl
Medium	=	6.6 – 9.4	µg /dl
High	=	12.5 – 17.5	µg /dl

Test Procedure Following test protocol was adopted for analysis of T4 level.

(i) 50 µl of serum samples , pre-treatment solution, and T4-anttiserum were added to the predilution well of sample cartridge. The pretreatment solution was used to remove the T4 from binding sites on TBG, prealbumin and albumin.

(ii) An aliquot of this predilution mixture was transferred to the cuvette and T4 fluorescein tracer was added to it. The T4 and labeled tracer compete for the sites on the antibody molecule.

(iii) The intensity of polarized fluorescent light was measured by Fluorescent polarization immunoassay optical assembly, of IMX immunology analyzer.

Reference range = 4.5 – 12.0 µg/dl.

Measurement of TSH

Serum TSH was measured using IMX TSH reagent kit manufactured by Abbott Laboratories. All reagents used in the method were provided in the kit.

Aliquots of same sera were used for testing for TSH level as mentioned earlier.

Reagents

(i) Mouse monoclonal anti-human TSH coated microparticles in TRIS buffer with protein stabilizer.

(ii) Goat anti-h TSH : Alkaline phosphatase conjugate in TRIS buffer with protein stabilizers, minimum concentration = 0.1 µg/ml.

(iii) 4-Methylumbelliferyl phosphate 1.2mM in AMP buffer.

(iv) Wash buffer containing surfactant.

(v) Calibrators: 6 calibrators containing recombinant TSH in TRIS buffer having following values were used to prepare a calibration curve against which the unknown samples were evaluated

A = 0.0 µIU/ml

B = 0.5 µIU/ml

C = 2.0 µIU/ml

D = 10.0 µIU/ml

E = 40.0 µIU/ml

F = 100.0 µIU/ml

Low, Medium and High level controls with known values of TSH were also processed at same time to validate the accuracy of results. These controls were provided along with the reagent kit and the expected recovery ranges for these controls were as below:

Low = 0.15 – 0.35 µIU/ml

Medium = 4.5 – 7.5 µIU/ml

High = 21 – 39 µIU/ml

Test Procedure

(i) 150 µl serum sample and anti-h TSH coated microparticles were delivered to incubation wells of reaction cells. The TSH present in sample binds to anti-h TSH coated microparticles forming antigen-antibody complex.

(ii) An aliquot of this reaction mixture containing antibody-antigen complex bound to microparticles was transferred to the glass fiber matrix.

(iii) The microparticles bind irreversibly to the glass fiber matrix. The matrix was washed with buffer to remove unbound material.

(iv) The anti-h TSH Alkaline Phosphatase conjugate was dispensed onto the matrix. This conjugate became bound with antigen-antibody complex.

(v) The matrix was washed again to remove unbound material.

(vi) The substrate, 4 Methylumbelliferyl phosphate was added to matrix and fluorescent product was measured by MEIA optical assembly of IMX immunology analyzer.

Reference Range 0.47 – 5.01 µIU/ml

Measurement of Anti TPO

Antithyroid peroxidase (TPO) antibodies are autoantibodies directed against thyroid peroxidase enzyme. This enzyme catalyzes the iodination of tyrosine in thyroglobulin during the biosynthesis of T3 and T4. Historically these antibodies were referred to as antimicrosomal antibodies because the antibodies bind to the microsomal part of the thyroid cells. Recent research has identified thyroid peroxidase as the primary antigenic component of microsomes. These antibodies are found in almost all cases of Hashimoto's disease and in the majority of cases of Graves' disease.

Serum anti-TPO was measured using anti-TPO kit from Abbott Laboratories. All the reagents were included in the kit.

Samples

The samples having abnormal values i.e., the values consistent with either hypo- or hyper-function of thyroid gland were isolated. 87 specimens having grossly abnormal hormonal levels were selected for further evaluation for element of autoimmunity, and detection of level of antibodies against thyroglobulin and thyroid peroxidase. The aliquots of these sera were processed accordingly.

Reagents

(i) Thyroid peroxidase (from human thyroid glands) coated microparticles in TRIS Buffer with protein stabilizer. Minimum concentration : 0.8 µg/ml

(ii) Goat anti-Human IgG : Alkaline phophatase conjugate in TRIS buffer.

(iii) Assay diluent in TRIS buffer.

(iv) Specimen dilution buffer in phosphate buffer.

(v) Calibrators: Six calibrators were used to calibrate the instrument and make a standard curve against which the unknown samples were read. Calibrators with following values were used.

A = 0.0 IU/ml

B = 8.0 IU/ml

C = 20.0 IU/ml

D = 100.0 IU/ml

E = 400.0 IU/ml

F = 1000.0 IU/ml

Negative and Positive controls were run along with each batch of samples to evaluate the validity of result. Controls having following values were used.

Negative Control	=	0.0	IU/ml
Positive Control	=	75.0	IU/ml

Test Procedure

The assay employed was based on Microparticle Enzyme Immunoassay .In this test reaction vessels having various compartments were used.

(i) Each specimen was diluted with specimen dilution buffer. Aliquot of this diluted sample was placed in incubation well of reaction vessel and TPO coated microparticles were added into it.

(ii) The Anti-TPO antibodies got bound to the TPO coated microparticles forming an antigen-antibody complex.

(iii) Assay diluent was added to the reaction mixture and an aliquot of the antigen antibody complex was transferred to the matrix cell of reaction vessel. The microparticles made an irreversible bond with the glass fiber matrix.

(iv) The matrix cell was washed to remove unbound material.

(v) The goat anti-human IgG Alkaline phosphatase conjugate was dispensed onto the matrix cells which combined with the Antigen-Antibody Complex.

(vi) The matrix cell was washed to remove unbound materials.

(vii) The substrate, 4-Methylumbelliferyl phosphate was added to the matrix cell and the fluorescent product formed was measured by MEIA optical assembly of AxSYM immunology analyzer.

Expected value in normal individual by this method comes out to be less then **12 IU/ml**.

Measurement of Anti-Thyroglobulin (Anti-TG)

The presence of anti –TG in patients with Hashimoto's thyroiditis was first demonstrated in 1956 by Roitt et al. using a precipitin reaction. Unlike anti-TPO , autoantibodies to thyroglobulin do not appear to be pathogenic and may simply be

indicators of disease. They have been found to be polyclonal in nature. In this test the IgG class of antibodies against thyroglobulin was measured quantitatively by Microparticle Enzyme Immunoassay (MEIA) in the sera, which were previously processed for T3, T4, TSH and anti-TPO, in an effort to assess the prevalence of autoimmune element in causation of thyroid disease.

Reagents

(i) Purified Thyroglobulin (human thyroid tissue extract) coated microparticles in TRIS buffer (minimum concentration 5.0 µg /ml).

(ii) Goat anti-Human IgG : Alkaline Phosphatase conjugate in TRIS buffer. (Minimum concentration 0.8 µg/ml).

(iii) Assay diluent in TRIS buffer.

(iv) Specimen Dilution buffer in phosphate buffer.

(v) Calibrators: Six standard calibrators were used to make a standard curve against which the concentration of anti-TG in study sample was measured. The values of the calibrators was as follows:

A	=	0.0	IU/ml.
B	=	25.0	IU/ml.
C	=	125.0	IU/ml.
D	=	250.0	IU/ml.
E	=	500.0	IU/ml.
F	=	1000.0	IU/ml.

(vi) Controls: Positive and negative controls with known values were also processed within each batch of the samples to assess the quality assurance of the assay.

The values of these controls were as follow.

Negative Control = 0.0 IU/ml.
Positive Control = 150.0 IU/ml.

Assay Procedure

The assay employed was based on Microparticle Enzyme Immunoassay (MEIA). In this test, reaction vessels having various compartments were used. The test was performed in following steps:

(i). 150 µl serum was required for assay; the sample was diluted in sample dilution buffer.

(ii) An-aliquot of diluted sample and TG- coated microparticles were transferred to incubation well of reaction vessel.

(iii) The anti-TG got bound to the TG coated microparticles forming an antigen-antibody complex.

(iv) Assay diluent was added to the reaction mixture and an aliquot of antigen-antibody complex was transferred to the matrix cells. The microparticles bound irreversibly to the glass fiber matrix of these cells.

(v) The matrix cell was washed to remove unbound material.

(vi) Goat anti-Human IgG Alkaline phosphatase conjugate was dispensed onto the matrix cell, which combined with the antigen-antibody complex.

(vii) The matrix cell was washed to remove unbound material.

(viii) The substrate, 4-Methylumbelliferyl phosphate was added to the matrix cell and the fluorescent product formed was measured by MEIA optical assembly of AxSYM immunology analyzer.

Expected Normal range = < 34 IU/ml.

Rheumatoid Factor (RF)

Many cases of autoimmune thyroid disease are associated with presence of antibodies directed against other organs or tissues. This correlation has been seen in cases of Rheumatoid arthritis, Addison's disease and type I diabetes. The detection of Rheumatoid factor was included in the study with a view to exclude the possibility that the body's immune system was reacting, excepting thyroid, against any other tissue. RF is found in sera of patients with Rheumatoid arthritis and is believed to be IgM antibodies directed against the patients own immunoglobulin G. This serological test was performed by Latex agglutination method by a kit manufactured by Omega-diagnostics Limited, (Omega House, Scotland, United Kingdom).

Principle of the test

The latex particles are coated with specially purified human gamma globulin as described by Singer., 1975. When the latex suspension gets mixed with serum containing elevated RF levels on a slide; clear agglutination is seen within two minutes.

Reagents / Material

1. Latex = Suspension of polystyrene latex particles (approximately 1.25%) coated with suitably modified crystallizing fragment (Fc) fraction of IgG in stabilizing buffer.
2. Positive Control = Serum containing rheumatoid factor antibodies.
3. Negative Control = Serum free of Rheumatoid factor antibodies.
4. Stirrers.
5. Plastic test cards.
6. Micropipettes capable of dispensing 50 µl.

Specimen collection and preparation

Sera already aliquoted were used to perform the test. Sera for the test were obtained after blood collection and clot reaction of venous blood, centrifuged at 10,000 rpm and clear

serum samples were stored in frozen state after appropriate numbering and labeling. Before subjecting to the test serum samples were thawed and allowed to reach the room temperature. (20 — 25°C) and were mixed gently prior to use. The test slides were thoroughly cleaned with detergent, and dried before use.

Assay Procedure

(i) All the reagents were allowed to reach room temperature before starting the test.

(ii) 50 µl of serum was transferred to the test circles on the slide.

(iii) Latex reagent was shaken, to mix thoroughly and then one drop of suspension was added to the test circle with the help of dropper provided.

(iv) Using a disposable stirrer, the drops on the test circles were mixed thoroughly, while spreading onto the entire test circle.

(v) The test slide was gently and evenly rocked and rotated for 2 minutes while agglutination on the circles was observed.

This two minutes procedure was examined under strong light source. Both positive and negative controls were simultaneously processed with the test samples. The samples showing a clear agglutination visible with unaided eye were positive, while negative result was indicated by no change in the latex suspension on the test slide.

All results were analyzed and represented as percent values.

RESULTS

RESULTS

Analysis of T3, T4, and TSH levels in the patients' blood is utilized to make the diagnosis of the thyroid disease. On the basis of results of the thyroid profile the patient is declared either hypothyroid or hyperthyroid.

In cases of hypothyroidism the T3 and T4 levels are decreased while those of TSH are raised; on the other hand a reverse pattern is seen in cases of hyperthyroidism. Although T3 has a greater biological potency, the major role is being played by the thyroxine circulating in the blood, the concentration of which is fifty times than that of T3. Clinically T4 measurement has long been recognized as an aid in the assessment and diagnosis of thyroid health status. Elevated T4 values are characteristically seen in cases of overt hyperthyroidism, while T4 levels are generally depressed in patients with overt hypothyroidism. T4 levels are altered by physiological or pathological changes in thyroxine binding protein (TBP) capacity. Thyroxin binding globulin (TBG) capacity has a pronounced effect on the concentration of thyroid hormones. Consequently, T4 levels may be elevated with increased levels of TBG, such as in pregnancy, administration of oral contraceptives or estrogen, infectious or chronic active hepatitis, biliary cirrhosis or congenital increase in TBG levels. Conversely, when TBG levels are decreased, such as in nephritic syndrome, androgen therapy, glucocorticoid therapy, major systemic illness or congenital decrease in TBG, T4 may be reduced. Autoimmunity against thyroid is one of the many causes that can lead to either hypothyroid or hyperthyroid states and tends to occur in a genetically predisposed population. About 300 patients (150 male and 150 female) having symptoms suggestive of thyroid pathology were included in the present study and their blood samples were analyzed to ascertain the level of T3, T4, and TSH as these are the primary parameters to assess the status of thyroid gland. Out of these 300 patients, 87 were found to have clearly abnormal levels of thyroid related hormonal profile; either in hypo or hyper level. Among these 44 patients had their hormonal levels consistent with hyperactive state of thyroid glands and 43 were diagnosed as hypothyroid patients (Table-1).

Ten healthy subjects presenting no signs and symptoms indicative of thyroid disease were also included in the study. Their blood samples were processed for T3, T4, and TSH estimation and were found to have values well within the reference range.

Patients having hyperthyroid hormonal level

The 44 patients which comprised about 50% of total abnormal cases showed hormonal levels consistent with hyperthyroidism. Their triiodothyronine levels were above 1.65 ng/ml and their thyroxine levels were above 12.0 µg/dl. While their TSH level was below 0.47 µIU/ml. Out of these 44 patients, 26 were females who comprised 59% of the total, and 18 were males comprising 41% of the total. This showed that this hyperactive state of thyroid gland was common in females than in males (Table-2).

Patients having hypothyroid hormonal level

Out of the 87 samples showing abnormal results for thyroid hormone levels 43 samples had levels consistent with hypothyroidism as their T3, T4 levels were well below the lower limit of the reference range and their TSH level was above the upper limit of reference range. T3 level were below 0.51 ng/ml, T4 levels were less then 4.5 mg/dl and their TSH levels were more than 5.01 µIU/ml. These 43 patients comprised of 50% of the patients selected for evaluation of anti thyroglobulin and anti thyroid peroxidase. Of these 43 patients 27 were females who were 62% of the total hypothyroid patients and rest of the patients were male which comprised 38% (Table-3).

Table 1 Patients having Hypothyroidism and Hyperthyroidism

	Total	Female	Male	Percentage (%)
Hypothyroid	43	27	16	62/38
Hyperthyroid	44	26	18	59/41

Figure 1

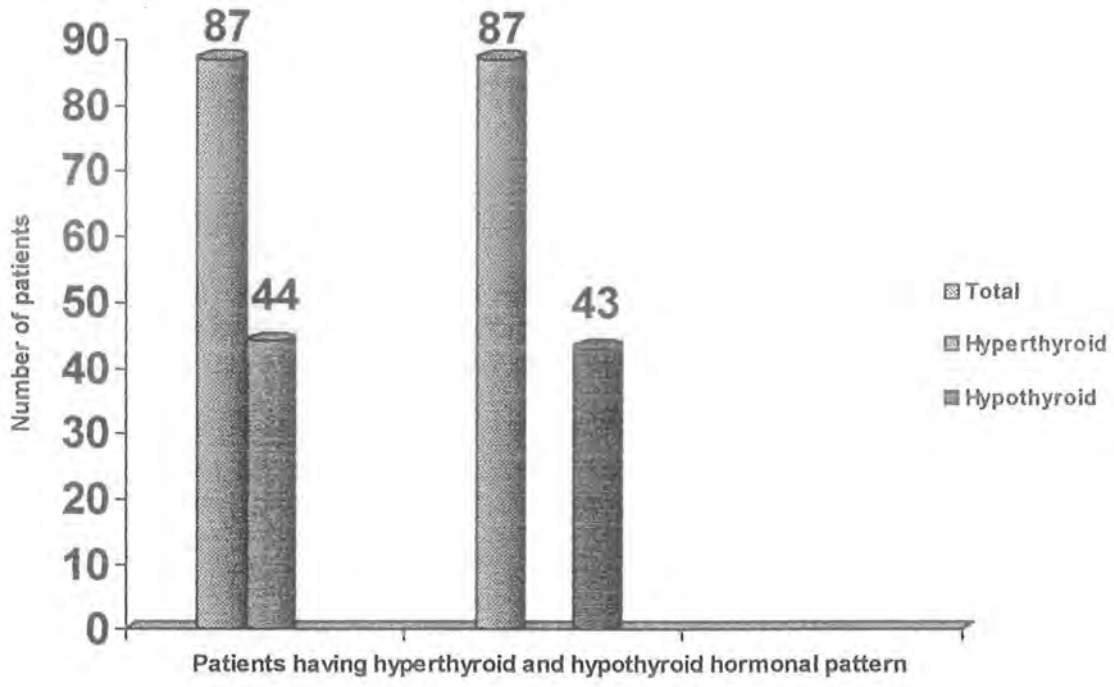


Table 2 Patients having Hyperthyroidism.

Test Result	Female	Male	Percentage(%)
High T3	26	18	59 / 41
High T4	26	18	59 / 41
Low TSH	26	18	59 / 41

Figure 2

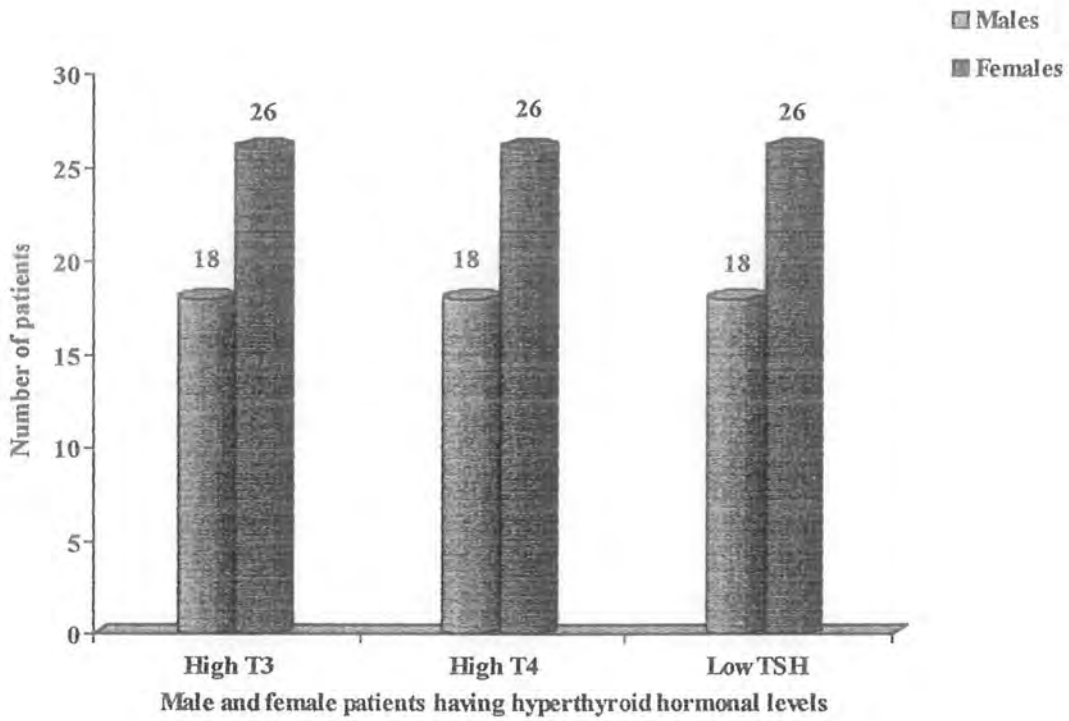
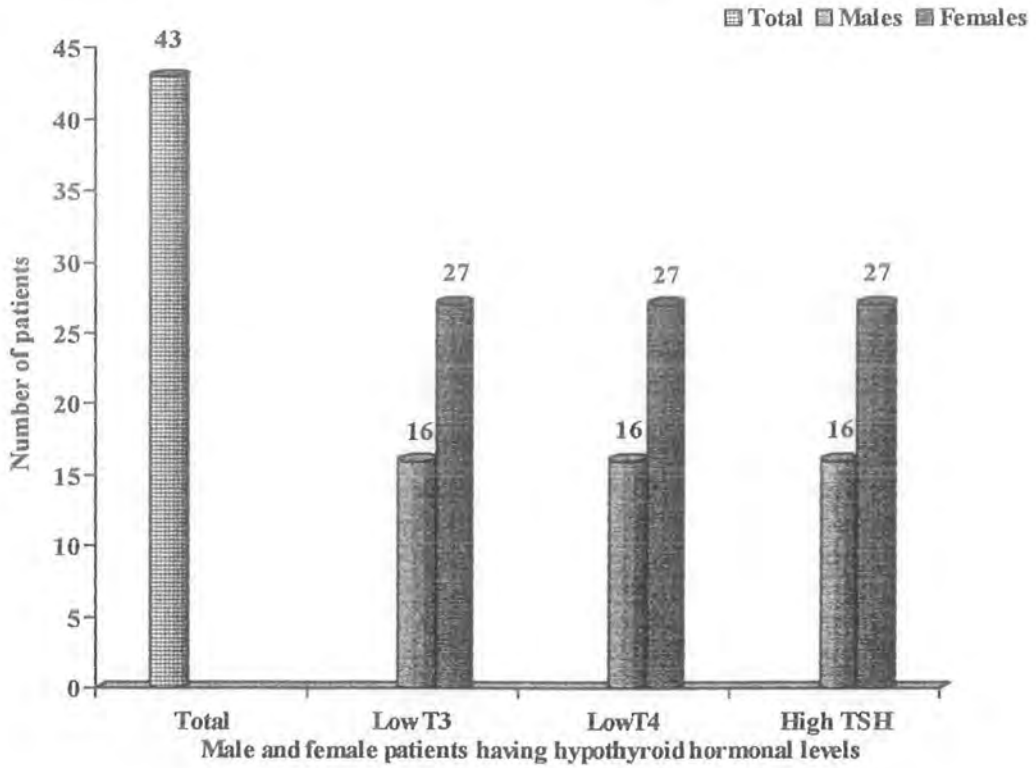


Table 3 Patients having Hypothyroidism.

Test Results	Total Patients	Females	Males	Percentage (%)
Low T3	43	27	16	62 / 38
Low T4	43	27	16	62 / 38
High TSH	43	27	16	62 / 38

Figure 3



Hyperthyroid patients positive for anti-TG

44 patients out of 87 were found to have thyroid hormone values consistent with hyperthyroidism. Sera of these hyperthyroid patients were subjected to analysis for detection of antibodies against thyroglobulin. Out of total 18 male patients, 11 were positive for anti-TG in their sera. This gave a percentage of 61%, while 39% did not show anti-TG in their sera. Of the hyperthyroid females, which were 59% of total hyperthyroid cases, 77% (20 in number) showed positive results for anti-TG and 23% (06 in number) were negative (Table-4).

Hypothyroid patients positive for anti-TG

Out of 43 patients which were found to be hypothyroid according to their T3, T4 and TSH levels 27 were female and 16 were male. Their samples were further evaluated for presence of antibodies against thyroglobulin. Of 16 male patients, 9 showed a positive result for anti-TG while 7 were negative. These ratios constituted a percentage of 56 and 44 respectively. Of 27 female patients which were 62% of the total hypothyroid cases 22 showed a positive result for anti-TG. This showed 81% positive cases while 19% patients showed negative result for anti-TG (Table-5).

Healthy controls positive for anti-TG

Sera of ten healthy persons were also included in the study to asses the prevalence of anti-TG in people having no signs and symptoms of thyroid disease. Sera of six persons yielded a positive result for anti-TG. In four samples the antibodies were not detected. Of the 5 male healthy controls 2 showed presence of anti-TG in their sera, which is 40% of these male controls. While 80% of the female controls showed a positive result for anti-TG and 20% were negative (Table-6).

Table 4 Hyperthyroid patients having Antibodies against TG

Patients	Number			Percentage (%)	
	T	+ve	-ve	+ve	-ve
Male	18	11	7	61	39
Female	26	20	6	77	23

T = Total number of patients ; +ve = Positive ; - ve = Negative

Figure 4

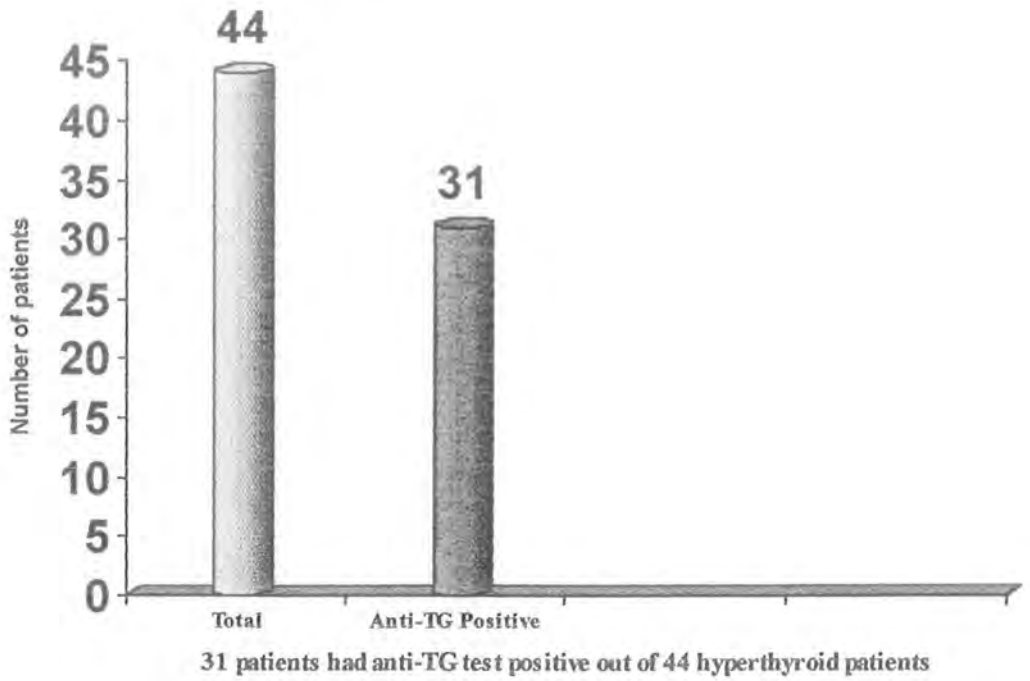
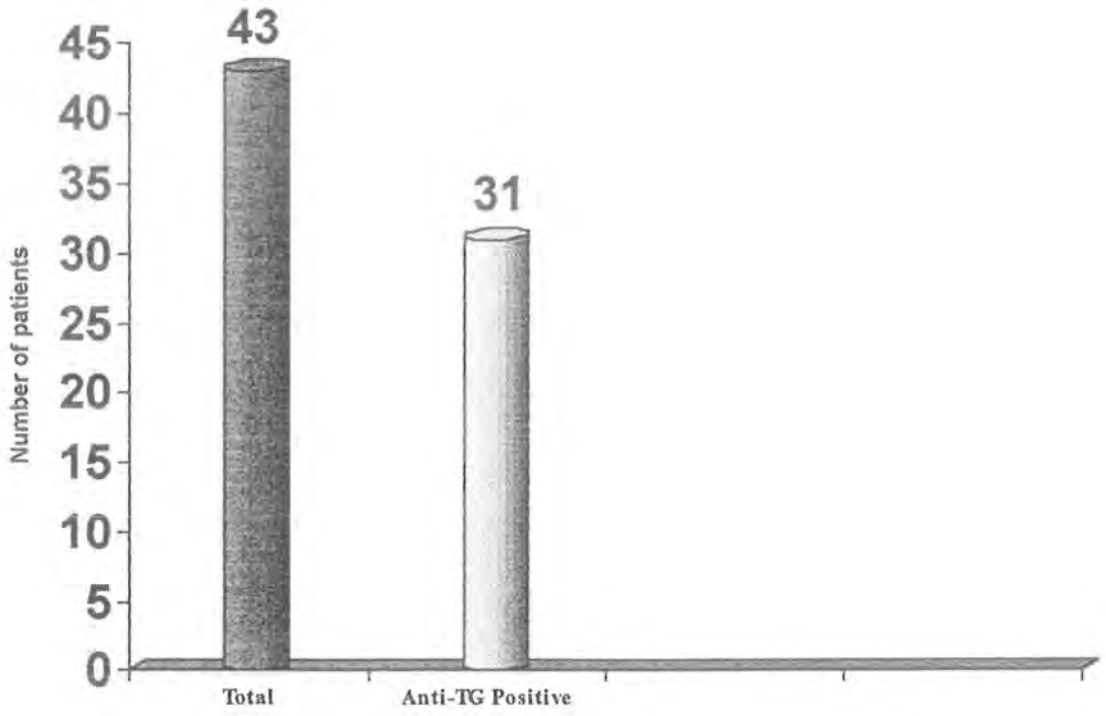


Table 5 **Hypothyroid patients having Anti-TG.**

Patients	Number			Average	
	T	+ve	-ve	+ve	-ve
Male	16	9	7	56	44
Female	27	22	5	81	19

T = Total number of patients ; +ve = Positive ; - ve = Negative

Figure 5



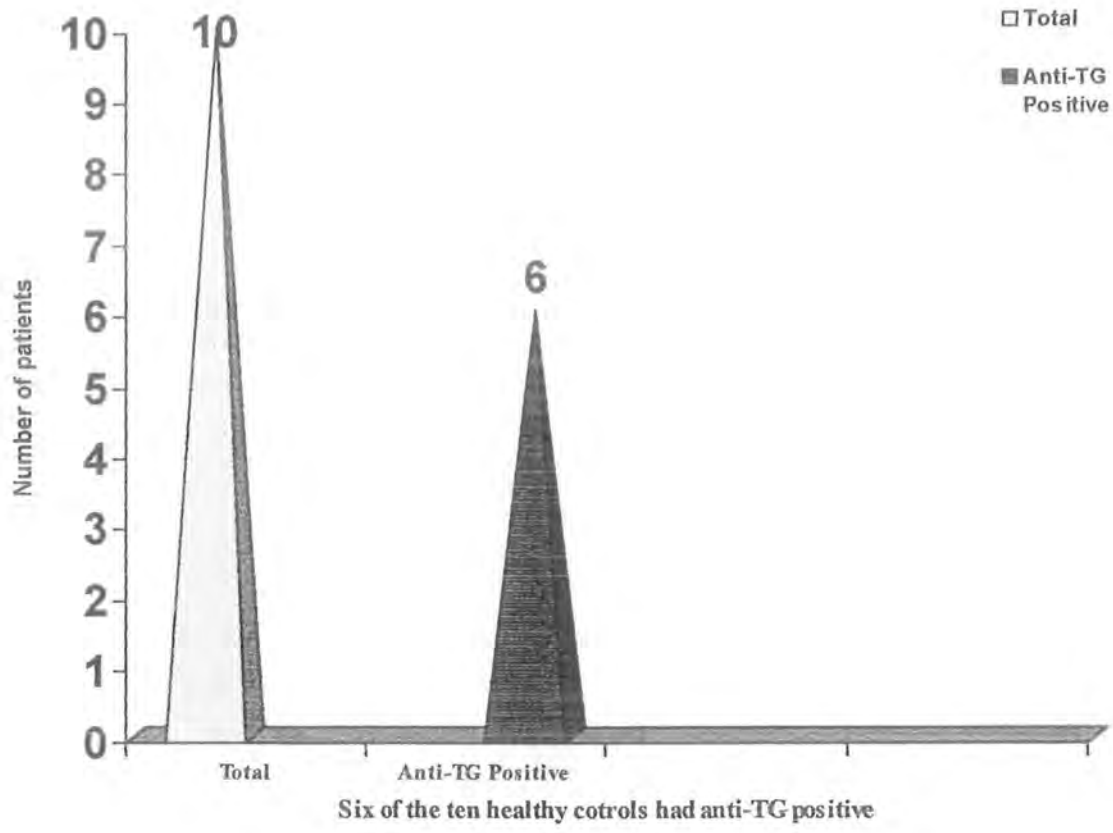
31 patients had anti-TG test positive out of 43 hypothyroid patients

Table 6 Euthyroid Controls having Anti-TG

Healthy Controls	Number			Percentage (%)	
	T	+ve	-ve	+ve	-ve
Male	5	2	3	40	60
Female	5	4	1	80	20

T = Total number of controls ; +ve = Positive ; - ve = Negative

Figure 6



Hyperthyroid patients positive for anti-TPO

Samples of 87 patients having abnormal thyroid function were tested for detection of anti thyroid peroxidase (anti-TPO). 44 patients having hyperthyroidism were first evaluated for presence of these antibodies. Out of these 44 patients 59% (26 patients) were females and 41% (18 patients) were males. Among 41% male hyperthyroid patients, 44% (8 patients) were positive for antibodies against TPO, while 56% were negative for anti-TPO. 26 samples belonged to female patients which was 59% of the total hyperthyroid cases. 13 female patients showed a positive result for anti-TPO, indicating 50% patients involvement in autoimmune process (Table-7).

Hypothyroid patients positive for anti-TPO

43 patients showed a thyroid hormonal profile consistent with hypothyroidism showing low levels of T3 and T4 whereas TSH levels were elevated. Of the 16 male patients in this hypothyroid group, 5 patients were positive and 11 were negative for anti-TPO, constituting respectively 31% and 69% of male patients. There were 27 female patients showing hypothyroid pattern. Out of these 7 were positive for anti-TPO while 20 were negative. This showed a percentage of 26% positive and 74% negative cases in female hypothyroid patients. (Table-8).

Healthy controls positive for anti-TPO

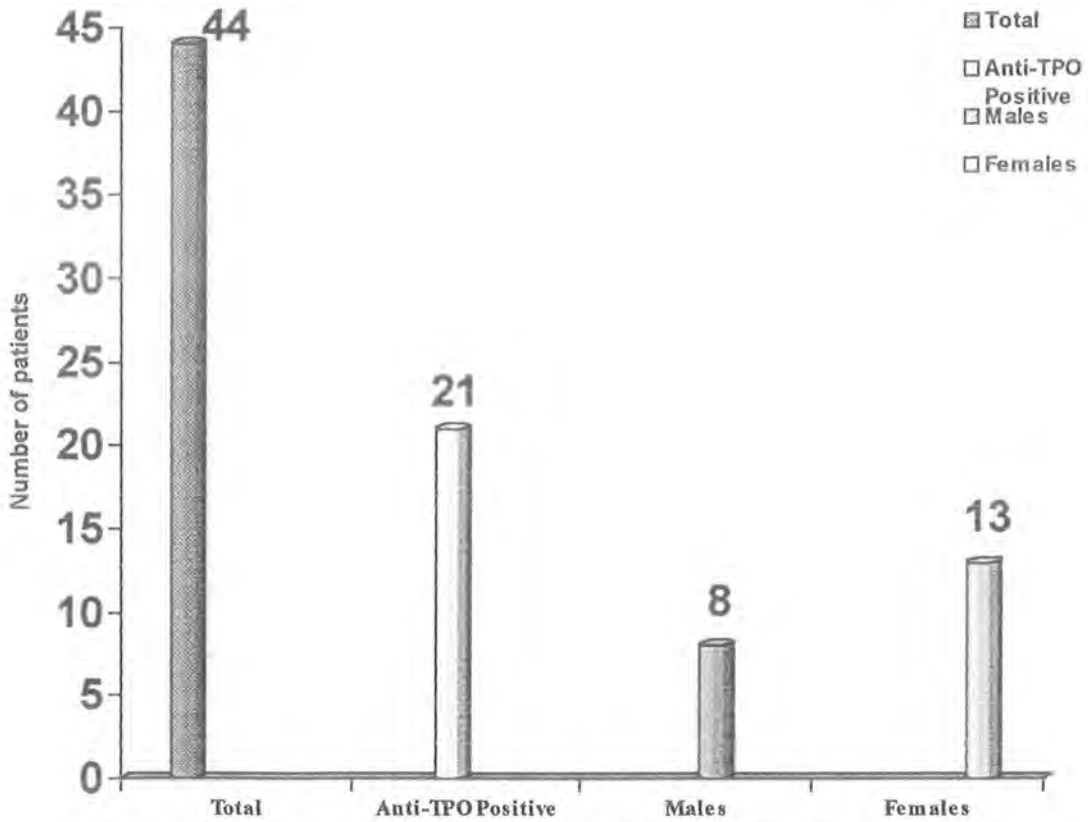
Sera of 10 healthy control persons having normal thyroid hormonal profile and without any signs and symptoms of thyroid pathology were also processed simultaneously for detection of anti-TPO to asses the element of autoimmunity against thyroid in normal individuals. It was noted that 3 persons were positive for anti-TPO which constituted 30% of total healthy persons included in study. While 70% were negative for anti-TPO. Of the 3 positive cases 2 were females and 1 patient was male. In this way females showed 40% positive result while males showed 20% positive result for anti-TPO (Table-9).

Table 7 Hyperthyroid patients having Anti-TPO

Patients	Number			Percentage (%)	
	T	+ve	-ve	+ve	-ve
Male	18	8	10	44	56
Female	26	13	13	50	50

T = Total number of patients ; +ve = Positive ; - ve = Negative

Figure 7



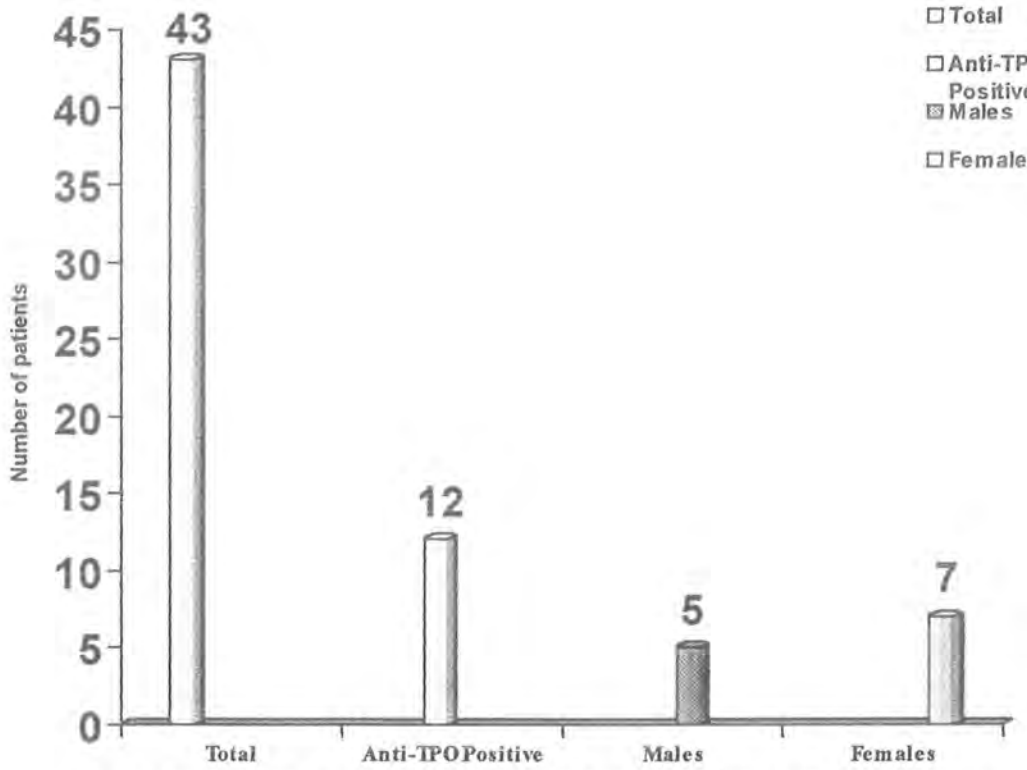
21 patients out of 44 hyperthyroid patients had anti-TPO positive. These included 8 males and 13 females.

Table 8 Hypothyroid patients having Anti-TPO

Patients	Number			Percentage (%)	
	T	+ve	-ve	+ve	-ve
Male	16	5	11	31	69
Female	27	7	20	26	74

T = Total number of patients ; +ve = Positive ; - ve = Negative

Figure 8



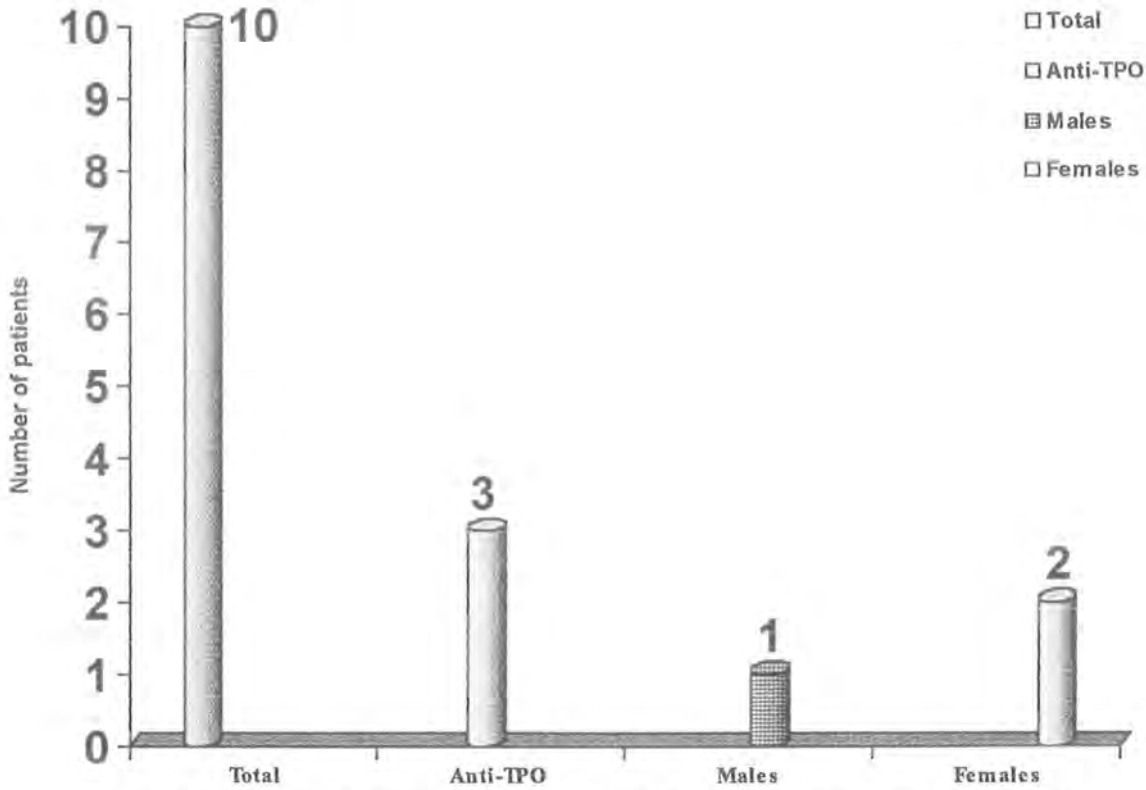
12 patients out of 43 hypothyroid patients had anti-TPO positive. These included 5 males and 7 females.

Table 9 Euthyroid Controls having Anti-TPO

Healthy Controls	Number			Percentage (%)	
	T	+ve	-ve	+ve	-ve
Male	5	1	4	20	80
Female	5	2	3	40	60

T = Total number of healthy controls ; +ve = Positive ; - ve = Negative

Figure 9



3 patients out of 10 hypothyroid Anti-TPO positive.These included 1 male and 2 females.

Hyperthyroid patients having positive RF

Sera of total 44 hyperthyroid patients were tested RF. Of these 44 patients, 26 were females and 18 were males. In 9 patients out of the 44 hyperthyroid patients RF was detected by latex agglutination method. The positive cases for RF were about 20%. Out of 18 male patients 4 were positive for RF, which gave a 22% positive value while 78% males were negative for RF. Out of 26 females 5 were positive for RF, which gave a 19% positive value and 81% female patients were negative for RF (Table-10).

Hypothyroid Patients having positive RF

Sera of 43 hypothyroid patients were tested for RF. Of these 27 were female patients and 16 were male patients. In total 10 patients showed a positive result for RF and this made about 23% of the total hypothyroid subjects. Of the 16 male patients 4 were having positive RF while 12 were negative making a 25% and 75% of male hypothyroid patients respectively. In the female 6 patients out of 27 showed positive result for RF while 21 were negative thus making 22% and 78% respectively (Table-11).

Healthy controls having positive RF

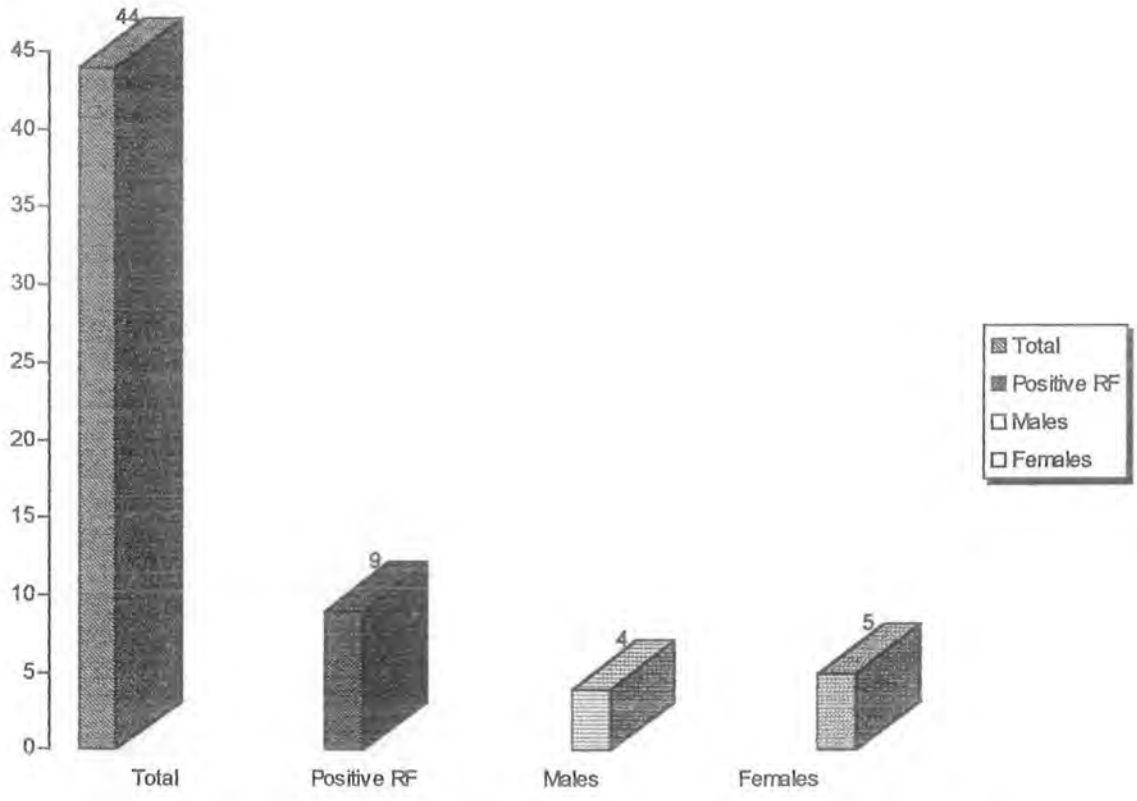
10 healthy subjects (5 males and 5 females) having thyroid hormone profile within normal limits were also included in the study to see the presence of antibodies against thyroid tissue and rheumatoid factor in their sera. 3 subjects out of these 10 were found positive for RF. Out of these 2 were females and 1 was male. This gave a value of 40% for female and 20% for male normal subjects (Table-12).

Table 10 Hyperthyroid patients having Positive RF

Patients	Number			Percentage (%)	
	T	+ve	-ve	+ve	-ve
Male	18	4	14	22	78
Female	26	5	21	19	81

T = Total number of patients ; +ve = Positive ; - ve = Negative

Figure 10



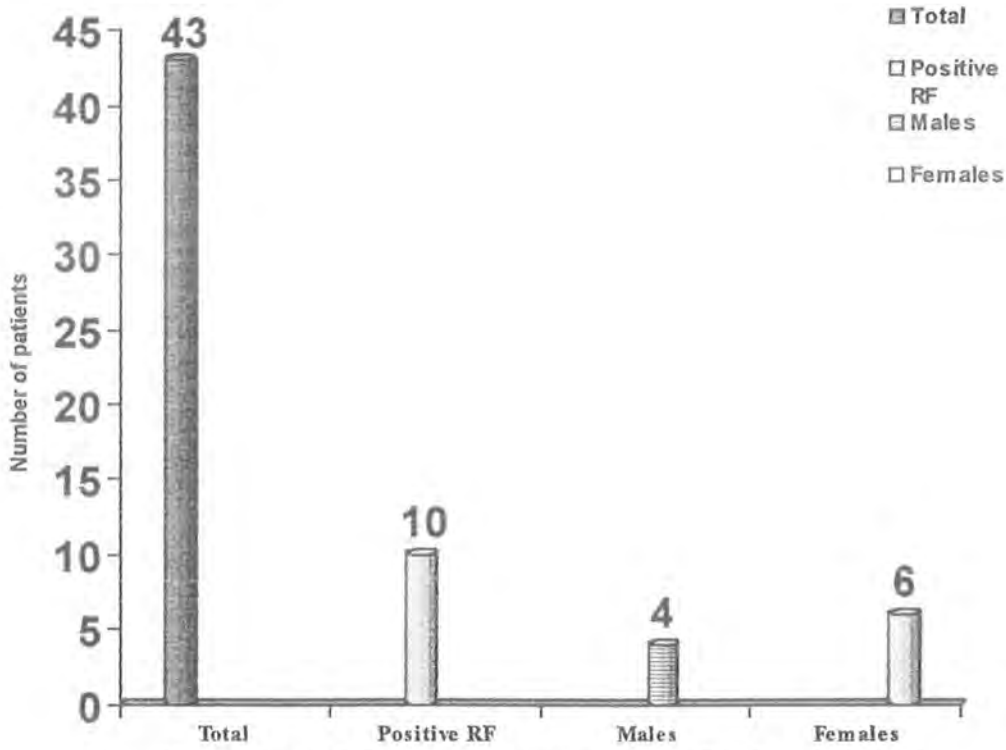
9 patients out of 44 hyperthyroid were positive for RF. These included 4 males and 5 females.

Table 11 Hypothyroid patients having Positive RF

Patients	Number			Percentage (%)	
	T	+ve	-ve	+ve	-ve
Male	16	4	12	25	75
Female	27	6	21	22	78

T = Total number of patients ; +ve = Positive ; - ve = Negative

Figure 11



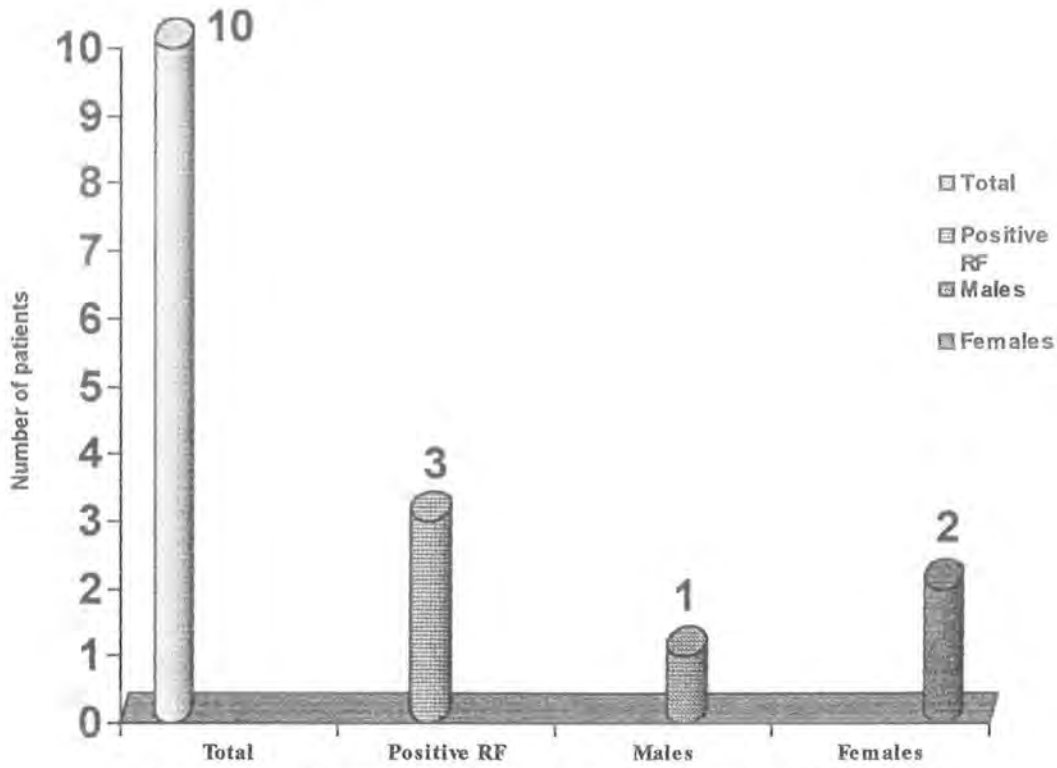
12 patients out of 43 hypothyroid were positive for RF.These included 4 males and 6 females.

Table 12 Healthy Controls having positive RF

Controls	Number			Percentage (%)	
	T	+ve	-ve	+ve	-ve
Male	5	1	4	20	80
Female	5	2	3	40	60

T = Total number of healthy controls ; +ve = Positive ; - ve = Negative

Figure 12



3 out of 10 healthy controls were positive for RF. These include 1 male and 2 females.

Hyperthyroid Patients positive for anti-TG having RF

44 patients with thyroid hormonal profile in hyperthyroid range were evaluated for anti-TG, anti-TPO and RF. Anti-TG was positive in 31 patients. Out of these 31 patients 6 patients were positive for RF. This gave an overall ratio of about 19% among the hyperthyroid patients having anti-TG in their sera. Out of these 6 patients 4 were female and 2 were male giving 18% and 20% ratio in their respective anti-TG positive group.(Table-13)

Hypothyroid Patients positive for anti-TG having RF

43 patients (27 females and 16 males) were showing hypothyroid levels of T3, T4 and TSH. 9 males and 22 females were positive for anti-TG. Their sera were tested for RF. Of these 43 patients 10 were positive for RF. Out of these 10 patients 4 were male and 6 were female. All of these RF positive patients also had anti-TG in their sera giving a 100% value.(Table-14)

Hyperthyroid Patients positive for anti-TPO having RF

Total 44 hyperthyroid patients (26 females and 18 males) were tested for anti-TPO. 13 females and 8 males patients showed a positive result for anti-TPO. Only 2 patients out of these 21 anti-TPO positive subjects were also positive for RF giving an overall about 9% value. Of these anti-TPO positive subjects having RF in their sera 1 was male and 1 was female. In their respective groups male showed a value of 12% and female showed a value of 7%.(Table-15)

Hypothyroidism Patients positive for anti-TPO having RF

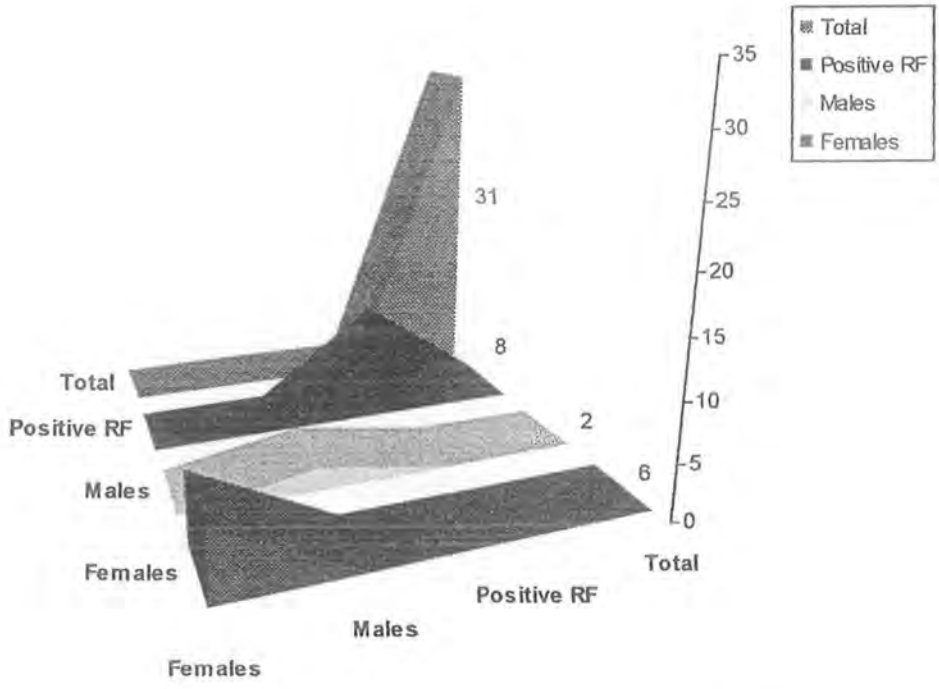
43 hypothyroid patients (16 males and 27 females) were evaluated for anti-TPO. 5 males and 7 females were positive for anti-TPO. Sera of 43 hypothyroid patients were also tested for RF. 10 patients were positive for RF. 8 patients out of these 10 subjects were also positive for anti-TPO giving an 80% ratio of RF positive cases who showed positive anti-TPO. Out of these 8 cases 3 were male and 5 were female patients. And in their respective groups of anti-TPO positive male and female patients they gave percentage of 60% for male and 71% for female patients who were positive for RF.(Table-16)

Table 13 Hyperthyroid Patients positive for anti-TG having RF

Patients	Number			Percentage (%)	
	T	+ve	-ve	+ve	-ve
Male	11	2	9	18	82
Female	20	6	14	30	70

T = Total number of patients ; +ve = Positive ; - ve = Negative

Figure 13



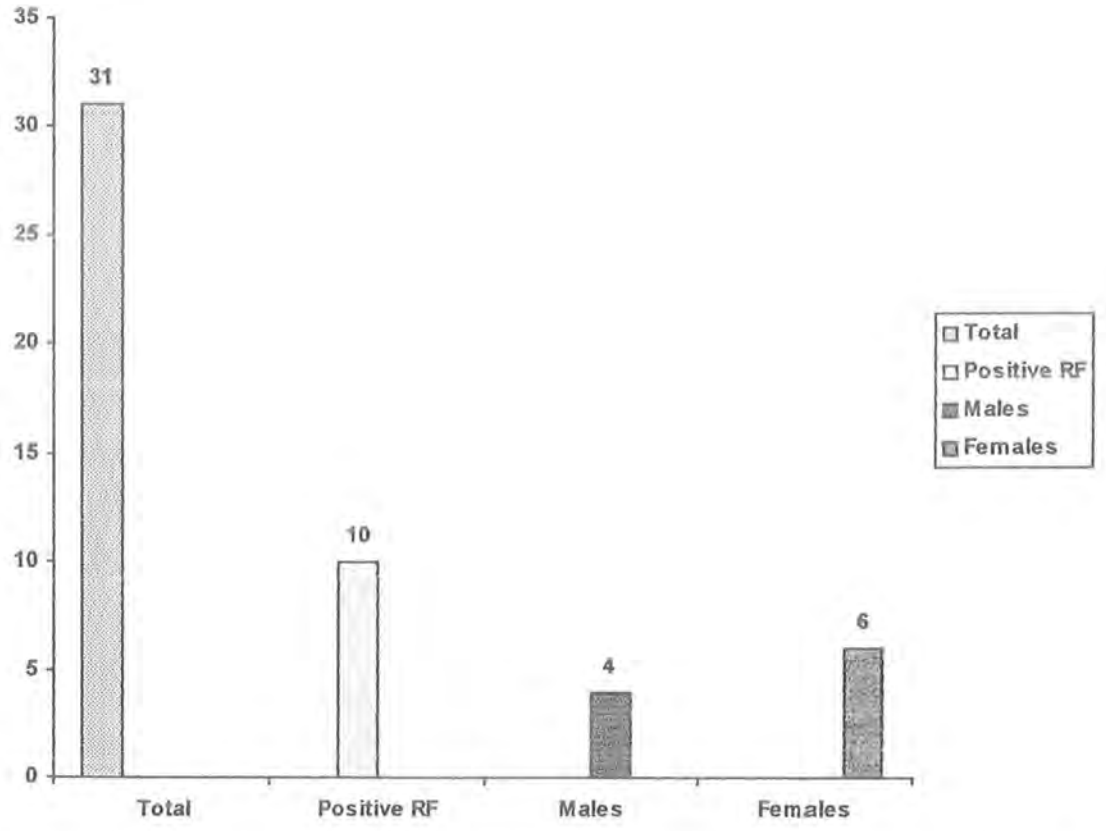
8 hyperthyroid patients positive for anti-TG were also positive for RF. These included 2 males and 6 females.

Table 14 Hypothyroid Patients positive for anti-TG having RF

Patients	Number			Percentage (%)	
	T	+ve	-ve	+ve	-ve
Male	9	4	5	44	56
Female	22	6	16	27	73

T = number of patients ; +ve = Positive ; - ve = Negative

Figure 14



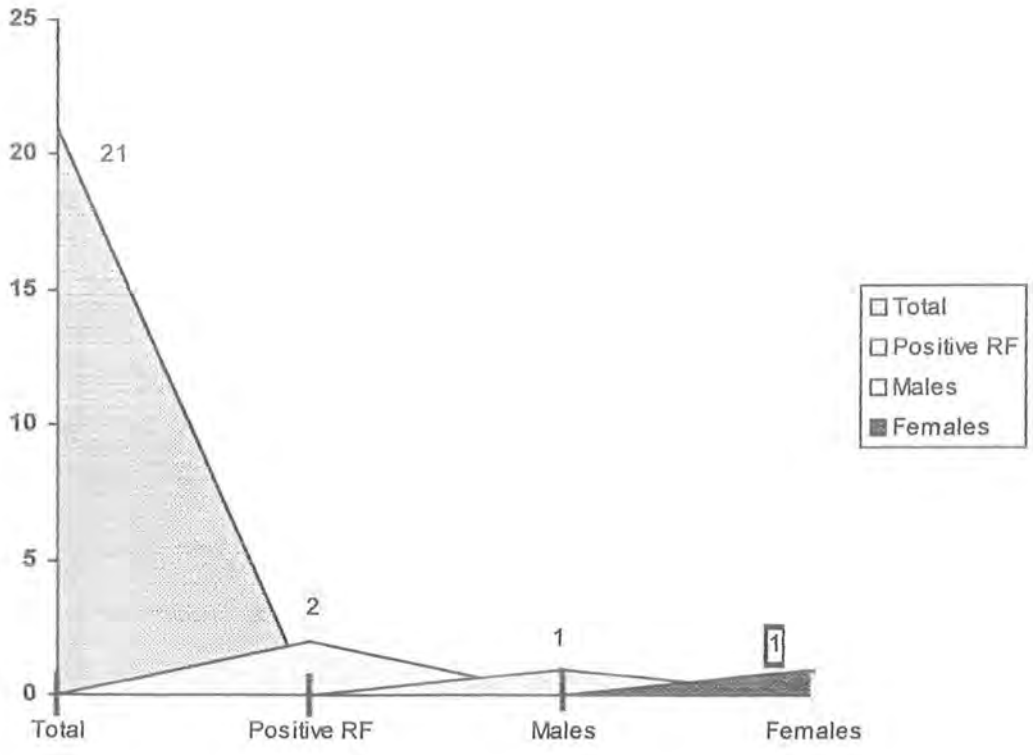
10 hypothyroid patients positive for anti-TG were also positive for RF. These included 4 males and 6 females.

Table 15 **Hyperthyroid Patients positive for anti-TPO having RF**

Patients	Number			Percentage (%)	
	T	+ve	-ve	+ve	-ve
Male	8	1	7	13	87
Female	13	1	12	8	92

T = number of patients ; +ve = Positive ; - ve = Negative

Figure 15



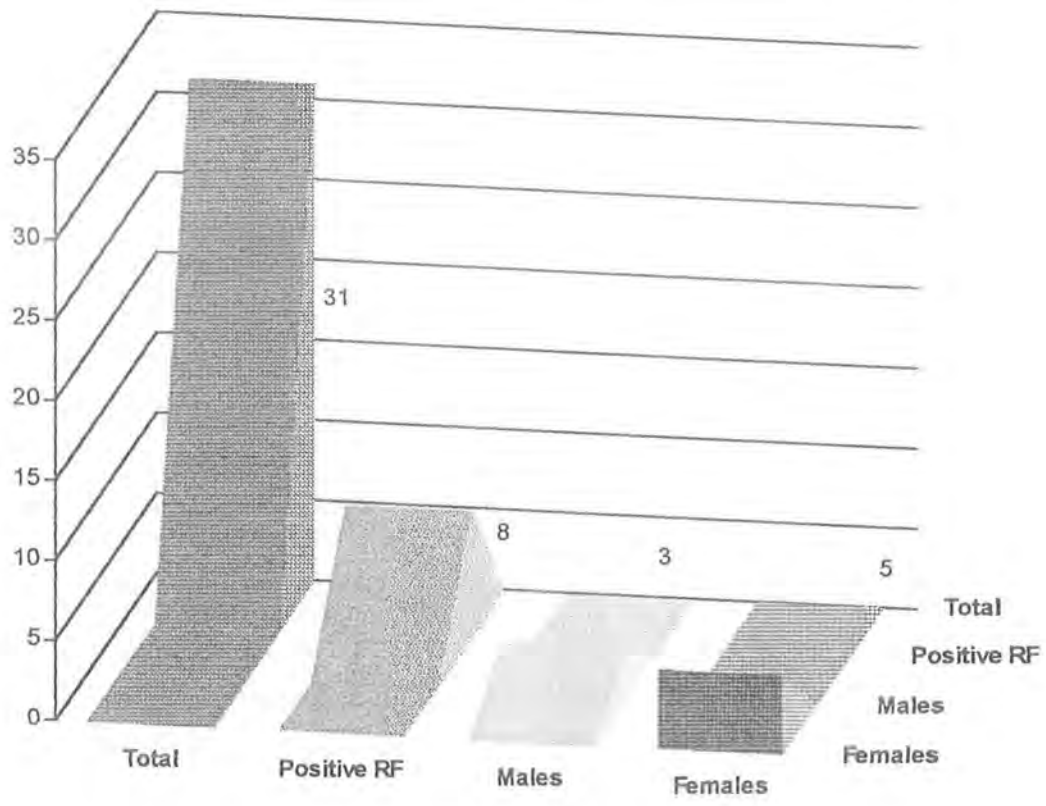
2 hyperthyroid patients positive for anti-TPO were also positive for RF. These included 1 male and 1 female

Table 16 Hypothyroid Patients positive for anti-TPO having RF

Patients	Number			Percentage (%)	
	T	+ve	-ve	+ve	-ve
Male	5	3	2	60	40
Female	7	5	2	71	70

T = number of patients ; +ve = Positive ; - ve = Negative

Figure 16



8 hypothyroid patients positive for anti-TPO were also positive for RF. These included 3 male and 5 female patients.

Hyperthyroid patients having, both, anti-TG and anti-TPO

A total number of 44 hyperthyroid patients (26 females and 18 males) were tested for presence of anti-TG and anti-TPO. 31 patients showed a positive test for anti-TG while 21 were positive for anti-TPO. Out of these 44 patients 20 were found to have a positive result for both anti-TG and anti-TPO. Of these 20 patients 12 were female and 8 were male. This showed a positive result for both anti-bodies in 44% male and 46% female patients.(Table-17)

Hypothyroid patients having, both, anti-TG and anti-TPO

Of the total 43 patients (16 males and 27 females) having hypothyroid pattern of thyroid profile 23 were positive for both anti-TG and anti-TPO. Of the 27 female patients 18 patients were having both antibodies in their sera, which gave a value of about 67%. On the other hand 5 male patients out of 16 gave a positive result for both antibodies giving 31% value.(Table-18)

Hyperthyroid Patients having anti-TG, anti-TPO and RF

Only 2 patients out of 44 hyperthyroid patients (26 females 18 males) which were evaluated for anti-TG, anti-TPO and RF showed a positive result for all the 3 parameters. These included 1 male and 1 female patient. This gave a value of about 4% for female and about 6% for male patients.(Table-19)

Hypothyroid Patients having anti-TG, anti-TPO and RF

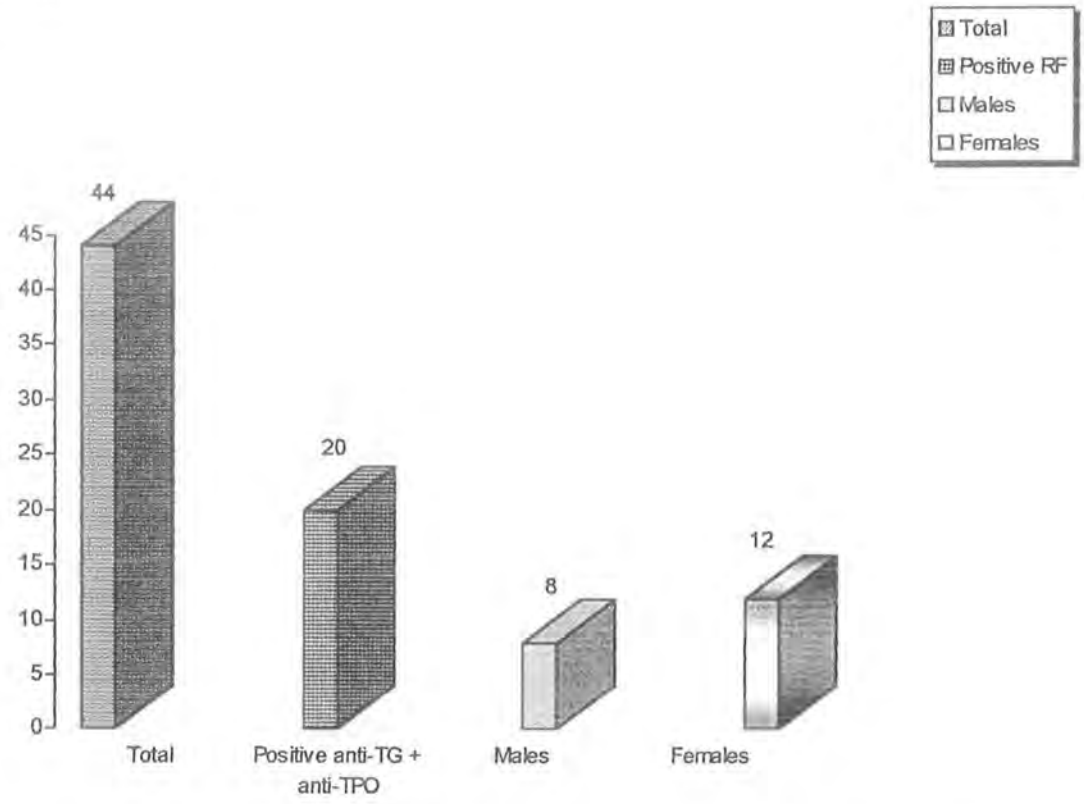
Of the 43 hypothyroid patients (16 males and 27 females) 8 showed a positive result for all the 3 parameters giving an overall value about 19%. Of the 27 females 5 showed positive result for all the 3 antibodies, which gave a value of about 19%. On the other hand 3 males showed all the 3 antibodies giving, again, a value of 19%.(Table-20)

Table 17 Hyperthyroid Patients positive for both anti-TG and anti-TPO.

Patients	Number			Percentage (%)	
	T	+ve	-ve	+ve	-ve
Male	18	8	10	44	56
Female	26	12	14	46	54

T = Total number of patients ; +ve = Positive ; - ve = Negative

Figure 17



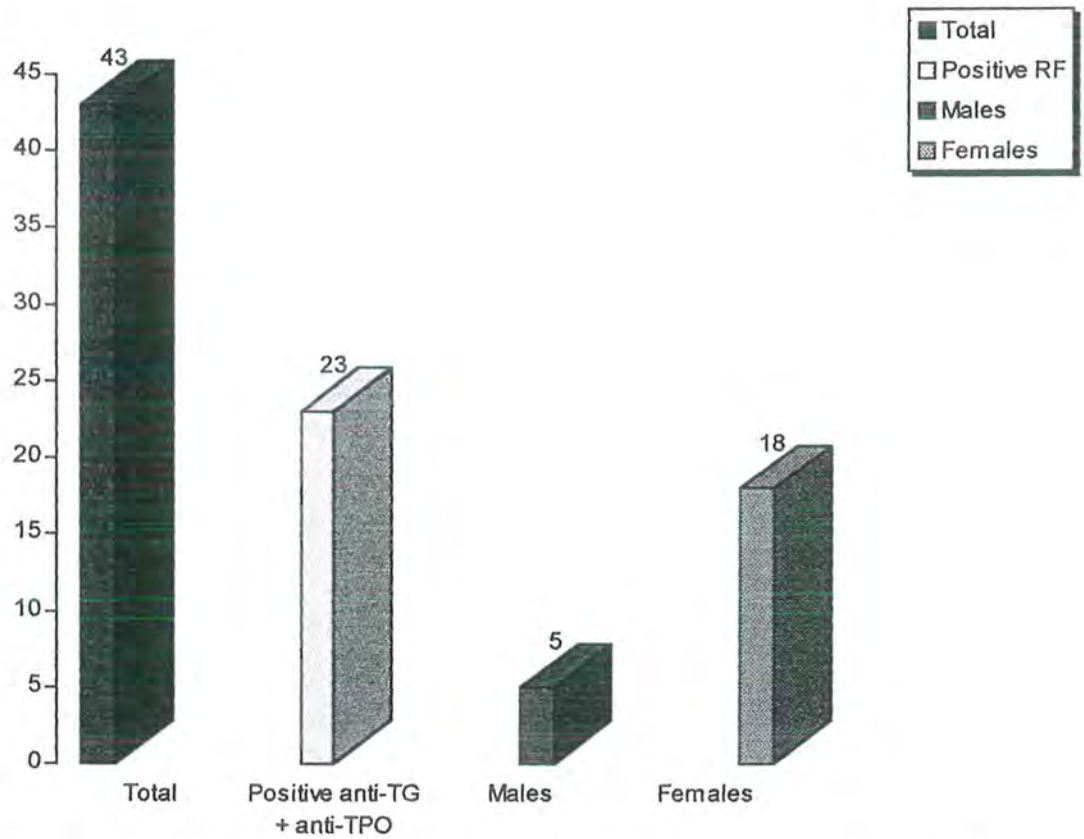
20 patients out of 44 hypothyroid patients had both anti-TG and anti-TPO in their sera. These included 8 males and 12 females.

Table 18 **Hypothyroid Patients positive for both anti-TG and anti-TPO.**

Patients	Number			Percentage (%)	
	T	+ve	-ve	+ve	-ve
Male	16	5	11	31	69
Female	27	18	19	67	33

T = Total number of patients ; +ve = Positive ; - ve = Negative

Figure 18



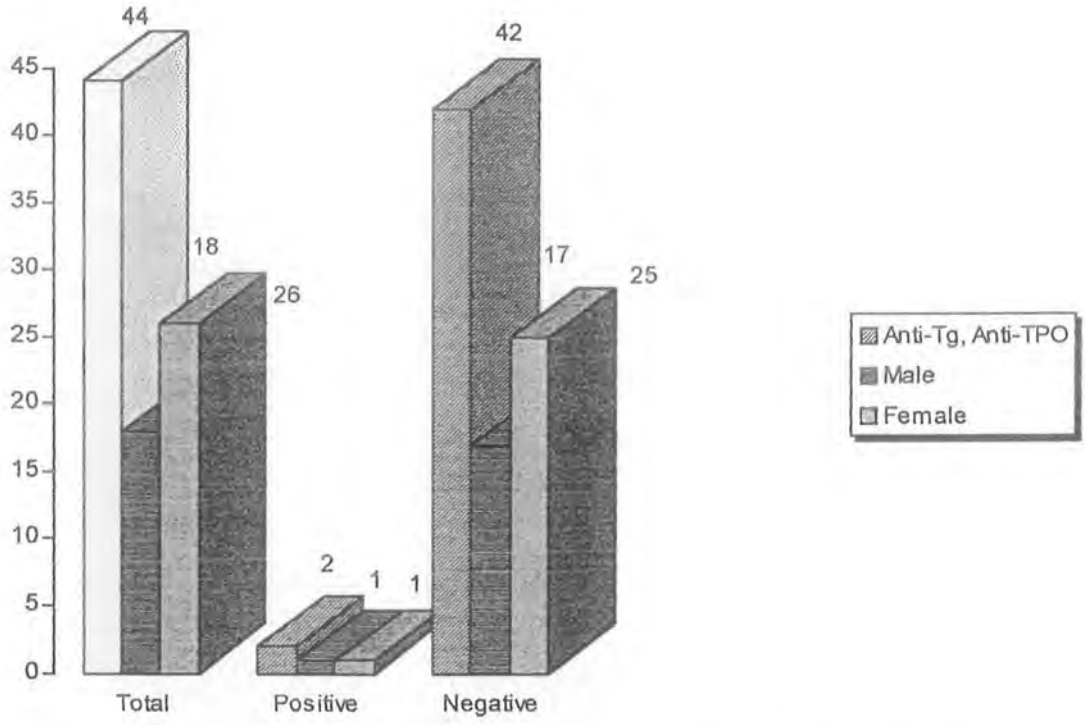
23 patients out of 43 hypothyroid patients had both anti-TG and anti-TPO in their sera. These included 5 males and 18 females.

Table 19 Hyperthyroid Patients positive for anti-TG, anti-TPO and RF

Patients	Number			Percentage (%)	
	T	+ve	-ve	+ve	-ve
Male	18	1	17	6	94
Female	26	1	25	4	96

T = Total number of patients ; +ve = Positive ; - ve = Negative

Figure 19



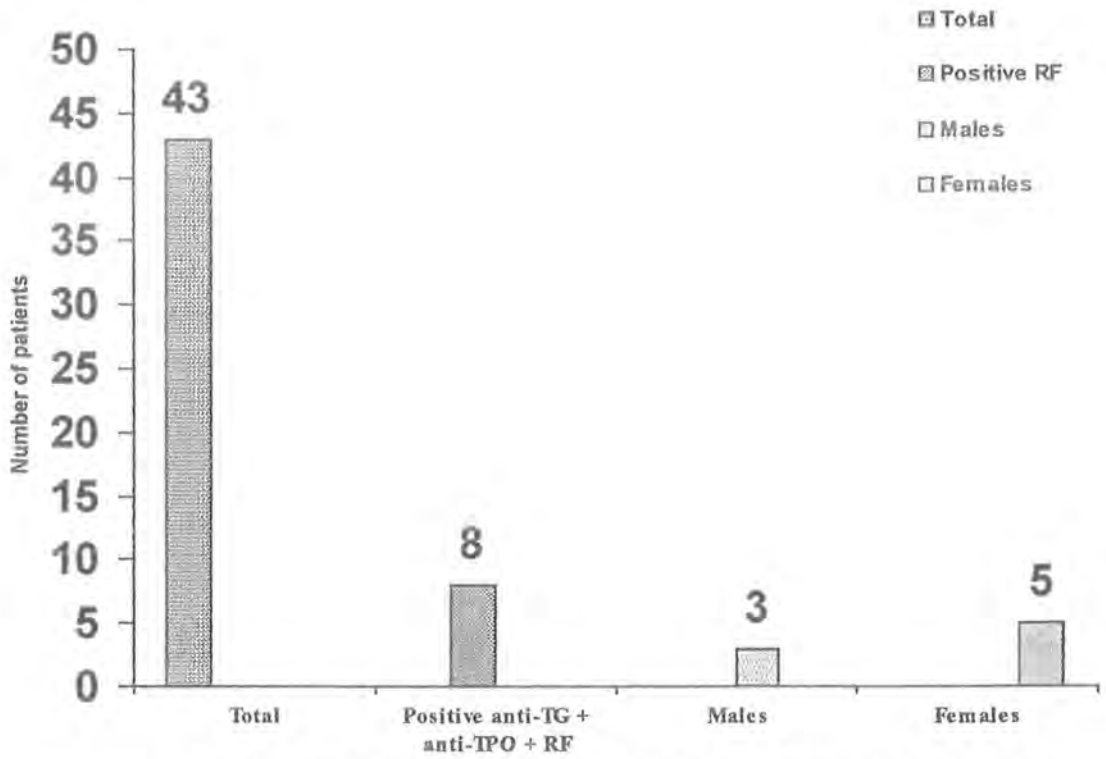
2 patients out of 44 hyperthyroid patients had all the three antibodies present in their sera i.e. anti-TPO and RF. These included 1 male and 1 female.

Table 20 Hypothyroid Patients positive for anti-TG, anti-TPO and RF

Patients	Number			Percentage (%)	
	T	+ve	-ve	+ve	-ve
Male	16	3	13	19	81
Female	27	5	22	19	81

T = Total number of patients ; +ve = Positive ; - ve = Negative

Figure 20



8 patients out of 43 hypothyroid patients had all the three antibodies present in their sera i.e. anti-TG,anti-TPO and RF.These included 3 males and 5 females.

DISCUSSION

DISCUSSION

The thyroid gland exerts a significant control over the rate of metabolism through secretion of hormones; T3 and T4. The thyroid hormones stimulate metabolism throughout the body, their major effect is to increase oxygen consumption via activating oxidative phosphorylation within mitochondria. Interaction of pituitary gland with TRH from the hypothalamus results in the synthesis and release of TSH. TSH interacts with receptors on the membranes of cells in thyroid gland to simulate production and release of T3 and T4. The amount of TSH released by pituitary is regulated through negative feed back control by the thyroid hormones (Felig , 1987). Hyperthyroidism is a medical term to describe the signs and symptoms associated with an overproduction of thyroid Hormones. This condition is caused by effect of excessive thyroid hormone secretion on the body tissues. Most symptoms that patients experience are same regardless of the cause. The body metabolism is increased; patient often feels hotter, can slowly lose weight despite eating more than normal.

The patient usually experiences fatigue and has trouble with sleep, trembling of hands, and hard or irregular heartbeat. Usually the symptoms of hyperthyroidism are so gradual in onset that patients do not realize symptoms until they become more severe. At this time the patients usually have deranged thyroid hormonal profile. Therefore their T3 and T4 are above normal and TSH levels are below normal or undetectable (Caplan et al, 1979).

In the present study, during clinical survey and screening of patients, 59% female patients and 41% male patients were found to have values of T3, T4 above the upper limit of reference range, while TSH values were below the normal reference range. These results are parallel to and supported by the study conducted by Larson (1972) and Marsden and Mc Kerron (1975). Deficiency of thyroid hormones is characterized by lethargy and a lowering of metabolism and is called hypothyroidism. The symptoms pointing to hypothyroidism are those attributed to low basal metabolic rate and include cold intolerance, decreased sweating, weight gain without increased caloric intake, bradycardia, slowness of movements, speech and thought, lethargy and sleepiness. Such a condition can result from disorders of pituitary or due to thyroid gland

itself (Brown, et al 1974). Patients with hypothyroidism often have joint and muscle aches, tenderness and stiffness especially in shoulders and hips, pain and stiffness in joints, swelling of knees or small joints of hands and feet. Patients may face attacks of pseudogout. This condition can result from disorders of pituitary or of thyroid gland itself. The major cause of hypothyroidism can be deficiency in iodine diet. Congenital hypothyroidism is an inherited deficiency of thyroid function that occurs in about one in every 6000 births (Teitz, 1996). T3, T4 and TSH levels of patients having symptoms of hypothyroidism are also disturbed. T3 and T4 levels fall below the normal limit while TSH levels are above the normal reference range (Surks et al, 1990).

While evaluating the results of thyroid profile in this study it was found that hormonal levels of 62% female and 38% male patients were found to be consistent with hypothyroidism. This revealed that the percentage of female patients is greater than the male hypothyroid patients. Similar findings have been reported by Burger and Quinn (1972), Sterling et al (1977) and Fagila et al (1979) while conducting studies on hypothyroid patients.

Autoimmune thyroiditis was first described by Hashimoto in 1912 and autoimmune thyroid disease associated with goiter is termed as Hashimotoe's thyroiditis. The presence of antibodies against thyroglobulin (anti-TG) in patients with this disease was first demonstrated by Roitt et al in 1956 . Antibodies to TG do not appear to be pathogenic and may simply indicate presence of disease. They have been found to be polyclonal and are heterogeneous with respect to heavy chain subclass (Laing,1983) and (McIntosh et al, 1997).

Anti TG antibodies are associated with cases of hypo- or hyperthyroidism and are frequently found in patients with other autoimmune diseases such as rheumatoid arthritis, pernicious anemia and type I diabetes (Walker et al.,1986 ; Ruf et al., 1994).

Anti TG are also detected in cases of thyroid carcinoma (Feldt-Rasmussen and Rasmussen, 1985; Schaadt et al., 1995). Low levels of anti TG are also found in asymptomatic individuals

particularly in the elderly and more often in women then men (Ericson et al 1985; Weetman and McGregor 1994). After screening and selecting the hyperthyroid and hypothyroid cases, aliquots of serum were evaluated for detecting the levels of Anti TG. 61% males and 77% females in the hyperthyroid group were found to have antibodies against TG in their blood.

In hypothyroid group of patients 56% males and 81% females demonstrated the presence of antibodies against TG in their sera. In contrast, patients having thyroid hormone level consistent with hyperthyroidism, 77% female and 61% male patients showed a positive result for the anti-TG. Serum aliquot of ten healthy individuals having T3, T4 and TSH values falling in normal range were subjected to Anti TG. It was found that sixty percent of normal controls had Anti TG in their sera. Although these findings are in accordance with the findings of Ericson et al (1985) and Weetman and McGregor (1994), but presently there was observed an overall increase in the percentage of anti-TG positive cases in healthy controls. This might be attributed to racial/and or dietary habits of individuals. Normally, TG appears in the peripheral blood in very small quantities amounting to 40 – 60 ng/ml. Due to the spill over of this prohormone into the circulation, it is exposed to immune system of the body that reacts by generating antibodies against the thyroglobulin (Van Herle et al, 1979). It is quite possible that this may be the cause of high prevalence of anti-TG in sera of normal individuals.

Higher levels of anti-TG are seen in areas of endemic goiter because of low iodine diet. In the present study, the anti TG has been found to be at higher level even in euthyroid controls. This may have a relation with moderately decreased iodine in diet of people living in this region of the country. Parallel findings have been reported by Pedersen and Knudsen (2003).
It was first demonstrated by Trotter et al (1957), that many patients with Hashimoto thyroiditis had detectable autoantibodies in their blood directed against a thyroid antigen distinct from thyroglobulin. This was subsequently proved by Roitt and Doniach (1958). This antigen was termed thyroid microsomal and since then it has been demonstrated that most if not all anti-thyroid microsomal autoantibodies recognize thyroid peroxidase, (Ruf et al., 1987). Contrary to autoantibodies against TG, antibodies against TPO fix compliment, anti-TPO are, potentially

deleterious and may have a pathogenic role in autoimmune thyroid disease (Khoury et al., 1981; Banga et al., 1985). The anti-TPO are demonstrated in most cases of postpartum thyroiditis and it has been found that the presence of autoantibody in early pregnancy is associated with a higher risk of asymptomatic postpartum hypothyroidism (Amino et al., 1981; Glinoer., 1998). It is common to find Anti TPO in the absence of antibodies to thyroglobulin; particularly in patients with small goiters and upto 64% cases of autoimmune hypothyroidism have been reported to be associated with anti-TPO alone (Nordyke et al., 1993).

In addition anti TPO are frequently found in patients with other autoimmune diseases such as Rheumatoid arthritis, Addison' disease and Type I diabetes (Scherbaum, 1987; Chang et al., 1998). Presently 20% hyperthyroid and 23% hypothyroid patients had demonstrated a positive result for rheumatoid factor. This finding was in accordance with the results of above studies.

Anti-TPO are also detectable at low level in about 20% of asymptomatic individuals in particular the elderly and more often in women than in men. (Rosenbaum and Davies, 1992; Mariotti et al., 1998).

Presently 44% males and 50% females belonging to hyperthyroid group were found to have antibodies against TPO while in hypothyroid group 31% male and 26% female patients had antibodies against TPO.

In control subjects 30% had antibodies, against TPO and 70% were negative for anti-TPO. The healthy controls positive for anti-TPO were also positive for anti-TG. Similarly 45% patients in hyperthyroid group and 53% patients in hypothyroid group were positive for both anti-TG and anti-TPO. The results obtained during the present study are further supported by the findings of Ruf et al., (1987); Doble et al., (1988) and Dually et al., (1991).
However, quite recently, it has been shown that another antibody newly discovered and named subsequently as anti-TGPO either present singly or in combination with anti-TG and / or anti-TPO may cause thyroid disease by acting against the thyroid tissue. (Multicentre study

published in. Eur J Endocrinol. 1999 Dec; 141(6): 563-9.). However this has to be evaluated with further experimentation.

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