THYROID FUNCTION PROFILE OF INFERTILE WOMEN IN ENUGU, AWKA AND NNEWI MUNICIPALITIES

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BY

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DEDICATION
This work is dedicated to my beloved wife Mrs. Esther Ngozi Anetoh
and kids for their encouragement and fervent prayers.
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ABSTRACT

Thyroid Hormone Profile of 217 infertile women aged 18 – 45 years were assayed using commercial Enzyme Linked Immunosorbent Assay (ELISA) kits. Thirty six healthy parous women within the same age group who had no history of reproductive problems were included as control. Out of the 217 infertile women studied 213 (98.16%) had normal serum T$_3$, FT$_4$ and TSH levels, a total of 4 (1.84%) had abnormal thyroid hormone profile. Out of these abnormal subjects 3 (1.38%) had raised serum TSH, normal T$_3$ and FT$_4$, while 1 (0.46%) had raised serum T$_3$ and FT$_4$ and suppressed TSH (overt hyperthyroidism). Thirty six women studied as control had their thyroid hormones within normal reference ranges. The mean value for T$_3$ (ng/ml) was 1.21 ($\pm$0.348) for patients and 1.18 ($\pm$0.322) for controls. Statistically, there was no significant difference between the means (P>0.05). For patients, the mean value of FT$_4$ test group (ug/dl) was 1.38 ($\pm$0.290), and for the control 1.42 ($\pm$0.324). There was no significant difference between the mean values (P>0.05). The mean value of TSH (uIU/ml) was 2.22, ($\pm$0.308) for infertile women and 1.57 ($\pm$0.700) for controls. Statistically, there was a significant difference between the mean values (P<0.05). The data obtained in this study suggests that routine screening for thyroid disorder on infertile women would yield few positive results and therefore not recommended as part of routine work up for all infertile women.
1.0 CHAPTER ONE

1.1 Introduction

Infertility is defined as inability of a couple to achieve pregnancy after one year of unprotected intercourse (Mosher, 1987). In sub-Saharan African, the prevalence of infertility is as high as 30-40% (Cates et al, 1983), and the female contribution is estimated to be 20-40%. In the United States of America, approximately 10% to 20% of couples are infertile (Speroff et al, 1989, Blackwell et al, 1999). Causes of infertility in women can be grouped in seven factor arrears: ovarian, pelvic, tubal, uterine, cervical, male, and unexplained. (Markham, 1991). However, with the advent of modern science, factors contributing to low conception rates are increasingly being better understood. Hyperthyroidism and hypothyroidism are among different causes of infertility in women (Mentere et al, 1981).

Thyroid hormones are essential and primary regulators of body metabolism. Imbalances can affect virtually every metabolic process in the body. Thyroid function has profound impact on overall health via modulation of carbohydrate, protein, and fat metabolism, vitamin utilization, hormone secretion, sexual and reproductive health and many other physiological parameters (Anderson and Manash, 2000).

Early reports have suggested a close association between infertility and hypothyroidism (Potter 1980, Bohnet 1991). Several reports have shown that severe hypothyroidism can give rise to hyperprolactinaemia, which can cause anovulation and infertility. (Synder et al 1973, Contrene et al, 1981). Some researchers also believe that women with untreated thyroid disease
who do conceive are at risk of their children being born with physical abnormalities as well as mental retardation (Law et al, 1998).

The prevalence of infertility associated with abnormal thyroid function among women in our society need to be evaluated. Most biochemical investigations on infertile women have been focused on hypothalamic-pituitary-gonadal axis, with less attention on thyroid abnormalities. The study will help to determine the prevalence of hyperthyroidism or hypothyroidism among infertile women in our society and suggest the need for thyroid evaluation as part of routine investigation of infertility.

1.2 **Aims and objectives**

(a) To determine the thyroid function status of infertile women in our society with a view to evaluating the prevalence of infertility associated with abnormal thyroid function.

(b) To determine the diagnostic utility of thyroid function screening as part of a routine workup for women with infertility.
CHAPTER TWO

LITERATURE REVIEW

2.1 Anatomy of thyroid gland

The thyroid is composed of two encapsulated lobes, one on either side of the trachea, connected by a thin band tissue, the isthmus, which gives the gland the appearance of a butterfly. The right lobe is somewhat larger than the left lobe. Sometimes a pyramidal lobe is found extending superiorly from the isthmus in the middle, indicating the embryology part along which the thyroid developed. In a healthy adult, thyroid weighs about 30gm. The thyroid develops as a thickening in the pharyngeal floor that elongates inferiorly as the thyroglossal duct and divides into two lobes as it descends through the neck.

The arterial supply of the thyroid is derived primarily from paired superior and inferior thyroid arteries. The venous drainage is more complex and variable, but usually there are paired superior, middle, and inferior thyroid veins. Normally, the parathyroid glands lie behind the upper and lower poles of each lobe of the thyroid.

Microscopically, thyroid tissue consists of spherical thyroid follicles. Each follicle is composed of a single layer of cuboidal follicular cells surrounding a lumen filled with a homogeneous material called colloid. When stimulated, the follicular cells become columnar and the follicles are depleted of colloid, when suppressed, the follicular cells become flat and colloid accumulates. The thyroid also contains parafollicular connective tissue, in less numbers, within thyroid follicles that produce calcitonin.
The Thyroid Gland

Diagram I: Thyroid Anatomy showing two encapsulated lobes one on either side of the trachea. (Windpipe)

2.2 Thyroid hormone biosynthesis

The important element involved in the synthesis of thyroid hormones is iodine, which is normally absorbed in the small intestine in form of iodides. Iodine transport to the follicular cells of the thyroid gland is the first and rate-limiting step in the synthesis process. An adequate supply of iodine is essential for normal thyroid function. The thyroid gland utilizes iodide more effectively when it is scarce, and less effectively when it is abundant. Much of this regulation is achieved via changes in TSH secretion, but iodide also participates in this process (Taurog 2000). When iodine intake is low, iodide transport and T₄ and especially T₃ synthesis are increased, even in the
absence of TSH. With increasing iodide availability, the synthesis of T₄ rises more than does that of T₃, but high iodide concentrations inhibit iodide oxidation and organification, and therefore the synthesis of both hormones decreases. Iodine excess also inhibits thyroglobulin production and thus release of T₄ and T₃ from the thyroid. This is a rapid effect, occurring within hours, and is the principal mechanism for antithyroid effect of inorganic iodine in patients with thyrotoxicosis (Taurog 2000). Iodine that is absorbed is distributed in the extracellular fluid, in which the iodine concentration is about 1μg/dl (0.08mmol/liter). The pool receives not only dietary iodine, but also iodine that is released from the thyroid gland and iodine derived from the peripheral deiodination of iodothyronines.

Iodide is transported into thyroid follicular cells against a chemical and electrical gradient; it then rapidly diffuses into the follicular lumen (Taurugo 2000). Iodide transport occurs at the basal membrane of the follicular cell, is mediated by a sodium iodide (Na/I) co-transporter, and is energy dependent and saturable (Dai, et al 1996). Iodide transport is stimulated by thyroid stimulating hormone (TSH), in addition, it is inhibited by excess iodide and increased by iodine deficiency, independent of TSH (Spitzweg et al, 2000). Ions of similar size, shape, and charge, such as perchlorate and pertechnetate can serve as substrate for transport system and therefore as competitive inhibitors of iodine transport.

In thyroid cells, iodide diffuses rapidly to the apical cell membrane, where it is transported to exocytotic vesicles fused with the membrane. This step is mediated by pendrin, a chloride-iodine transport protein. In these vesicles,
iodide is very rapidly oxidized and then covalently bound (organified) to some of the tyrosyl residues of thyroglobulin (Taurog, 2000). Tyrosines, another principal raw material are provided from large glycoprotein scaffold called thyroglobulin, which is synthesized by thyroid epithelial cells and secreted in the lumen of the follicular colloid, is essentially a pool of thyroglobulin.

Fabrication of thyroid hormone is conducted by the enzyme thyroid peroxidase, an integral membrane protein present in the apical membrane of the thyroid epithelial cells. Thyroid peroxidase catalyzes iodination of tyrosines on thyroglobulin (also known as organification of iodide). The oxidation or organification process results in mono or diiodination of about 15 of the 134 tyrosine residues of thyroglobulin (Scott et al 1999). Thyroxine (T₄) is formed by the coupling of two diiodotyrosyl residues and triiodothyronin (T₃) is formed by coupling of one moniodotyrosyl residue and one diiodotyrosyl residue within individual thyroglobulin molecules.

Each of the processes described above is stimulated by thyroid-stimulating hormone (TSH) from the anterior pituitary gland. Binding of TSH to its receptors on thyroid epithelial cells stimulates synthesis of iodine transport, thyroid peroxidase and thyroglobulin. The magnitude of the TSH signal also set the rate of endocytosis of colloid, high concentrations of TSH lead to faster rate of endocytosis, and hence, thyroid hormone release into the circulation. Conversely when TSH levels are low, rates of thyroid hormone synthesis and release diminish. (Taurog, 2000)
2.3 Chemistry of thyroid hormones

Thyroid hormones are composed of a phenyl ring attached via ether linkage to tyrosine and contain from one to four iodine atoms. T₄ is 3,5,3’,5’-tetraiodothyronine, whereas T₃ is 3,5’,3’-triiodothyronine, having one less iodine atom on its outer ring. Deiodination of the inner ring of T₄ results in formation of 3, 3’,5’-triiodothyronine (reverse T₃). Diodothyronine, or T₂, can exist in three forms, two iodine atoms on the outer ring (3’, 5’-T₂), two on the inner ring (3,5-T₂) or on each ring (3,3’-T₂). Oxidative deamination and decaboxylation of T₄ result in the formation of tetraiodothyroacetic acid; triiodothyroacetic acid is formed from T₃ in the same way.

Of the compounds, only T₄, T₃, tetraiodothyroacetic acid, and triiodothyroacetic acid have biological activity. The latter two are produced in such small amount that they contribute little if at all to thyroid hormone action in vivo.
Diagram 2.2: Structural formulae of various iodothyronines and iodothyronine analogs.
2.4 **Thyroid hormone production and metabolism**

Once inside the thyroid epithelial cells, iodide is transported to the apical membrane, and thyroid peroxidase, an integral membrane enzyme, catalyzes sequential reaction in thyroid hormone production. Thyroid peroxidase first oxidizes iodide to iodine and then iodinates tyrosines on thyroglobulin to produce monoiodothyrosine and diodothyrosine. Thyroid peroxidase finally links two iodinated thyrosines to produce T\(_4\), and T\(_3\) (Perron *et al.*, 2001).

The total daily production rate of T\(_4\) is 80-100ug (100-130nmol), all derived from thyroidal secretion. The extrathyroidal T\(_4\) pool contains 800-1000ug (1000-1300nmol), most of which is extracellular. The T\(_4\) turnover rate is 10 percent per day (serum half-life 6.7 days). Thus, some T\(_4\) remains available for several weeks in the absence of any thyroidal secretion. Approximately 80 percent of the T\(_4\) secreted each day is metabolized by deiodination, with about 40 percent being converted to T\(_3\) and 40 percent to rT\(_3\). The remaining 20 percent is metabolized by conjugation with sulphate and glucuronide, oxidative deamination and decarboxylation to form tetraiodothyroacetic acid, and ether link cleavage (Leonard and Koehrle, 2000). Deiodination of T\(_4\) to T\(_3\) leads to increased biological activity. The extrathyroidal conversion of T\(_4\) to T\(_3\) is regulated by a variety of factors, so that production of T\(_3\), the most active thyroid hormone, may be altered independently by changes in pituitary-thyroid function.

T\(_3\) is produced primarily (80 percent) by extrathyroidal deiodination of T\(_4\); the remainder comes from the thyroid. The total daily production rate is 30-40ug (45-60nmol) (Engler and Burger, 1984). The extrathyroidal T\(_3\) pool contains about 50ug (75nmol), most of which is intracellular. T\(_3\) is much
more rapidly degraded than is T₄, its turnover rate being about 75 percent per day. Hence, alterations in T₃ production rapidly alter its availability. T₃ either as such, or after being sulfated, is metabolized primarily by deiodination to 3',3'-diodothyronin. A small amount is deiodinated to 3', 5'-diodothyronine (Pinna et al, 1997), and the remainder (about 10 percent) is metabolized to triiodothyroacetic acid.

The daily production of rT₃ is 30-40ug (45-60nmol); more than 95 percent is produced at extrathyroidal sites. It is cleared from the circulation even more rapidly than is T₃. Most is deiodinated by type 1 diodinase to 3',3'-deiodothyronin, but some is deiodinated to 3',5'-diodothyronine or metabolized by nondeiodinative pathway (Engler and Bunger, 1984).

*Diagram 2.3: Outline of a thyroid follicle and the major steps in thyroid hormone biosynthesis and release.*

_I- denotes inorganic iodine; MIT, monoiodotyrosine; and DIT, diiodotyrosine.*
2.5 Regulation of thyroid hormone production

Thyroid hormone production is regulated in two ways; first, thyroidal $T_4$ and $T_3$ biosynthesis are stimulated by Thyroid Stimulating Hormone (TSH). The secretion of TSH in turns is inhibited by circulating $T_4$ and $T_3$ and is stimulated by thyrotropin releasing hormone (TRH). Second, extrathyroidal $T_3$ production from $T_4$ is regulated by a variety of factors and the effect of these regulating factors differ in different tissues. The first mechanism provides a sensitive defense against alteration in thyroid secretion. The second provides for rapids alterations in tissue to nonthyroidal illness that probably constitute an important adaptation to illness (Santini, et al 1996). The sensitivity of the thyrotroph cells to changes in serum $T_4$ and $T_3$ concentrations is great, so that very small changes have large effects on TSH secretion, when the changes in serum $T_4$ and $T_3$ are due to changes in thyroid secretion.

Diagram 2.4: Control of thyroid hormone synthesis and secretion
2.6 Thyroid stimulating hormone (TSH)

Thyroid-Stimulating hormone (TSH), also known as thyrotropin, is a 28-kDa glycoprotein that is synthesized and secreted by the cells in the anterior pituitary called thyrotrophs. It is composed of two non-covalently linked peptide subunits, an alpha subunit and a beta subunit, and contains about 15 percent carbohydrate. The alpha subunit of TSH is the same as that of luteinizing hormone, follicle-stimulating hormone, and chorionic gonadotropin. The beta subunit is unique, and therefore confers the TSH receptor specificity. (Grossmann et al, 1997), but both subunits are required for biological activity.

The synthesis of each subunit is directed by separate mRNAs that are coded by separate genes on different chromosomes. After synthesis, the subunits are glycosylated and linked together to form TSH, which is then packaged into granules. TSH stimulates virtually every aspect of thyroid hormone biosynthesis and secretion. It also stimulates many steps in intermediary metabolism and the expression of many genes in thyroid tissue, and it causes thyroid hyperplasia and hypertrophy. The actions of TSH are initiated by its binding to specific plasma membrane receptors (Rapport and Chazenbalk 1998). Binding of TSH to the receptor activates the G-protein, which stimulates adenyl cyclase, increasing cyclic AMP formation, which in turn stimulates several protein kinases.

TSH stimulates most if not all aspects of intrathyroidal iodide and thyroglobulin metabolism (Taurog, 2000; Vassart and Dumont, 1992). The stimulation of iodide transport is slow, requiring 24 hrs of exposure to TSH, and is due to the production of additional transport units. The TSH –induced
increase of iodothyroine formation is rapid and occurs in the absence of measurable increases in $\text{H}_2\text{O}_2$ production, or thyroid peroxidase activity. Stimulation of colloid endocytosis and thyroglobulin proteolysis is even more rapid, occurring within minutes after TSH exposure. TSH stimulation of thyroidal deiodinase activity increases the proportion of $\text{T}_3$ that is secreted, thus helping to conserve iodine stores (Laurberg, 1984).

Physiologically, TSH stimulates the secretion of thyroglobulin as well as that of $\text{T}_4$ and $\text{T}_3$, although more slowly (Ramirez et al, 1997). It is more effective in raising serum $\text{T}_4$ and $\text{T}_3$ concentrations when its secretion is pulsatile than when it is continuous (Shulkin et al, 1986). TSH stimulates many other factors of thyroid cell function. It increases oxygen consumption, glucose and fatty acid utilization, and the content of NADPH, which is utilized for $\text{H}_2\text{O}_2$ generation as well as iodotyrosine and perhaps $\text{T}_4$ deiodination. (Vassart and Dumont, 1992). A clinically obvious and important chronic action of TSH is stimulation of thyroid hypertrophy hyperplasia, i.e. goiter formation. This growth reflects the ability of TSH to stimulate the synthesis of DNA, RNA, and structural proteins (Dumont et al, 1992).

$\text{T}_4$ and $\text{T}_3$ directly inhibit TSH secretion, and deficiency of $\text{T}_4$ and $\text{T}_3$ increases it. The sensitivity of the thyrotrph cells to changes in serum $\text{T}_4$ and $\text{T}_3$ concentrations is great, so that very small changes have large effects on TSH secretion, when the changes in serum $\text{T}_4$ and $\text{T}_3$ are due to changes in thyroid secretion. Both $\text{T}_4$ and $\text{T}_3$ participate in this regulation, which serves to maintain thyroid secretion within very narrow limit. (Leonard and Koehrle, 2000). $\text{T}_4$ and $\text{T}_3$ inhibit TSH secretion by decreasing both the
biosynthesis and release of TSH and, to a lesser extent, by decreasing the secretion of TRH. (Shupnik et al 1989)

The determination of serum or plasma levels of TSH is recognized as a sensitive method in the diagnosis of primary and secondary hypothyroidism (Leonard and Koehrle, 2000). Although the concentration of TSH in the blood is extremely low, it is essential for the maintenance of normal thyroid function. The release of TSH is regulated by Thyroid Releasing Hormone (TRH) produced by the hypothalamus. The level of TSH and TRH are inversely related to the level of thyroid hormones.

### 2.7 Thyrotropin releasing hormone (TRH)

TRH is synthesized as a 26-kDa protein (proTRH) that contains five copies of the sequence glutamine-histidine-proline-glucine formed by proteolytic cleavage sites. TRH is formed from proTRH by the action of peptidases followed by cyclization of the glutamine residue to form a pyroglutamyl residue (Wu and Lechan 1997).

TRH stimulates TSH secretion by binding to receptors on thyrotroph cells membrane that are linked to phospholipase C. The phosphoinositides formed by the action of this enzyme stimulate the release of calcium from intracellular storage sites; the increase in cytosol calcium concentration in turn stimulates exocytosis of TSH (Gershengorn 1996). The number of TSH receptors on the thyrotroph cells is down regulated by TRH and by thyroid hormones (Schomberg and Bauer, 1995).

TRH secretion is probably pulsatile, accounting from the pulsatility of TSH secretion, and surges of TRH secretion are responsible for the surges of TSH
secretion that occur in newborn infants and during cold exposure in some subjects. More important, TRH is required for maintenance of normal TSH secretion, and it determines the set point about which thyroid hormones regulate TSH secretion (Schomberg and Bauer, 1995).

2.8 Serum binding proteins
Very little of the T4 and T3 in the circulation is free, more than 99.95 percent of the T4 and 99.5 percent of the T3 are bound in reversible physiochemical equilibrium to several serum proteins. These proteins are thyroxine-binding globulin (TBG), transthyretin (TTR-formally called thyroxine binding prealbumin (TBPA)], albumin, and lipoprotein. In normal subjects, approximately 75 percent of the T4 in serum is bound to TBG, 10 percent to TTR, 12 percent to albumin, and 3 percent to lipoproteins (Robins, 2000). Approximately 0.05 percent of the total serum T4, or about 2ng/dl (26pmol/liter), is free. For serum T3, the respective values are approximately 0.5 percent and 0.5ng/dl (7pmol/liter). Because so much of the T4 and T3 in serum is bound, changes in serum concentrations of binding proteins have a large effect on serum total T4 and T3 concentrations and fractional T4 and T3 metabolism, but they do not alter the serum free T4 and T3 concentration or the absolute rates of T4 and T3 metabolism (Robins, 2000). It is the serum free T4 and T3 concentrations that determine the hormone biological activity. The overall effect of the binding proteins is to maintain serum free T4 and T3 concentrations within narrow limits, yet ensure that the hormones are continuously and immediately available to tissues.
They have, therefore, both storage and buffer functions. The storage function serves to facilitate the uniform distribution of T₄ and T₃ within tissues, particularly large solid organs. In the longer term, if thyroid secretion ceases, the T₄ stored in serum serves to delay the appearance of thyroid deficiency for weeks, whereas if only free T₄ were available its supply would be exhausted within hours. Conversely the binding proteins serve to buffer the free T₄ and T₃ concentrations in serum from sudden increases in thyroid secretion or release from extrathyroidal tissue (Mendel et al 1987).

Diagram 5: Origin, circulating forms and degradation of T₃ and T₄. Note: only liver is shown as a site of conversion to T₃, but conversion occurs in many tissues.
2.8.1 Thyroxine binding globulin (TBG)
Thyroxine binding globulin (TBG) is a 54-kDa glycoprotein that is synthesized in the liver. It has one binding site for T₄, while the affinity constant of TBG for T₄ is high; T₃ binds to TBG considerably less avidly (Robins 2000). The serum TBG concentration in normal subjects is about 1.5mg/dl (0.27umol/liter). This amount is capable of binding about 20ug T₄ (26nmol) but only about one-third of the TBG in serum normally contain T₄.

2.8.2 Transthyretin: (TTR)
Transthyretin (TTR) is a 55-kDa tetrameric protein composed of four identical subunits. It is synthesized in the liver, pancreatic islets, and choroids plexus. Each molecule has two identical T₄ binding sites, but occupation of one site by T₄ greatly decreases the affinity of the second site for T₄. The affinity constant of TTR for binding of T₄ is about 7x10⁷m⁻¹, its affinity for T₃ is considerably less (Mendel et al 1987).

2.8.3 Albumin
Albumin (66-kDa) has one strong binding site and several weaked binding sites for T₄. Because only about 12 percent of the T₄ in serum is bound to albumin changes in serum albumin concentration have relatively little effect on serum T₄ concentrations.

2.8.4 Lipoproteins
A small percentage (3-5 percent) of the T₄ and T₃ in serum is bound to lipoprotein. Among them, high density lipoproteins, mostly their apoprotein
A-I component, bind the most T₄ and T₃, but some are bound to the apoprotein- B 100 component of low density lipoprotein and very-low density lipoprotein.

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<th>Proteins</th>
<th>Affinity constant m⁻¹</th>
<th>Serum concentration mg/dl</th>
<th>Serum umol/l</th>
<th>T4 Binding capacity ug/dl mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroxin</td>
<td>1x10⁻¹⁰ 5x10⁵</td>
<td>1.5</td>
<td>0.27</td>
<td>5</td>
</tr>
<tr>
<td>Binding Globulin</td>
<td>7x10⁻⁷ 2x10⁷</td>
<td>25</td>
<td>4.6</td>
<td>2</td>
</tr>
<tr>
<td>Transthyretin</td>
<td>7x10⁻⁷ 2x10⁷</td>
<td>25</td>
<td>4.6</td>
<td>2</td>
</tr>
<tr>
<td>Albumin</td>
<td>7x10⁻⁵ 1x10⁵</td>
<td>4000</td>
<td>640</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 2.1: Properties of Serum Thyroid Hormone Binding Protein

2.9 **Cellular hormone entry and tissue response**

Serum free T₄ and free T₃ are available for cellular uptake at any instant, and the rates of dissociation of T₄ and T₃ from their binding proteins are so rapid that additional T₄ and T₃ rapidly become available. The primary mechanism of cellular uptake of T₄ and T₃ in most tissues is via diffusion, its rate being determined by the gradient in free hormone concentration between the serum and intracellular fluid (Mendel and Weisiger 1990). Other factors, such as vascular flow and permeability and intracellular binding, also are important in determining T₄ and T₃ entry into cells.
Specific nuclear receptors (T3 Nuclear Receptor) in most tissues mediate most if not all of the physiologic actions of thyroid hormone (Anderson and Marish 2000). These receptors are members of a super family that includes the receptors of steroid hormones, Vitamin D, and retinoid. T₃ enters nuclei directly from the cytosol by diffusion and then binds to the receptors, no cytosol T₃-binding protein is required to facilitate entry of T₃ into nuclei. The T₃ nuclear receptors are acidic proteins that bind T₃ much more avidly than T₄, and in vivo nearly all the nuclear-bound hormone is T₃. T₄ can thus be considered largely if not completely a prohormone for T₃ (Anderson and Marish, 2000).

Notwithstanding the fact that T₃ is the biologically active thyroid hormone, the effects of hypothyroidism in animals are reversed better by combinations of T₃ and T₄ than either alone (Escobar-Morreale et al 1995, Escobar-Morreale, et al 1996). Similarly, in patients with hypothyroidism receiving adequate doses of T₄, substitution of 12.5ug T₃ for 50ug T₄ has beneficial effect on neuropsychological function without altering serum TSH concentrations. (Bunevicius et al, 1999). These differences probably result from differences in cellular entry of the two hormones and the differential effects on deiodinase activity. They also suggest that the small (20 percent) thyroidal contribution of T₃ to overall T₃ production is important.
Diagram 2.6: Schematic outline of extracellular and intracellular translocations of \( T_4 \) and \( T_3 \) and their actions in target cells.

2.9.1 Effect of thyroid hormones on protein synthesis

\( T_3 \) stimulates the synthesis of many structural proteins, enzyme, and hormones. (Jameson and De Groot, 1995). The consequences of this action are most obvious in the decreased neural and somatic growth that accompanies thyroid deficiency in infants and children. The increase in protein synthesis is due primarily to increased gene transcription, but proliferation of the ribosomal constituents involved in protein synthesis, increased translational efficiency, and perhaps increased amino acid transport may also be involved.
2.9.2 Thermogenic action
Energy released by the oxidation of substrate is stored by the formation of ATP or liberated as heat. Thyroid hormone stimulation of thermogenesis can be demonstrated both in vivo and in isolated tissues such as muscle, liver, and kidney (Guernsey and Edelman, 1983). The degree of stimulation correlates with the number of $T_3$ nuclear receptors in most tissues, except in the brain, which contains receptors but in which $T_3$ has no thermogenic action.

2.9.3 Cardiovascular action
Thyroid hormones increases heart rate, cardiac contractility and cardiac output. They also promote vasodilation, which leads to enhanced blood flow to many organs. The increase in contractility is at least in part due to increased transcription of the myosin heavy-chain alpha gene, leading to an increase in a myosin isoform and contracts rapidly, and in calcium ATPase, which accelerates muscle relaxation (Ojamaa and Klein, 1998).

2.9.4 Action on lipids
Thyroid hormones stimulate both lipogenesis and lipolysis, thereby providing increased quantities of fatty acids for oxidation to generate the ATP used for thermogenesis and other energy consuming action (Beylot et al, 1991).
2.9.5 Action on carbohydrate metabolism
Thyroid hormones stimulate almost all aspects of carbohydrate metabolism, including enhancement of insulin-independent entry of glucose into the cells and increased gluconeogenesis and glycogenolysis to generate free glucose (Moller 1996).

2.10 Thyroid disorders
Thyroid disorders may either be in form of under or excessive hormone secretion, or may present as goiter, which is an enlargement of the gland caused by diffuse enlargement or due to one or several nodules in the gland. In considering the prevalence of thyroid dysfunction, a distinction needs to be made between so-called subclinical and overt abnormality; a distinction that is based on laboratory rather than clinical criteria. Before reliable tests of thyroid function became available, several thyrotoxicosis and hypothyroidism were sometimes life-threatening disorders, but thyroid storm or myxodema coma now have become uncommon, a change that is probably the result of widespread diagnostic testing in clinical practice.
The Whickham study, first reported in 1977 from an iodine replete region in Northern England (Tumbridge, 1977), showed a prevalence of 1.9-2.7% overt thyrotoxicosis and 1.4-1.9% overt hypothyroidism in women, with progressive increase with age; prevalence in male was much lower. Estimates of subclinical dysfunction were 4-5 folds higher, with about 10% of women over 50 showing an increase in serum TSH, again with progressive increase with age (Tumbridge, 1997)
2.10.1 Hyperthyroidism (thyrotoxicosis)

The term hyperthyroidism, although often used synonymously with thyrotoxicosis, refers to excessive T\textsubscript{3} and T\textsubscript{4} synthesis and secretion by the thyroid gland. Thyrotoxicosis strictly is the clinical, physiologic, and biochemical syndrome that result when tissues are exposed to excessive concentration of thyroid hormone.

Hence hyperthyroid patients have thyrotoxicosis, but thyrotoxicosis has causes other than hyperthyroidism. (Hefand and Redfern, 1998, Vanderpump et al, 1995).

Thyrotoxicosis is usually categorized as overt or subclinical. Overt thyrotoxicosis is defined as high serum T\textsubscript{4} and T\textsubscript{3} concentrations and low serum TSH concentrations, most patients have symptoms and signs of thyrotoxicosis. Subclinical thyrotoxicosis is defined as normal serum T\textsubscript{4} and T\textsubscript{3} concentrations and low TSH concentrations, most patients have no symptoms and signs of thyrotoxicosis. (Okamura et al, 1997)

Various studies have shown that among patients with overt thyrotoxicosis, women outnumber men by 4-8 to 1, largely independent of the cause. The cause is Graves’ disease in 60 to 85 percent, toxic nodular goiter in 10 to 30 percent, toxic thyroid adenoma in 20 to 50 percent, and some type of thyroiditis in the remainder (Williams et al 1993, Berglund et al 1998, Brownlie and Wells, 1999). The frequency of toxic multinodular goiter and toxic adenoma varies the most, being higher in areas of lower (but not truly deficient) iodine intake (Williams et al, 1993). Most patients with Graves’ disease are 30 to 60 years old, whereas those with toxic multinodular goiters or toxic thyroid adenoma are 40 to 70 years old (Reinwein and Benker 1998)
Thyrotoxicosis is caused by unregulated release of T₄ and T₃ from the thyroid gland, or the ingestion of excessive amounts of T₄, T₃ or both. It may be caused by increased T₄ and T₃ synthesis and release due to intrinsic thyroid disease, excessive TSH or theoretically excessive TRH secretion, or the production of other thyroid-stimulating hormones, such as TSH receptors-stimulating autoantibodies and choronic gonadotropin. It may also be caused by destruction of thyroid tissue, with release of stored T₄ and T₃. Compensatory increases in T₄ and T₃ secretion otherwise called secondary hyperthyroidism may occur in patients who have accelerated degradation or increased urinary excretion of T₄ and T₃ or peripheral resistance to the actions of T₄ and T₃ but they do not have thyrotoxicosis (Nicolof et al, 1992). Increased extrathyroidal T₄ conversion to T₃ should not cause thyrotoxicosis, because any substantial increase in extrathyroidal T₃ production would be expected to decrease TSH secretion and therefore decrease thyroidal T₄ and T₃ secretion. (Nicolof et al, 1992)

Some patients with thyrotoxicosis have high serum T₃ concentration and normal serum T₄ concentrations. This is called T₃ thyrotoxicosis; in these patients, T₃ production is increased relative to that of T₄ even more than is the case in the usual patients with thyrotoxicosis. It is most common in patients whose thyrotoxicosis is due to a toxic adenoma or Graves’ disease, but it may be due to hyperthyroidism of any cause. The abnormality responsible for it is probably relative intrathyroidal iodine deficiency (Engler and Burger 1994). Few patients with thyrotoxicosis have high serum T₄ but normal T₃ concentration (T₄ thyrotoxicosis). In addition to thyrotoxicosis, these patients have another serious illness or have recently received a drug
that inhibits extrathyroidal conversion of T4 to T3. The biochemical findings of T4 thyrotoxicosis therefore are as a result of excessive thyroidal T4 and T3 secretion concomitantly impaired extrathyroidal conversion of T4 to T3 (Caplan et al, 1990).

The common symptoms and signs of thyrotoxicosis include nervousness, palpitation, profuse sweating, fatigue and weakness, loss of weight despite increased appetite, and in some, an increased protrusion of eyeballs (exophthalmos) (Martin and Deam 1996, Trivalle et al, 1996). The frequency and severity vary substantially among patients, and clinical severity is poorly correlated with serum T4 and T3 concentrations. None is specific for thyrotoxicosis, although the combination of increased appetite and weight loss is nearly so. Factors that influence the clinical severity of thyrotoxicosis include the rate of onset, the age of the patient, and the vulnerability of different organ system to excess thyroid hormone action. (Philip, 2001). Thyroid enlargement of some type is common, in Graves’ disease; both thyroid lobes are usually diffusely and more or less symmetrically enlarged.

2.1.2 Graves’ disease

Graves’ disease is an autoimmune disease characterized by the presence of antibodies that stimulate thyroid hormone synthesis and secretion. These antibodies are IgG immunoglobulin that binds to the extracellular domain of the TSH receptors on thyroid follicular cells, thereby activating the receptors; they are therefore TSH-stimulating antibodies that cause
hyperthyroidism by mimicking the action of TSH. (Weetman and McGregor 1994)

TSH receptors-stimulating antibodies stimulate thyroid growth as well as thyroid function, acting via receptors linked to adenyl cyclase (Rees et al, 1998). Some of the antibodies also stimulate the phospholipase C-Phosphoinositide pathway in thyroid cell; patients whose serum activates this pathway tend to have larger goiter than those whose serum does not. (Di Cerbo et al, 1995)

Many patients with Graves’ thyrotoxicosis have high serum concentrations of other antithyroid antibodies. They include antibodies against thyroid peroxidase, thyroglobulin, and Na/I cotransporter. (Moris and Berget 1997). In terms of quantity of antibody protein, the concentration of TSH receptor-stimulating antibodies are much lower than the concentrations of antithyroid peroxidase antibodies. (Jaume et al, 1997)

2.1.3 Hypothyroidism

Hypothyroidism is the clinical, physiologic, and biochemical syndrome that results from decreased thyroid hormone production by the thyroid gland. Hypothyroidism is usually categorized as overt or subclinical. Overt hypothyroidism is defined as low serum T4 and T3 concentrations and, in nearly all patients, high TSH concentrations; most patients with overt hypothyroidism have symptoms and signs of hypothyroidism. Subclinical hypothyroidism is defined as normal serum T4 and T3 concentrations and high serum TSH concentrations, most of these patients have no symptoms and signs of hypothyroidism. (Tunbridge et al, 1977), Canaris et al, 2000).
Hypothyroidism is common; the prevalence rate of overt hypothyroidism is about 1 per 1,000 in community survey and 2 to 5 per 1,000 in patients seeking medical care (Helfand and Redfern 1998). The prevalence rate of subclinical hypothyroidism is 20 to 100 per 1,000 persons in community (Tunbridge et al 1977, Canaris et al, 2000, Helfand and Redfern 1998, Vanderpump et al,1995). The higher rates occur in older women, most of whom have high serum antithyroid peroxidase antibody concentrations (Vanderpump et al, 1995). The ratio of women to men with either overt or subclinical hypothyroidism ranges from 2 to 8 to 1 (2-8:1) and increases with age. Hypothyroidism may develop suddenly or gradually, it may be transient or persistent, and it may be of little importance or life threatening (Myxedema coma)

The daily rate of \( T_4 \) production in patients with overt hypothyroidism is about 25 percent of normal. The daily production rate of \( T_3 \) is decreased less, and the proportion of \( T_3 \) that originates in the thyroid is increased and the proportion that originates in extrathyroidal sites is decreased. (Nicolof et al 1992, Bianchi et al, 1984)

There are several defenses against hypothyroidism, the most important is TSH. Its secretion increases in response to very small decreases in thyroid secretion. In patients with little thyroid damage or iodine deficiency, the increase in TSH secretion may restore thyroid hormone secretion to normal or near normal, in so doing causing only thyroid enlargement, TSH stimulates the secretion of \( T_3 \) in preference to \( T_4 \) in two ways; it stimulates the synthesis of \( T_3 \) more than that of \( T_4 \), and it stimulates thyroidal deiodinase activity (Laurberg, 1984; Ishii et al, 1983). Therefore, thyroidal
T3 secretion decreases less than that of T4, and the thyroidal contribution to overall T3 production increases. Other mechanisms that minimize the development of hypothyroidism include an increase in the fraction of T4 that is converted to T3 at extrathyroidal sites, particularly in tissues rich in type 2 deiodinase (Lum et al, 1984). When hypothyroidism is caused by thyroid disease, it is called primary hypothyroidism, this occurs in more than 95 percent of the patients, when the hypothyroidism is caused by pituitary or hypothalamic disease it is called secondary hypothyroidism or central hypothyroidism. (Lum et al, 1984). The clinical manifestations of hypothyroidism are highly variable, depending on the cause, duration, and severity. The spectrum of hypothyroidism extends from subclinical hypothyroidism to overt hypothyroidism to myxedema coma. The features of hypothyroidism include slowing down of metabolism, mental dullness, weakness and fatigue, dry skin, cold intolerance, weight gain, infertility, monorrhagia, galactorrhoea periorbital edema and delayed reflexes. (Zulewski et al, 1997) Older patients have fewer symptoms and signs, and in both young and old the correlation between clinical and biochemical manifestations of hypothyroidism is poor (Doucet and Trivalle, 1994) Spontaneously occurring hypothyroidism (e.g. that due to chronic autoimmune thyroiditis) usually develops very slowly, and as thyroid secretion declines, the resulting rise in TSH secretion limit further decline in thyroid secretion. Thus, the symptoms and signs of hypothyroidism usually develop very slowly.
2.1.4 Chronic autoimmune thyroiditis (Hashimoto’s Disease)

Chronic autoimmune thyroiditis is the most common cause of hypothyroidism in persons living in regions of iodine sufficiency (Weetman et al, 1990). Also known as chronic lymphocytic thyroiditis, it exists in an atrophic (non goitrous) form and a goitrous form. The atrophic form has been called idiopathic hypothyroidism, primary myxedema, or primary thyroiditis, and the goitrous form Hashimoto’s disease. The two forms differ clinically only in the presence or absence of goiter.

Among patients with either form of chronic autoimmune thyroiditis, nearly all have high serum antithyroid peroxidase antibody concentrations, and most have thyroglobulin and Na/I co-transporter antibodies (Mariotti et al, 1990, Endo et al 1996, Raspe and Costagliola, 1995).

Likes Graves’ disease, thyroid cells from patients with goitrous autoimmune thyroiditis express HLA class II molecules. Therefore, the thyroid cells, along with other antigen-presenting cells, may activate T helper cells, which then stimulate B cells to produce the different antibodies. (Raspe and Costagliola 1995). This leads to destruction of the thyroid cells and, eventually thyroid failure (hypothyroidism). The enlargement of the gland in these cases is the consequence of increased TSH, an attempt to restore the supplies of thyroid hormone to normal.
2.1.5 Sub-clinical thyroid disease

With the advent of serum thyrotropin (TSH) radioimmunoassay in the 70s, the entity of mildly elevated TSH and normal serum thyroid hormone levels are recognized, the introduction of the second and third generation sensitive TSH in the 1980s identified the entity of subclinical hyperthyroidism in which serum TSH is suppressed and serum thyroxine $T_4$ and triiodothyronine $T_3$ levels are normal and subclinical hypothyroidism in which serum T4 and T3 are normal and serum TSH elevated. (Vanderpump et al, 1996)

2.1.6 Subclinical hyperthyroidism

Subclinical hyperthyroidism is defined as a persistently suppressed serum thyrotropin with normal thyroxine and triiodothyronine in patients who do not have symptoms (Kek, et al, 2003). This condition reflects the fact that before the clinical features of thyrotoxicosis is apparent, the thyrotrophs usually respond to minor increments in thyroid hormone concentrations, which remain within the normal range, by switching off the production and secretion of thyrotropin (Snyder and, 1972). An absence of symptoms was once part of the definition of subclinical hyperthyroidism, but it is now understood that subtle symptoms or signs of thyrotoxicosis may be present. (Toft, 2001)

Subclinical hyperthyroidism can be caused by the same thyroid disorder that results in clinical hyperthyroidism. Suppressed thyrotropin levels may occasionally result from non-thyroidal causes. The most common cause of
subclinical hyperthyroidism is excessive thyroid hormone therapy (Kek, et al, 2003). Recent Colorado Thyroid Disease Prevalence study involving 25,862 subjects showed a prevalence of 2.1%, Josept et al studies a representative population of 17,353 people aged 12 and above and found that the prevalence of subclinical hyperthyroidism was only 7%. Subclinical hyperthyroid subjects with initial suppressed but detectable TSH levels tend to return to normal on follow-up while those with undetectable TSH remained unchanged with a small risk of progression to frank hyperthyroidism (Parle et al 1991). The rate of progression to overt hyperthyroidism has been estimated to be 5% per year with subjects with autonomous thyroid adenoma and nodular goiter (Wiesinga, 1995).

The clinical significance of subclinical hyperthyroidism thus relates to three risk factors, progression to overt hyperthyroidism, cardiac effects and skeletal effects. The American Association of Clinical Endocrinology had recommended treatment in patients with symptoms of hyperthyroidism, atrial fibrillation, or unexplained weight loss and also women with osteopenia (AACE Thyroid Task Force 2002).

However, Biondi et al, (2001) and Sgarbi et al, (2000) suggested early treatment of persistent endogenous subclinical hyperthyroidism not only in older but also young and middle-age patients to improve their quality of life and avoid consequences of long term exposure of cardiovascular system to small increase in the thyroid hormone (Samuel, 1998).

In view of the relatively high prevalence of unrecognized hyperthyroidism in older adults, especially women, an expert panel of the American Thyroid Association has recommended routine screening of adults for thyroid disease
by measurement of serum thyrotropin (Ladenson *et al*, 2000), such screening will inevitably identify patients with undetectable serum thyrotropin concentrations but normal thyroxine and triiodothyronine concentrations, although such findings are low. (Toft 2001).

### 2.1.7 Subclinical hypothyroidism

Subclinical hypothyroidism is the term used to describe patients with normal thyroxine (T4) and raised thyroid stimulating hormone (TSH) concentration who do not have symptoms (Vanderpump *et al*, 1996).

The findings of slightly elevated TSH and normal thyroid hormone level do not necessarily imply the presence of subclinical hypothyroidism. Several medications and conditions are known to cause an elevation in TSH. Such drugs such as sulfonylureas, Lithium, amiodarone, ethionamide, phenylbutazone, aminoglutethimide, and iodine can interfere with thyroid hormone production or release and secondarily result in a slight elevation of TSH. Large population studies have suggested that the prevalence of subclinical hypothyroidism is much higher in women than in men and increases with age (Kek *et al* 2005). In the Whickham Survey, TSH levels above 6miu/l were approximately three times more common in females (7.5%) than in males (2.8%) and occurred more frequently in females over 45 years of age. TSH levels also showed a progressive increase with age in women but not in men (Wang and Crapo 1997).

There is also a strong association between positive antithyroid antibodies and elevated TSH. Generally the prevalence of elevated TSH level parallels that of antibody positively (Wang and Crapo 1997). A high prevalence of
antibodies was found in a UK study where antibodies were present in 81% of those with TSH concentration over 10μl/l, 46% of those with TSH over 5μl/l and less than or equal to 10μl/l and only in 5.7% of those whose TSH concentration was less than 0.5μl/l (Parle et al, 1991). Interestingly, the National Health and Nutrition Examination Survey III (NHANES) survey found a significant association between anti-thyroid peroxidase antibody with hypo or hyperthyroidism and not thyroglobulin antibody (Hallowell et al, 2002).

After 20 years of follow-up of subjects in the Whickham Survey, the risk of overt hypothyroidism was found to be 4.3% per year in women with elevated TSH and antithyroid antibodies at baseline. This is a 38 times increased risk over normal women. Moreover, an isolated elevation in TSH or presence of antithyroid antibodies alone at baseline According to a 2004 consensus statement from American Thyroid Association, the American Association of Clinical Endocrinologists, and Endocrine Society, the also confirmed an increased risk of overt hypothyroidism (2.6% per year and 2.1% per year respectively) (Vanderpump et al, 1995). Progression to hypothyroidism was noted to be more common in those with initial TSH values greater than 10μl/l and in those with positive anti-thyroid antibodies (Parle et al, 1991).

The potential benefits and risks of therapy for subclinical hypothyroidism have been debated for two decades. The positive advantage of treating subclinical hypothyroidism generally include, firstly, preventing the progression to overt hypothyroidism. Secondly, thyroxine therapy may improve the serum lipid profile and thereby potentially decrease the risk of
death from cardiovascular causes. Finally, treatment may reverse the symptom of mild hypothyroidism, including psychiatric and cognitive abnormalities (Cooper 2001).

Because the majority of persons with subclinical hypothyroidism have few symptoms or none at all, routine population screening has been advocated (Danese et al 1996). Population screening has not been endorsed unanimously, because the benefits of subsequent therapy have not been established in prospective clinical trials (Cooper 2001). Using a decision and cost-effectiveness model, it was calculated that screening women older than 35 years of age every five years would cost $9,200 per quality-adjusted year of life. Although screening is controversial Allan et al believed that it is warranted every five years in women older than 35 years of age, given the high prevalence, potential consequences, and cost of treatment of the disorder. (Allan et al, 2000)

Sub clinical hypothyroidism is determined on the basis of serum TSH level. normal range of TSH concentrations falls between 0.45-4.5mu/l. Patients with levels greater than 10mu/l are considered to have overt hypothyroidism, and patients with serum TSH values between 4.5mu/l and 10mu/l belong to sub clinical hypothyroidism class.

2.11 Hyperthyroidism and pregnancy
Numerous hormonal changes and metabolic demands occur during pregnancy, resulting in profound and complex effects on thyroid function. As thyroid disease are, much more prevalent in women during the child bearing period(than in men), it is not surprising that thyroid disorders such
as Grave’s disease, hypothyroidism, chronic thyroditis, etc are relatively common in pregnancy. The major cause of hyperthyroidism in women of childbearing age is Graves’ disease, even though the frequency of the disorder is relatively low, occurring in only 0.5 to 2 per 1000 pregnancies (Glinor 1997). It constitutes an important clinical entity that has been the subjects of several excellent reviews in recent years (Becks and Burrow 1991; Mestman et al 1995).

When the diagnosis of Graves’ disease has not been established before the start of pregnancy, the disorder is not always readily suspected clinically, mainly because the symptoms and signs of mild to moderate hyperthyroidism may be mimicked by the hypermetabolic state of normal pregnancy (Innerfield and Hollander 1977).

The increased hormone-binding capacity of the serum (due to the rise in TBG in the first trimester) tends to decrease the free fraction of the thyroid hormones, and hence the free hormone concentrations (Amino et al 1982). Also due to the immune suppression associated with the pregnancy state, there is a progressive decrease in the titer of thyroid-stimulating antibodies, as gestation progresses. If hyperthyroid is not detected and adequately treated, fetal repercussion are observed with significantly higher frequency of preeclampsia, premature labour, low birth weight, fetal and prenatal loss (Mastman et al 1995).

2.12 Hypothyroidism and fertility
There is a known association between hypothyroidism and decreased fertility, which, in most cases, is associated with primary ovulatory
disturbances and not with abortion (Glinor 1997). Hypothyroidism causes anovulation, and, most women with hypothyroidism are diagnosed with the condition for the first time during fertility evaluation. Observations in human species are confirmed by animal investigations showing an association between experimentally induced hypothyroidism and menstrual cycle dysfunctions (Peterson 1994). Normal thyroid hormone blood levels are essential for growth and development of tissues and for the maintenance of tissue and organ function. Changes in thyroid hormone levels can adversely affect fertility, pregnancy outcome, and postnatal development in humans and animals; with major effects on growth, hearing, mental acuity, and reproductive system development and function in the offspring. (Gloria et al, 2004).

Women with hypothyroidism tend to have low serum estradiol concentrations, because hepatic production of sex hormone-binding globulin is decreased (Brenta, et al, 1999), but their serum free estradiol concentrations are normal. The secretion of estradiol and progesterone is decreased, as is their clearance. Pulsatile FSH and LH secretion is usually normal but there is no ovulatory surge. (Tamosi et al, 1997) The women therefore tend to have anovulation and irregular cycles, infertility, and excessive menstrual bleeding.

Among women with hypothyroidism who do become pregnant, the frequency of preeclampsia, placental abruption, and post partum hemorrhage is increased, and the risk of abortion, stillbirth, or premature delivery are increased (Glinoer, 1997, Davis and Leveno, 1998). In successful pregnancies, the growth of the fetus and the subsequent growth and
development of the child may be slowed if the mother is not treated (Glinoer et al, 1991)

In male, hypothyroidism is the common disorder of testicular function presenting with either impairment of testosterone production or androgen deficiency, which is nearly always associated with an impairment of spermatogenesis and erectile dysfunction. (Philip and Lawrence, 2001). Serum testosterone concentration may be low because production of sex hormone-binding globulin is decreased. Serum free testosterone, FSH and LH concentrations are usually normal, but may be low. (Jannini et al 1995, Donnelley and White, 2000)

2.13 Hypothyroidism and pregnancy outcome
Hypothyroid women who become pregnant carry an increased risk for obstetrical complications such as intrauterine fetal demise, gestational hypertension, placental abruption, and poor perinatal outcome. (Wasserstrum and Anania 1995). There are indications that thyroid hormone administration greatly improves, although it does not entirely suppress the frequency of these abnormalities (Greenman et al, 2001). In general, infants of hypothyroid mothers appear healthy without evidence of thyroid dysfunction. In infants born to hypothyroid mothers, some studies have indicated the risk of a higher perinatal mortality and congenital malformations, and there is also evidence for an increased frequency of low birth weight (Montore et al, 1981, Lowe 1991, Mestman et al, 1995), and a concern about potential long-lasting pyschoneurological impairment in the progeny (Man et al, 1991).
Hypothyroidism in adult female humans and rats is associated with altered menstrual and estrous cycles and interference with gestation, usually during the first trimester (humans) or first half (rodents) of pregnancy. In humans, increased abortion and stillbirths are noted, whereas in rodents, increased abortion, stillbirths, and reduced litter sizes are observed. Hypothyroidism in adult female humans and rats is associated with altered level of LH (Gloria et al, 2004). The most common cause of primary hypothyroidism in young women is chronic autoimmune thyroiditis, which occurs in both goitrous and atrophic forms. The incidence of hypothyroidism in infertile women has been estimated to be less than 1 per cent. Stephen et al (1999) reported a prevalence rate of 2.3 per cent in 704 women with at least one year of infertility (Stephen et al, 1999).

Thyroid hormones are critical for development of the fetal and neonatal brain, as well as for many other aspects of fetal growth. Hypothyroidism in either the mother or fetus frequently results in fetal disease. Serum $T_4$ and $T_3$ concentrations increase progressively throughout the first trimester of pregnancy to values 30-50 per cent higher than those in non pregnant women and in men, and then remain constant during the second and third trimester (O’Leary et al, 1992, Glinoer, 1997). The increase is due primarily to a 75-100 percent increase in TBG concentrations. Pregnancy increases the need for iodine, because maternal thyroid hormone synthesis and renal iodide clearance are increased and some iodide is lost to the fetus. (Berghout and Wiesinger, 1998).

Thyroid deficiency during the latter thirds of gestation and the first months after delivery can result in mental retardation and sometimes neurological
deficits. During the middle and last trimesters, thyroid hormone is supplied by both the mother and the fetus but mostly the mother. This is most evident in the fate of infants with sporadic congenital hypothyroidism. Most of these infants are normal at birth, and even among those with no thyroid secretion, umbilical-cord serum thyroxine concentrations at birth are 25 to 50 per cent of normal; an indication that substantial transplacental passage of maternal thyroxine has taken place (Vulsma et al, 1989). However, if infants with congenital hypothyroidism are not identified and treated very soon after birth, when they become dependent on their own thyroid secretion, they will become permanently mentally retarded. Hypothyroidism in newborns (known as congenital hypothyroid) occurs in one in every 3,000 to 4,000 births, making it the most common hormonal disorder in infant. In 90 percent of these cases, it persists throughout life (Utiger 1999). These observations are the underpinnings of the neonatal screening programs that have largely eliminated congenital hypothyroidism as a cause of mental retardation in many countries.

The Importance of adequate maternal thyroid secretion is also evident from the studies in regions of endemic iodine deficiency. When dietary iodine intake is low, both pregnant women and their fetuses have poor thyroid function throughout gestation (Glinor, 1997). The consequences of combined maternal and fetal hypothyroidism for the infants are not only mental retardation but also neurological defects - spasticity, ataxia, and deaf-mutism that do not occur in infants with sporadic congenital hypothyroidism. When the iodine deficiency is less severe, the infants have only mental retardation (Delange 1994). These abnormalities can be
prevented by increasing the mother’s intake of iodine, but it must be increased at the beginning of the second trimester, if not sooner. (Xue et al, 1994).

Haddow et al (1999) provided additional evidence that harm to the fetus results from hypothyroidism in pregnant women in the United States (Haddow et al, 1999). Among 62 children whose mothers had hypothyroidism in the second trimester of pregnancy, the full-scale IQ score and the scores on several subtests at seven to nine years of age were slightly lower than in 124 children, whose mothers had normal thyroid function during pregnancy, and more had difficulties in school or had repeated a grade. Many of the women with hypothyroidism had high serum antithyroid peroxidase antibody concentrations, which indicated chronic autoimmune thyroiditis, and many later had clinically apparent hypothyroidism. (Pop et al, 1999, Man et al, 1991, Haddow et al, 1999). Their results led Haddow et al to suggest that pregnant women be screened for hypothyroidism, by measurement of serum thyrotropin. In surveys of a total of nearly 14,000 pregnant women in Japan, Belgium, and the United States, 0.3 percent, 2.2 percent, and 2.5 percent, respectively, had high serum thyrotropin concentrations (Glinor, 1997, Kamijo et al, 1990). Most of these women had subclinical hypothyroidism (high serum thyrotropin and normal serum thyroxine concentrations), rather than overt hypothyroidism (high serum thyrotropin and low serum thyroxine concentrations), and many had evidence of chronic autoimmune thyroiditis. Thus, the frequency of hypothyroidism varies among pregnant women in different countries.
Based on recent evidence of significant intellectual impairment in the offspring of women who were even mildly hypothyroid early in pregnancy, testing of thyroid function may become the standard practice as early as possible in pregnancy, or in women who intend to become pregnant (Smallridge and Ladenson, 2001). Early diagnosis and treatment is crucial, because foetal brain development in the first trimester is influenced by maternal thyroid function and follow-up studies indicate significant intellectual impairment in the offspring of women who are mildly hypothyroid during pregnancy (Pop et al, 1999; Haddow et al, 1999).

It is likely that both chronic autoimmune thyroiditis and iodine deficiency contribute to the occurrence of hypothyroidism in pregnant women in many countries. Before routine screening of hypothyroidism in pregnant women is introduced, efforts should be made to increase dietary iodine intake, by ensuring, for example, that all prenatal vitamins and, indeed, all vitamin products contain iodine, by increasing the amount of iodine added to salt, or by adding iodine to other foods. The beneficiaries would be not only pregnant women and their offspring, but everyone (Utiger, 1999).

### 2.14 Autoimmune thyroid disease and pregnancy

Autoimmune antibodies have been reported in apparently healthy population and are observed more frequently in women during their reproductive years (Geva et al, 1997). It has been suggested that immunological factors may play an important role in the reproductive processes of fertilization, implantation and placental development (Billingham and Head 1981). Women have a high degree of immunological responsiveness, which is
reflected by their increased susceptibility to non-organ specific and organ specific autoimmune antibody (Chiovato et al, 1993). Such increased susceptibility is supported by the fact that thyroid antibodies have been associated with an increased risk for pregnancy loss (Stagnaro-Green et al, 1990). Recent studies have suggested an association between autoimmune factors and reproductive wastage (Gleicher et al, 1989).

The association between thyroid autoimmunity and the risk of miscarriage has recently been examined in three comprehensive review articles. In the review by Poppe and Glinor published in 2003, the available information from thirteen studies comparing the risk of a miscarriage with the presence (versus the absence) of thyroid autoimmunity clearly led the authors to the conclusion that thyroid autoimmunity (without overt thyroid dysfunction) was significantly associated with a 3-5 fold increase in overall miscarriage rate (Poppe and Glinor 2003). In more recent review by Stagnaro-Green and Glinor published in 2004, a more detailed classification was carried out by examining separately (a) the association between miscarriage and thyroid autoimmunity (five studies); (b) the association between recurrent miscarriage and thyroid autoimmunity (seven studies), and finally (c) the association between early pregnancy loss after in vitro fertilization and thyroid autoimmunity (five studies). Overall, and with few exceptions all studies documented a statistically significant relation between thyroid autoimmunity and increased pregnancy loss (Stagnaro-Green and Glinor, 2004).
2.15 Evaluation of thyroid function

Evaluation of thyroid function is not simple because it depends on interaction among several glands—the hypothalamus, the anterior pituitary and the thyroid. No single test is really suitable for a definite diagnosis of thyroid function. A combination of tests is usually adopted for appropriate diagnosis. Over the past forty years, improvements in the sensitivity and specificity of thyroid test methodologies have dramatically impacted the clinical strategies for detecting and treating thyroid disorders. In the 1950s only one thyroid test was available—an indirect estimate of the serum total (free + protein bound) thyroxine (TT₄) concentration, using the protein bound iodine (PBI) technique (Helfand and Redfern, 1998). Since 1970, technological advances in radioimmunoassay (RIA) and immunometric assay (IMA) methodologies have progressively improved the specificity and sensitivity of the methods (Landenson et al 2000). Currently, serum-based methods are available for measuring both total (TT₄ and TT₃) and free (FT₄ and FT₃) thyroid hormone concentrations. (Tunbridge and Vanderpump, 2000).

Measurements can also be made of the thyroid hormone binding proteins, Thyroxine Binding Globulin (TBG), Transthyretin (TTR)/Prealbumin (TBPA) and Albumin, as well as for the pituitary thyroid stimulator, Thyrotropin (thyroid Stimulating hormone, TSH) and the thyroid hormone precursor protein, Thyroglobulin (Tg). (Stunkey et al, 2001, Jenkins and Weetman, 2002).

There is some controversy on the relative value of clinical and laboratory evaluations of thyroid function. O’Reilly (2000) has expressed the view that
clinical criteria are being side-lined while biochemical assessments lack specificity. However, this author considered TSH and T4 individually, rather than in the feedback relationship between trophic hormone and the target gland product that is fundamental to endocrine diagnosis. In response to this position, several authorities emphasized the lack of sensitivity of clinical features in detecting thyroid disease, while conceding that optimal assessment requires both clinical and laboratory input (Price and Weetman, 2000; Toft and Beckett, 2000). Both overt thyrotoxicosis and hypothyroidism can have important consequences before the usual clinical features are obvious, and clinicians often fail to recognize diagnostic features even when they are present (Eggertsen, et al, 2001).

2.15.1 Total hormone measurements (TT\(_4\) and TT\(_3\))

Technically it has been easier to develop methods to measure the concentrations of total (free + protein bound) thyroid hormone (TT\(_4\) and TT\(_3\)) that circulate at nanomolar concentrations, in contrast to the free hormones (FT\(_4\) and FT\(_3\)) that are measured in picomolar range. The protein binding iodine (PBI) tests of the 1950s were replaced first by competitive protein binding methods in the 1960s, and later superseded by radioimmunoassay (RIA) methods in the 1970s (Lumberg 1999). Currently TT\(_4\) and TT\(_3\) concentrations are primarily measured by non-competitive immunometric assay (IMA) methods that use radioactivity, enzymes, fluorescence or chemiluminescent molecules as signals (Ritter et al, 1993). The diagnostic accuracy of total hormone measurements would equal that of free hormone if all patients had similar binding protein concentrations.
Unfortunately serum TBG abnormalities that distort the total /free hormone relationship are commonly encountered in clinical practice. Additionally some patients have abnormal thyroid hormone binding proteins or autoantibodies that render total hormone measurements unreliable (Nordyke, 2000). Consequently, TT₄ and TT₃ measurements are rarely used as stand-alone tests, but are employed in conjunction with a binding protein estimate test (i.e. thyroid hormone binding ratio (THBR) to form a free hormone index.

2.15.2 Free hormone measurement (FT₄ and FT₃)
In accord with the free hormone hypothesis, it is believed that the minute free fraction of hormone (0.02% versus 0.2%, FT₄ versus FT₃, respectively) is responsible for the biologic activity of thyroid hormones at cellular level (Nordyke, 2000). It follows that free hormone measurement will better reflect the physiologic effects of the hormone than total hormone measurements, especially when binding proteins are abnormal. (Nelson et al, 1994)

Free hormone measurements (FT₄ and FT₃) are made either by two-test index methods or single-test methods that include reference techniques that employ physical separation of free from bound hormone, and immunoassay “sequestration” method that are usually automated. The single-test methods are either standardized with gravimetric preparations or use calibrators with values assigned by a reference method. Most clinical laboratories now use one step competitive immunoassay that measure free hormones as a function of fractional occupancy of hormone-antibody binding sites in the reaction
mixture (Danese, 1996). The impetus of free hormone test development has been high frequency of binding-protein abnormalities encountered in clinical practice.

Considerable confusion still surround abnormalities of pregnancy and non-thyroidal illness (Stockigt, 2000; Stathatus and Wartofsky the nomenclature of free hormone tests and controversy continues regarding the technical validity of measurements themselves, and their clinical utility in conditions associated with binding protein 2003).

2.15.3 Serum thyroxine concentrations (T4)

Serum $T_4$ concentrations are high in patients with thyrotoxicosis and those with high serum concentration of TBG and other binding proteins. Rarer causes of high serum $T_4$ concentrations include the presence of autoantibodies that bind $T_4$ and decrease the $T_4$ clearance. Thus, the serum $T_4$ concentrations can be high in patients who are euthyroid or even hypothyroid.

Conversely, serum $T_4$ concentrations are low in patients with hypothyroidism. They are also low in patients who have low serum concentrations of TBG and other binding proteins or who are receiving drugs that inhibit the binding of $T_4$ to TBG, patients receiving $T_3$, and those who have serious nonthyroidal illnesses.

Misinterpretation usually can be avoided by simultaneous determination of serum thyroid hormone binding capacity or direct measurement of serum free $T_4$. 

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2.15.4 Serum triiodothyronine concentration (T3)
Serum T₃ concentrations, like those of T₄, are altered by changes in thyroid secretion and changes in serum thyroid hormone-binding proteins. In patients with thyrotoxicosis serum T₃ concentration usually are increased to a greater degree than are serum T₄ concentrations; in patients with hypothyroidism, they are more often within the normal range than are serum T₄ concentrations.
The major, if not only, indication for measurement of serum T₃ is in patients with clinical manifestations of thyrotoxicosis who have normal serum T₄ concentration (Chopra, 1998). Its determination has uncovered a variant of hyperthyroidism in thyrotoxic patients with elevated T₃ levels and normal T₄ often referred to as T₃ toxicosis (DeGroot 1990).

2.15.5 Serum free thyroxine concentrations (FT4)
Measurements of serum free T₄ are gradually replacing measurements of serum free T₄ index in clinical laboratories. The immunoassay for serum free T₄ provide accurate estimates of serum free T₄ concentrations in patients with nonthyroidal illness and those with abnormalities in binding proteins, particularly those that use a T₄ analog that may not bind to the abnormal binding protein (Nelson et al, 1994).
Alterations in the concentration of serum binding proteins will generally result in a corresponding change in total T₄ concentrations while the physiologically active free T₄ level remain largely unchanged in euthyroid individual. Therefore, determination of free T₄ concentration provides a more accurate assessment of thyroid status than total T₄ measurement.
Elevated free T4 concentrations are indicative of hyperthyroidism and low levels are indicative of hypothyroidism. The choice of free T4 measurement in preference to total T4 is to improve the diagnostic accuracy for detecting hypo- and hyperthyroidism in patients with thyroid hormone binding abnormalities that compromise the diagnostic utility of total hormone measurements.

2.15.6 Serum free triiodothyronine concentration (FT3)
Serum free T3 concentrations can be measured directly or indirectly (as the serum T3 index) in the same way as used in measuring serum free T4 concentrations. Measurement of serum free T4 has no clinical advantage and is therefore rarely indicated (Chopra, 1998).

2.15.7 Serum thyrotrophin measurement (TSH)
Measurement of serum TSH provides a very precise indication of availability of T4 and T3 to the pituitary in normal subjects and patients with thyroid disease (Bauer and Brown, 1996). For case finding among patients not suspected of having any abnormality of thyroid secretion or evaluating clinically euthyroid patients with thyroid nodular disease, measurement of serum TSH is the most appropriate test. Very few patients with normal serum TSH concentrations have abnormal thyroid secretion (Nordyke et al, 1998).

Serum TSH concentrations in normal subjects range from approximately 0.3 to 3.0 mu/liter. Serum TSH concentration are high in patients with hypothyroidism when it is due to thyroid disease and are normal or low
when it is due to hypothalamic or pituitary disease. Serum TSH concentrations are low or undetected in patients with thyrotoxicosis, excepting those few patients with TSH-dependent thyrotoxicosis. (Bauer and Brown 1996). The change in serum TSH concentration in patients with thyroid disease is continuous, so they range from very high in patients with overt hypothyroidism to very low in patients with overt thyrotoxicosis. Changes in serum T<sub>4</sub> and T<sub>3</sub> concentrations of as little as 15 to 25 percent caused by changes in thyroid secretion are sufficient to rise or lower serum TSH concentration from within to outside the normal range. On the other hand, in patients with a normal thyroid gland in whom TSH secretion is not normally inhibited by excess T<sub>4</sub> and T<sub>3</sub>, for example those with TSH-secreting pituitary adenomas or those with generalized resistance to thyroid hormone, serum TSH concentration in the upper range of normal are sufficient to raise T<sub>4</sub> and T<sub>3</sub> concentrations to as well above their respective normal ranges. Because of the sensitivity of TSH secretion to small changes in thyroid secretion, an abnormal serum TSH concentration usually indicates the presence of an abnormality in thyroid secretion. Conversely, a normal serum TSH concentration usually provides strong evidence against the presence of any abnormality of thyroid secretion. (Nordyke et al, 1998).

### 2.15.8 Serum thyroglobulin concentrations

Thyroglobulin is a molecule produced by the thyroid cells and stored in the thyroid colloid. The primary function of thyroglobulin is the storage and synthesis of thyroid hormones. Thyroid hormones are synthesized on thyroglobulin, which subsequently serves in the synthesis of T3 and T4
Serum thyroglobulin concentrations are high in patients with thyrotoxicosis, except patients with thyrotoxicosis caused by exogenous thyrotoxicosis. (Spencer et al 1998). Thus, measurements of serum thyroglobulin can be used to differentiate spontaneously occurring thyrotoxicosis from exogenous thyrotoxicosis.

In hypothyroid patients, measurements of serum thyroglobulin can be used to distinguish those who have no thyroid tissue from those in whom thyroid hormonogenesis is abnormal but thyroid tissue is present.

Serum thyroglobulin measurements are most useful in the follow-up of patients with thyroid carcinoma who have undergone thyroidectomy and radioiodine ablation of any remaining tissue (Spencer et al, 1998).

2.15.9 Serum thyroid hormone-binding ratio (THBR)

Because nearly all the T\textsubscript{4} in serum is bound to TBG or other proteins, the extent of protein binding must be determined to interpret the result of serum T\textsubscript{4} measurements properly. This is done most simply by determination of the thyroid hormone-binding capacity, also known as the T\textsubscript{3}–resin uptake.

The test measures the number of unoccupied protein binding sites for T\textsubscript{4}. In normal serum, the unoccupied protein-binding sites, primarily TBG, take up about 55 to 75 percent of the labeled T\textsubscript{3} and 25 to 45 percent is bound to the resin uptake. Thus, a serum T\textsubscript{4} value must be interpreted in conjunction with the THBR. If both the THBR and the serum T\textsubscript{4} concentration are high or if both are low, thyroid secretion is altered. If two results are discordant, an abnormality of binding protein is likely.
The product of the serum T₄ value and THBR is the serum free T₄ index. Serum free T₄ index value usually correlate well with the values obtained by direct measurement of serum free T₄.

2.15.10 Thyrotropin-releasing hormone stimulation test
TRH testing is usually done by intravenous administration of 400 or 500ug of TRH. In normal subjects, serum TSH concentration increase two to eight fold to peak values of 5 to 25mu/liter, 20-30 minutes after TRH administration (Utiger 1986).

2.15.11 Test for antithyroid antibodies in serum
The recognition that autoimmunity is a major cause of thyroid dysfunction has led to the development of tests for thyroid autoantibodies-thyroid peroxidase antibodies (TPOAb), thyroglobulin antibodies (TgAb) and TSH receptor antibodies (TRAb). Autoimmune thyroid disorder is characterized by the presence of antithyroid antibodies, specifically antithyroglobulin and antithyroid peroxidase. Thyroglobulin (Tg) is a molecule produced by the thyroid cells and stored in the thyroid colloid. Thyroid peroxidase is an enzyme responsible for iodination of tyrosine residues along with coupling of iodinated residues to form thyroid hormones. All patients with autoimmune thyroid disease have T-cells in their blood and within the thyroid gland, which recognize the specific thyroid molecules-thyroglobulin, thyroid peroxidase and thyroid stimulating hormone receptor. Some of the T-cells are able to kill “self” thyroid cells and activate B-cells to secrete antibodies, which bind to these same thyroid molecules (O’Conner
Antibodies to thyroid peroxidase, formally known as thyroid microsomal antigen, are present in high concentrations in the serum of most patients with chronic autoimmune thyroiditis, especially those with the goitrous form of the disease (Hashimotos disease) and many patients with Graves’ disease. (Moriotti et al, 1990). In Hashimoto’s disease, the goiter is caused by an accumulation of white blood cells and fluid in the thyroid gland, which leads to destruction of the thyroid cells and eventually thyroid failure and decreased thyroid hormone production (hypothyroidism). Low concentration of these antibodies also are found in patients with most other thyroid diseases; patients with other autoimmune diseases, such as pernicious anaemia, idiopathic Addison’s disease, and type I diabetes mellitus, and in normal subjects, particularly elderly women. (Moriotti et al 1990, Szabolcs et al, 1995, Vander pump et al, 1995).

2.16 Laboratory Diagnosis of Thyroid Disorder

2.16.1 Diagnosis of thyrotoxicosis

The biochemical hallmarks of thyrotoxicosis are high serum total and free T4 and T3 concentration and low serum TSH concentration. (Trzepecz et al, 1989) The correlation between the clinical and biochemical severity of thyrotoxicosis is poor, and therefore judgment of its severity should be based on clinical findings.

The initial step should be measurements of serum TSH and serum free T4, either as the serum free T4 index or serum free T4 concentration. If the serum free T4 value is high and serum TSH concentration is low, the diagnosis of
thyrotoxicosis is confirmed and no further tests are needed. If the serum free $T_4$ value is normal and serum TSH concentration is low, the patient may have $T_3$ thyrotoxicosis or subclinical thyrotoxicosis. The two possibilities can be distinguished by measurement of serum $T_3$. Also, a normal serum free $T_4$ concentration and suppressed serum TSH concentration is suggestive of suclinical hyperthyroidism.

A high serum free $T_4$ value and a normal serum TSH concentration indicate the presence of familial dysalbuminemic hyperthyroxinaemia (FDH), TSH dependent thyrotoxicosis, or generalized resistance to thyroid hormone. Patients with familial dysalbuminemic hyperthyroxinaemia are enthyroid, and the diagnosis can be confirmed by finding a normal or nearly normal serum $T_3$ concentration, a low THBR, and high serum free $T_4$ index values and serum $T_4$ concentrations and normal serum TSH concentration in family members. (Utiger, 1986)
Serum TSH
+
Serum free T4 (FT4)

TSH

FT4

TSH normal

FT4 normal

Thyrotoxicosis

T3-toxicosis

FDH

Normal

TSH normal

FT4 or normal

FT4 normal

Subclinical Thyrotoxicosis

(FDH- Familial Dysalbuminemic Hyperthyroxaemia)

Table 2.2 Laboratory Diagnosis of Thyrotoxicosis
2.16.2 Diagnosis of hypothyroidism

Measurement of serum TSH is an indispensable test for the recognition of primary hypothyroidism and the differentiation of primary form central (secondary) hypothyroidism, whether caused by pituitary or hypothalamic disease (Kateuo et al 1985). Serum TSH concentrations are high in all patients with overt primary hypothyroidism and, by definition, in all patients with sub clinical hypothyroidism. The initial step should be measurements of serum TSH and free T4 index or serum free T4. If the serum TSH concentration is high and the serum free T4 value is low, the diagnosis of overt hypothyroidism is confirmed. If the serum TSH concentration is normal or low and the serum free T4 value is low, the diagnosis is central (secondary) hypothyroidism or nonthyroidal illness. Because most of the T3 in serum is produced as a result of extrathyroidal deiodination of T4, serum T3 concentrations are usually low in patients with hypothyroidism. However, measurement of serum T3 is neither a specific nor sensitive test for hypothyroidism. (Staub and Althaus, 1992).
Table 2.3  Clinical interpretation of thyroid function tests

<table>
<thead>
<tr>
<th></th>
<th>TSH</th>
<th>T₃</th>
<th>Free T₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Subclinical hypothyroidism</td>
<td>↑</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Subclinical hyperthyroidism</td>
<td>↓</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Overt hypothyroidism</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Overt hyperthyroidism</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>T₃ thyrotoxicosis</td>
<td>N</td>
<td>or ↑</td>
<td>N</td>
</tr>
</tbody>
</table>

(N = Normal, ↑ = Increased level, ↓ = Decreased level)

2.16.3  Choice of initial test

The definitive diagnosis of thyroid disease should always be made using the typical relationship between trophic hormone and target gland secretion that define endocrine dysfunction. (Ercan-Fang et al, 2000; Stockigt, 2000). In contrast, case finding studies generally begin with a single test. Previous discussion about the relative value of TSH and free T4 measurement as single initial tests has swung in favour of TSH because of its superior sensitivity in detecting the earlier stages of thyroid disease (Danese et al, 1996). Thus, the assessment of untreated subjects now begins with measurement of TSH alone, with free T4 and T3 assay added only if TSH is abnormal; it is self-evident that serum TSH loses its diagnostic value when pituitary function is abnormal, serum free T4 then becomes front runner test (Wardle et al, 2001)
A number of current guidelines recommend the use of TSH as the first line-test for detecting both hypo- and hyperthyroidism in ambulatory patients with stable and intact hypothalamic/pituitary function (Samuel et al, 2003). This strategy is considered more cost effective than a panel approach (TSH+FT4 or FT4 +FT3) but necessitates the use of TSH assay with functional sensitivity below 0.02mIU/l (Maxon and Smith, 2003). Third generation sensitivity (<0.02mIU/l) is critical for detecting subnormal TSH values, since less sensitive second generation assays are prone to produce falsely negative (normal range) values for sera with subnormal TSH concentrations (Jenkins, 2002). There are some clinical situations in which assessment of thyroid function will give a high prevalence of abnormalities that cannot be interpreted with certainty. Notably, glucocorticoids and dopaminergic agents have a potent effect to suppress TSH secretion (Surks and Sievert, 1995), while TSH is also frequently subnormal in starvation or caloric deprivation. Transient increase above normal can occur in euthyroid subjects during recovery from critical illness. (Stockigt, 2001). A serum free T4 estimate will generally follow from abnormal TSH value, but during critical illness, free T4 estimate often show non-specific abnormalities. This lack of specificity is the basis for a recommendation against routine assessment of serum TSH and free T4 during acute illness in the absence of risk factors or clinical features, suggestive of a thyroid disorder (Stockigt, 1996). If serum TSH is used as a single initial test for case finding, a value outside the reference interval should lead to estimation of serum free T4 on the same sample. This requires an algorithm of protocol, which should also include
measurement of serum T3 if TSH is suppressed, in order to identify T3 toxicosis.

Guidelines from American College of Physicians for detection of thyroid disease now recommend routine measurement of TSH in women over 50 years, the group most likely to have overt or sub clinical thyroid dysfunction (Helfed and Redfern, 1998). This approach depends on the application of TSH assay sufficiently sensitive to detect the earliest stages of either thyrotoxicosis or primary hypothyroidism. A normal TSH value in ambulatory patients without associated disease of pituitary dysfunction has a high negative predictive value in ruling out both primary hypothyroidism and thyrotoxicosis (Danese, 1996), which suggests that free T4 need only be routinely estimated if TSH is abnormal. However, it was further suggested that after a normal TSH value, re-testing is probably not required for 5 years (Denase, 1996). Recent American Thyroid Association guideline extend these indications to recommend that all adults have their serum TSH concentrations measured, beginning at the age of 35 and every 5 years thereafter (Maxon and Smith, 2003). Almost all developed countries now have routine neonatal programs for congenital hypothyroidism using heel prick filter paper blood spot. The value of such programs is clear, but it is notable that neonatal screening is not yet routine in numerous developing countries where the prevalence of neonatal hypothyroidism may be high (LaFranchi, 1999), often associated with iodine deficiency (Bhatara et al, 2002).
Table 3.1  Thyroid function profile – clinical classification

<table>
<thead>
<tr>
<th>Reference Range</th>
<th>T&lt;sub&gt;3&lt;/sub&gt; 0.6– 1.8 ng/ml</th>
<th>FT&lt;sub&gt;4&lt;/sub&gt; 0.8–2.0 ug/dl</th>
<th>TSH 0.4–6.0 IU/ml</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6–1.8</td>
<td>0.8 – 2.0</td>
<td>0.4 – 6.0</td>
<td></td>
<td>Euthyroid</td>
</tr>
<tr>
<td>&gt; 1.8</td>
<td>&gt;2.0</td>
<td>&lt;0.4</td>
<td></td>
<td>Overt Hyperthyroidism</td>
</tr>
<tr>
<td>&lt;0.6</td>
<td>&lt;0.8</td>
<td>&gt;6.0</td>
<td></td>
<td>Overt Hypothyroidism</td>
</tr>
<tr>
<td>0.6 – 1.8</td>
<td>0.8 – 2.0</td>
<td>&lt; 0.4</td>
<td></td>
<td>Subclinical Hyperthyroidism</td>
</tr>
<tr>
<td>0.6 – 1.8</td>
<td>0.8 – 2.0</td>
<td>&gt; 6.0</td>
<td></td>
<td>Subclinical Hypothyroidism</td>
</tr>
</tbody>
</table>

Standard reference from where the results were interpreted and classified. Adapted from Philip 4<sup>th</sup> Edition (2001) Endocrinology and Metabolism.
CHAPTER THREE

3.0 Materials and method

3.1 Subjects
Between February 2005 and November 2006, 217 women between the ages of 18 and 45 years attending fertility clinic in UNTH, Enugu, Nnamdi Azikiwe University Teaching Hospital, Nnewi and Regina Caeli Hospital, Awka, were screened for thyroid dysfunction. The Clinicians attending to the patients were first approached with the proposal for the study which embodied the aims and objectives and the importance of the study. The recruitment criteria were based on those women who were living with their husbands and having regular, unprotected sex for one year or more without pregnancy. The consultant Gynecologists recommended those who certified the inclusion criteria after reviewing their folder. Patient’s consent was obtained before the test. The results of the tests were made available to the Clinicians for the benefit of the patients.

Thirty six parous women within the same age limit were also enlisted as control.

3.2 Sample collection
Three milliliters of blood samples were collected from the patients and introduced into plain dry test tube, samples were centrifuged for 5 minutes at 3,000 revolutions per minute and serum removed into clean dry bottle.

Samples were assayed in batches by Enzyme-Linked Immunosorbent Assay (ELISA) procedure using DRG International (USA) Kits.
All abnormal results were confirmed by repeat testing.

The following tests were performed on each sample

Triiodothyronine (T₃). - Reference range 0.6 – 1.8ng/ml

Free Thyroxine (FT₄) - Reference range 0.8 – 2.0ug/dl

Thyroid Stimulating Hormone (TSH) - Reference range 0.4 – 6.0uIU/ml

**Assay Procedure** – As directed by the manufacturer of the kits.

### 3.3 Principle of T₃ ELISA test

The principle of T₃ ELISA procedure is based on Competitive Enzyme Immunoassay. A second antibody (goat anti-mouse 1gG) is coated on microtiter wells. A measured amount of patient’s serum, a certain amount of mouse monoclonal anti - T₃ antibody, and a constant amount of T₃ conjugated with horseradish peroxidase are added to the microtiter wells and incubated for 60minutes. During incubation, the mouse anti- T₃ antibody is bound to the second antibody on the wells, and T₃ and conjugated T₃ compete for the limited binding sites on the anti- T₃ antibody.

After the 60 mins incubation at room temperature, the wells were washed 5 times with water to remove unbound T₃ conjugate.

A solution of Tetramethylbenzidine (TMB) reagent (Substrate) was then added and incubated for 20 minutes, resulting in the development of blue colour.

The colour development was stopped with addition of stop solution, (1N HCL), and the absorbance was measured spectrophotometrically using microplate Reader at 450nm.
The intensity of the colour formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled T\textsubscript{3} standards assayed in the same way.

### 3.4 Principle of FT\textsubscript{4} ELISA

The Free T\textsubscript{4} test is also a solid phase competitive enzyme immunoassay. Patients serum sample, standards, and thyroxine-enzyme conjugated working reagent are added to wells coated with monoclonal T\textsubscript{4} antibody, Free T\textsubscript{4} in the patients specimen and the T\textsubscript{4} labeled conjugate compete for available binding sites on the antibody.

After 60 minutes incubation at room temperature, the wells are washed with water to remove unbound T\textsubscript{4} conjugate. A solution of H\textsubscript{2}O\textsubscript{2}/TMB is then added and incubated for 20mins, resulting in the development of blue colour. The colour development is stopped with the additions of 3N HCL (stop solution), and the absorbance measured spectrophotometrically at 450mm with microplate reader.

The intensity of the colour formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabelled FT\textsubscript{4} in the sample.

### 3.5 Principle of TSH ELISA

The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determination on the intact TSH molecule. Mouse monoclonal anti-TSH antibody is used for solid phase immobilization (on
the microtiter wells). A goat anti TSH antibody is in the antibody-enzyme (Horseradish peroxide) conjugate solution.

The test sample is allowed to react simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies.

After 60 minutes incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB reagent is added and incubated for 20 minutes, resulting in the development of blue colour. The colour development is stopped with the additions of stop solution, changing the colour to yellow which is measured spectrophotometrically at 450nm wavelength using microplate reader. The concentration of TSH is directly proportional to the colour intensity of the sample.

3.6 Assay procedure

1. All reagents and samples were allowed to attain room temperature.
2. The required number of microtitre wells were secured and placed in the strip holder.
3. 100 ul of standard samples and controls were placed into appropriate wells.
4. 100 ul of enzyme conjugate reagent was added in each well.
5. The wells were mixed thoroughly and incubated for 60 minutes at room temperature.
6. The wells were washed 5 times with distilled water and drained with absorbent paper to get rid of any residual water.

7. 100 ul of TMB reagent (substrate) was added into each well and incubated for 20mins at room temperature.

8. 100 ul of stop solution was added into each well to stop the reaction.

9. Using Hyperion Microreader 4 plus, the tests were read at 450 nm wavelength.

3.7 Statistical methods used

The following statistical methods were done.

1) Comparison of T3, FT4 and TSH values of the test group and the control to determine if there is any significant difference. Since the tests were measured in different units, we used t-test to compare each of the independent test values at 0.05 level of significance.

2) Computation of confidence interval to determine if the upper and lower bounds of the control and test groups are within or outside the standard reference ranges.

3) Computation of correlation (r) values of various test parameters to determine their relationship.
CHAPTER FOUR

4.1 Results
Out of 217 infertile women studied 213 (98.16%) had normal serum T3, FT4 and TSH levels (euthyroid), a total of 4 (1.84%) had abnormal thyroid hormone profile. Out of these abnormal subjects 3 (1.38%) had raised serum TSH, normal T3 and FT4, while 1 (0.46%) had raised serum T3 and FT4 and suppressed TSH (overt hyperthyroidism). Thirty six women studied as control had their thyroid hormones within normal reference ranges.

The mean value of T3 test group (ng/ml) was 1.21 for patients (± 0.348), and 1.18 (± 0.322) for controls. Statistically, there was no significant difference between the means (P> 0.05). The mean value of FT4 (ug/dl) was 1.38 (±0.29) for patients and 1.42 (± 0.324) for controls. There was no significant difference between the mean values (P>0.05). The mean value of TSH (uIU/ml) was 2.22 (± 1.308) for patients and 1.56 (± .0.70) for controls. Statistically, there was a significant difference between the mean values (P<0.05) (see table and graphs).

The confidence interval for both the control and test group of all the parameters were within their standard reference ranges. The control of T3 (1.07-1.28) and FT4 (1.31-1.53) had wider confidence interval when compared with their test groups- T3 (1.17-1.26), FT4 (1.34-1.42). The confidence interval of TSH showed smaller range and the upper bound of the control (1.32-1.80) is much smaller than the lower bound of the test group (2.05-2.40) (see table and graphs).
All correlations showed weak negative relationship with the pairs at 0.05 level of significant. T3 and FT4 (r = -0.048; P=0.445), FT4 and TSH (r = -0.107; P=0.088), but T3 correlated positively –significantly with TSH = (r= -0.138; P= 0.028) (*see table and graphs*).

Table 4.1. **Summary of result obtained**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients Studied</td>
<td><strong>217</strong></td>
</tr>
<tr>
<td>Normal serum T3, FT4, TSH</td>
<td><strong>213</strong> (98.16%)</td>
</tr>
<tr>
<td>Raised TSH with normal T3, FT4</td>
<td><strong>3</strong> (1.38%)</td>
</tr>
<tr>
<td>Low TSH with raised T3, FT4</td>
<td><strong>1</strong> (0.46%)</td>
</tr>
<tr>
<td>Control- Normal TSH, T3, FT4</td>
<td><strong>36</strong> (100%)</td>
</tr>
</tbody>
</table>
Table 4.2  Comparison of T3, fT4, and TSH values of patient and controls and the control

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>t-value</th>
<th>df</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test group</td>
<td>217</td>
<td>1.212</td>
<td>0.348</td>
<td>0.636</td>
<td>251</td>
<td>0.525</td>
</tr>
<tr>
<td>Control</td>
<td>36</td>
<td>1.175</td>
<td>0.322</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>FT4</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test group</td>
<td>217</td>
<td>1.377</td>
<td>0.290</td>
<td>-0.809</td>
<td>251</td>
<td>0.419</td>
</tr>
<tr>
<td>Control</td>
<td>36</td>
<td>1.419</td>
<td>0.324</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TSH</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test group</td>
<td>217</td>
<td>2.222</td>
<td>1.308</td>
<td>2.960</td>
<td>251</td>
<td>0.003</td>
</tr>
<tr>
<td>Control</td>
<td>36</td>
<td>1.561</td>
<td>0.700</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significant at p<0.05
Table 4.3  **Confidence Interval of test group and the control**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lower – Upper Bounds</th>
<th>Standard Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T3</strong> (ng/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>1.17 – 1.26</td>
<td>0.6 – 1.8</td>
</tr>
<tr>
<td>Control Group</td>
<td>1.07 – 1.28</td>
<td>0.6 – 1.8</td>
</tr>
<tr>
<td><strong>FT4</strong> (ug/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>1.34 – 1.42</td>
<td>0.8 – 2.0</td>
</tr>
<tr>
<td>Control Group</td>
<td>1.31 – 1.53</td>
<td>0.8 – 2.0</td>
</tr>
<tr>
<td><strong>TSH</strong> (uIU/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>2.05 – 2.40</td>
<td>0.4 – 6.0</td>
</tr>
<tr>
<td>Control Group</td>
<td>1.32 – 1.80</td>
<td>0.4 – 6.0</td>
</tr>
</tbody>
</table>
### Table 4.4: Correlations

<table>
<thead>
<tr>
<th></th>
<th>T3 (ng/ml)</th>
<th>FT4 (ug/ml)</th>
<th>TSH (uIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation</td>
<td></td>
<td>r = -0.138</td>
<td></td>
</tr>
<tr>
<td>P = value</td>
<td></td>
<td>0.028</td>
<td></td>
</tr>
<tr>
<td><strong>FT4</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation</td>
<td>r = -0.048</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P = value</td>
<td>0.445</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TSH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation</td>
<td>r = -0.107</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P = value</td>
<td>0.088</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Correlation is significant at 0.05 levels.
Figure 4.1: Correlation of FT$_4$ and T$_3$ of infertile women

$r = -0.048$, $P > 0.05$
Figure 4.2: Correlation of T3 and TSH of infertile women.

$r = -0.138$, $P<0.05$
Figure 4.3 - Correlation of FT4 and TSH of infertile women.

$r = -0.107, P>0.05$
5.0 CHAPTER FIVE

5.1 DISCUSSION

Our data suggests that screening for thyroid disorder as part of routine infertility evaluation may yield few positive results. We evaluated 217 infertile women and found a prevalence of 1.84%. Out of these abnormal subjects, 1.38% had evidence of sub-clinical hypothyroidism, while 0.46% had evidence of overt hyperthyroidism. The confidence interval of T3 and FT4 tests were found within the confidence bound of the control group; this indicates that they are not very sensitive tests for evaluation of thyroid disorder. The TSH test showed a narrow confidence interval, the upper bound of the control (1.32) is lower than the lower bound of the test group (2.40). This indicates that the TSH test is a sensitive assay for thyroid evaluation as a patient with a test value higher than the upper bound of the control will readily indicate thyroid dysfunction (sub clinical thyroid disorder), even when T3 and FT4 values are within their respective reference ranges. It further confirms that determination of serum or plasma TSH level is a sensitive method for diagnosis of thyroid disorder.

Correlations coefficient (r ) values were negative for all the pairs (T3 vs FT4, T3 vs TSH , and  FT4 vs TSH). The correlation was not significant for T3 vs FT4 and FT4 vs TSH (P>0.05) but was significant for T3 vs TSH (P<0.05). It is a well known fact that the secretion of TSH is inhibited by serum T3 and FT4 levels by negative feedback mechanism (Rapport and Chazenbalk, 1998), the weak negative correlation implies that as serum T3 and FT4 levels increase, the serum TSH decreases and vice versa. Furthermore, T3 (which binds more avidly to thyroid receptor) is produced principally (80%) from
deiodination of T4 at extrathyroidal level, the negative correlation of T3 with T4 implies that T3 and T4 tests are independent (non-linear) and cannot be substituted for each other in evaluation of thyroid disorder, hence patients with T3 thyrotoxicosis have high serum T3 but normal FT4 concentration (Nicoloff et al, 1972). In this situation, the increase in production rate of T3 is characteristically greater than those of T4. Conversely, a few patients with T4 thyrotoxicosis have high serum T4 but normal T3 concentration (T4 Thyrotoxicosis, Caplan et al, 1980).

The data obtained in this study is similar to those reported by various study groups: Stephen et al (1999) screened 704 infertile women and found 16 (2.3%) elevated TSH levels (hypothyroidism). Shavler et al (1994) reviewed 444 women and found 3 (0.7%) evidence of clinical or sub clinical hypothyroidism. In a report of Strickland et al (1999), 8 of 210 (4%) infertile patients were found to have sub clinical hypothyroidism. Bohnet et al (1991) studied 150 patients with anovulation and reported 20 (13.3%) sub clinical hypothyroidism. Glinor (1995) investigated 1,990 pregnant women who attended prenatal clinic for the first time between June 1990 and December 1992 and found 41 with elevated TSH, thus yielded an overall prevalence of 2.2%.

Most women with thyroid disorder are diagnosed with the condition for the first time during a fertility evaluation. Glinor, (1994) reported that women with thyroid hypotrophy who incidentally become pregnant presumably have a sufficient functional reserve for thyroid gland to function adequately before gestation (hence allowing them to become pregnant), but not after establishment of the pregnant state. Because majority of persons with sub
clinical hypothyroidism have few symptoms or not at all, routine population screening has been advocated (Danese et al, 1996). Some experts go further, then and propose that screening be done on any woman who is planning a pregnancy to help determine those who may be at risk for hypothyroidism and if needed, begin treatment as early as possible during the critical first trimester. Based on recent evidence of significant intellectual impairment in the offspring of women who were even mildly hypothyroid early in pregnancy, testing of thyroid function may become standard practice on women who intend to become pregnant or as early as possible in pregnancy (Smallridge, 2001). However, population screening has not been endorsed unanimously, because the benefits of subsequent therapy have not been established in prospective clinical trials (David, 2001).

In view of high cost of immunoassay reagents used for estimation of thyroid hormones and given the fact that routine thyroid screening will yield few positive results, we do not recommend thyroid function screening as part of routine workup for women with infertility. However, in view of the grave consequences of underlying thyroid diseases on reproductive outcome, and because it has been established that greater percentage of cases of infertility associated with abnormal thyroid function are in sub clinical class (they have few symptoms or not at all), we suggest inclusion of thyroid function screening using “initial test model” (i.e. single test) by measuring serum TSH level (only) in isolated cases. Stephen et al (1999) suggested that in view of low percentage rate of positive result that thyroid function be evaluated only when infertility is accompanied with ovulatory dysfunction. TSH assay has historically been defined by its clinical sensitivity-the ability
to discriminate between hyperthyroid and euthyroid values (Mariotti, 1990). This model is cost effective and will help identify those women whose infertility are due to abnormal thyroid function. If the screening and classification of patient with potential thyroid disease is to be based on serum TSH, there should be consensus on the reference range for the parameter. If the serum TSH is used as a single initial test for case-finding, a value outside the reference range should lead to estimation of serum free T4 on the same sample, if FT4 is within the reference range, then measurement of T3 is indicated in order to identify T3 thyrotoxicosis. Stockigt (2000) concluded that a normal TSH concentration has high negative predictive value in ruling out primary thyroid disease; this assay has become increasingly used as the single initial test for thyroid function (Beckett and Toft, 2003), with further assay done routinely if serum TSH is outside the reference range to distinguish between overt and sub clinical thyroid disorder. However, consensus is yet to be achieved on the reference range for the TSH. Monzani (2003) reported that the upper limit of the ‘normal range’ for serum TSH is dependent on the analytical method and on the study population. There is need to establish our local reference range for various parameters of thyroid function tests to increase the sensitivity of the results especially the TSH which is used for classification of patients with potential thyroid disorder. Stephen et al, (1999) screened 701 infertile women by evaluating their serum TSH level using 0.45-4.09 mIU/ml as reference range. A higher upper reference limit is cited for most current TSH assay kits (0.4-6.0mIU/ml). Knudsen et al (2002) reported that TSH reference interval should be established from 95% confidence limit of log
transformed value of at least 120 rigorously screened normal euthyroid volunteers who have no detectable thyroid antibodies, no personal or family history of thyroid dysfunction and no visible or palpable goiter, for this reasons the author suggested a reference interval of 0.4-4.0mIU/L. The new TSH upper limit of 2.5mIU/L is supported by the data from Whickham follow-up that found an increased risk of hypothyroidism in individuals with serum TSH<2.0mIU/L

5.2 Conclusion
This work shows that routine screening for thyroid disorder on infertile women will yield few abnormal results, therefore, we do not recommend thyroid function screening as part of routine work up for all infertile women. However, if infertility is accompanied with anovulation, thyroid function should be evaluated by measuring the serum TSH level, a value outside the reference range should lead to estimation of serum FT4 and T3.

5.3 Limitations of the study
Thyroid hormone assay is very expensive and therefore can hardly be employed as routine test for women during fertility evaluation. Consensus is yet to be achieved on the reference range for the parameters for effectiveness of laboratory assay for case finding and screening.
5.4 **Recommended for further study**

We recommend large population study to determine our local reference range for various parameters of thyroid hormone assay. We also recommend antithyroid antibody assay in future evaluation studies to determine the prevalence of autoimmune antibody.
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