A STUDY ON THYROID FUNCTION STATUS IN NEWLY DIAGNOSED POLYCYSTIC OVARIAN SYNDROME PATIENTS

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BONAFIED CERTIFICATE

This is to certify that this dissertation work entitled "**A Study on Thyroid Function Status in Newly Diagnosed Polycystic Ovarian Syndrome Patients**'' is the original bonafide work done by **Dr. R. Amirtha Jansirani,** Post graduate student, Institute of Biochemistry, Madras Medical College, Chennai under out direct supervision and guidance.

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DECLARATION

I, **Dr. R. Amirtha Jansirani,** solemnly declare that the dissertation titled **"A Study on Thyroid Function Status in Newly Diagnosed Polycystic Ovarian Syndrome Patients"** is the bonefied work done by me at Institute of Biochemistry, Madras Medical College under the expert guidance and supervision of Director and Professor **Dr. K. Ramadevi,** M.D., Institute of Biochemistry, Madras Medical college. The dissertation is submitted to **The Tamilnadu Dr. M.G.R Medical University** towards partial fulfilment of requirement for the award of M.D., degree (Branch XIII) in Biochemistry.

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INDEX

ABBREVIATION

PCOS	Polycystic Ovarian Syndrome
GnRH	Gonadotropin Releasing Hormone
LH	Luteinizing Hormone
FSH	Follicular Stimulating Hormone
BMI	Body Mass Index
W/H Ratio	Waist/Hip ratio
SCH	Subclinical Hypothyroidism
T4	Thyroxine (Tetra-iodothyronine)
T3	Tri-iodothyronine
fT4	Free T4 (Thyroxine)
fT3	Free T3 (Tri-iodothyronine)
Tg	Thyroglobulin
rT3	Reverse Tri-iodo thyronine.
TRH	Thyrotropin Releasing Hormone.
TR	Thyroid hormone Receptor.
TSH	Thyroid Stimulating Hormone.
SHBG	Sex Hormone Binding Globulin.
Na/I symport	Sodium- iodide transport
DAG	Diacyglycerol
PL-C	Phospholipase-C
IP3	Inositol triphosphate.
Ca++	Calcium.
BMR	Basal Metabolic Rate
H_2O_2	Hydrogenperoxide.
OH	Hydroxyl group
KDa	KiloDalton

INTRODUCTION

The most common cause for physical and psychological stresses in post pubertal young women are menstrual disorders and infertility.

- A survey conducted by World Bank in 2013 showed that the fertility started dropping 10 years back with 17% decline every year from 2000 which was reported in a very popular magazine of India.
- In another famous journal survey, 10% of urban Indian couples in reproductive age group are infertile.

Most females with irregular menses ignore this complaint till they are criticized as infertile women. The leading causes for menstrual irregularity are Polycystic ovarian syndrome and Hypothyroidism. Polycystic ovarian syndrome is the most common endocrine disorder among the reproductive age group women [1, 2, 3, 4] and it races with other metabolic disorders resulting in complications if left untreated. PCOS is a complex disorder with chronic anovulation, hyperandrogenism and hyperinsulinemia with multiple cysts in ovaries [5]. Western population studies show the prevalence of PCOS to be about 4-8% among young women [3]. In India studies show the prevalence of 5-10% [1]. Even though the definition of PCOS is not fully established, they usually present with different complaints from simple acne to complicated infertility based on their response to the available androgen. Most of them are obese and are at risk of developing Diabetes mellitus, dyslipidemia, metabolic syndrome, cardiovascular disorders, carcinoma of endometrium or cervix [6,7, 8].

Hypothyroidism is another complicated disorder found particularly in young reproductive age group women. Hypothyroid patients may present with simple weakness and fatigue to complicated infertility. Apparently healthy young females selected as controls in studies were found to have hypothyroidism incidentally and that too in a higher percentage. This implies that many healthy looking women may have subclinical or occult and even overt hypothyroidism that has remained undiagnosed. They are identified only after developing complication or following a routine health check up. Several women with menstrual disorder or infertility attending gynaecology department were retrospectively analysed to have polycystic ovaries and / or elevated serum TSH. PCOS and hypothyroidism both together or individually adds the risk for infertility and menstrual irregularities. The burden of infertility in India is in the ascending trend and the expenditure per birth in infertile PCOS women has risen tremendously. Studies conducted in combining these two disorders show that they are significantly related. The ultimate finding in PCOS is hyperandrogenism which is aggravated in the presence of hypothyroidism. Screening for hypothyroidism in PCOS patients in our population gives a better idea about the epidemiology of this disorder and the importance of evaluating thyroid function status in them. A study conducted by Sridhar et al. also supports the concept of treating hypothyroidism first in patients having both these disorders and has found positive results by doing so. These facts and findings from various literatures stimulated us to conduct a cross sectional study about thyroid function status in newly diagnosed PCOS patients by using a single and best indicator of thyroid function status- Thyroid Stimulating Hormone (TSH).

REVIEW OF LITERATURE

Polycystic ovary Syndrome (PCOS) is the most common endocrine disorder of women in reproductive age group[1,2,3,4]. It is a heterogeneous group of disorder with Hyperandrogenism (Ovarian), Hyperinsulinemia & chronic anovulation. The most common cause of chronic anovulation and hyperandrogenism is Polycystic ovarian syndrome. The term Poly cystic ovary is actually a misnomer, as there are only multiple unruptured immature follicles. In 1935, Irving F. Stein and Michael L. Leventhal first published a case series of about 7 women with amenorrhoea, hirsutism, bilaterally enlarged ovaries, which was later known as **POLYCYSTIC OVARIAN SYNDROME (PCOS) [5].** The definition of PCOS has undergone several revisions and still inconclusive [6].

There are different nomenclatures given for this syndrome [7]:

1. Polycystic Ovarian Syndrome - PCOS
2. Chronic Hyper-androgenic Anovulation - CHA.
3. Functional Ovarian Hyper-androgenism - FOH

The presentation of PCOS in women varies from simple acne to infertility and may even be associated with disorders like Diabetes mellitus, Hypothyroidism, Dyslipidemia. This shows that the individual's response to androgen varies i.e ethnic variation exists [8]. They are at risk of developing Coronary artery disease, Metabolic syndrome, Type 2DM, Endometrial cancer [9,10,11]. More than 50% of the PCOS women are obese but can also present as lean individuals. Obese PCOS women have higher risk of developing Insulin resistance & Infertility than lean PCOS women.

However lean PCOS women have their mean hormones (Androgens, Testosterone, Estradiol) levels higher than their age matched controls.

This multi-endocrine disorder is more commonly found among teen aged girls to early thirties i.e in the early phase of reproductive age of women. Even though Hyper Insulinemia, elevated LH/FSH ratios are not included in criteria for diagnosing PCOS, many women with this syndrome were found to have associated elevation of fasting Insulin, raised LH/FSH ratio. Co-existence of Hypothyroidism in PCOS is found in many young girls with menstrual irregularities and infertile women.

Thyroid hormone sensitivity by ovaries could be explained by the presence of thyroid receptors in human oocytes.[12]

PREVALENCE STATISTICS:

TABLE : 1

World [14,15]	3.7-8.6%
Europe[13]	6.5-8%
India [1]	5-10%
Adolescent girls in India (15-18yrs)[14, 15]	9.13%
Young women in India (18-25 yrs)[14,15]	3.7%

- In UnitedStates, 10% of women visiting Gynaecology out Patient department were diagnosed to have PCOS and black women with PCOS showed defect in insulin receptor at higher level than white women [2].
- Even 20% of normal menstruating women also showed cystic ovarian morphology by UltraSonogram [17].

There exists a difference in ethnic response to the same androgen level & that seems to be the cause for different level of presentations (acne to Infertility) with same androgen level [2,8]. Among pre-menopausal women, about 10% of them with glucose intolerance were related to Insulin resistance in PCOS. This suggests that regardless of the age PCOS women are at risk of developing NIDDM [22,23]. The percentage of PCOS in infertile women has increased to 30%. [14, 18] It is also interesting to see that 75% of the women with secondary amenorrhoea fulfil the Rotterdam diagnostic criteria for PCOS.[19, 20, 21].

PATHOGENESIS OF POLYCYSTIC OVARIAN SYNDROME:

The ultimate outcome of the disease is the development of follicles on the ovary due to chronic anovulation. The core culprit for anovulation is **Hyperandrogenism,** other factors includes **Genetic & Environment.** Patients with PCOS were found to have menstrual abnormalities from the onset of menarche indicating that they have failed to establish a regular menstrual cycle from the onset of puberty. Along with cystic ovary, hirsutism, male patterned baldness, skin lesion like Acne, seborrhoea are some of the features of Hyperandrogenism [24, 25, 26] leading to Polycystic Ovarian Syndrome. The exact or the central pathogenesis is not clearly known until now. The alteration in ovarian morphology (cystic) may be due to disturbance at any one of the following level:

1) Hypothalamo-Pituitary-Ovarian axis.
2) Ovary (local)
3) Systemic disorder.

In Polycystic ovarian syndrome, the site of pathology is at ovary.

OVARY:

Anatomy & development:

Ovary, the female gonad, lies in ovarian fossa, one on either side of the lateral Pelvic wall. In nullipara, the long axis of the ovary is vertical but in multipara It is horizontal. In young girls before puberty the ovaries are Greyish pink & smooth. After puberty, it becomes Grey & have uneven surface. Each Ovary has 2 poles, 2 borders, 2 surfaces. It is divided into outer cortex & inner medulla. After puberty, the Cortex at any time shows various stages of development of Graffian follicles. Medulla has no anatomical and physiological significance. The fallopian tube is proximal to ovary. Ovum released during every menstrual cycle is picked up by the fallopian tube. If fertilisation takes place embryo is formed if not normal menstruation occurs [27,28].

Ovary is formed from genital ridge which is formed from the coelomic epithelium. Cords of cells proliferates from this germinal epithelium (coelomic) and forms mesoderm (Sex cords). Primordial germ cells of yolk sac, migrates to ovarian region & give raise to OOCTES. Sex cords are broken into small masses which is enveloped by primordial germ cells that gives rise to PRIMORDIAL FOLLICLE. Later, sex cords are replaced by cortical cords which give rise to FOLLICULAR CELLS of ovary. Developmental arrest of the ovarian follicle may result in the formation of one or more small cyst called ovarian cysts. Occasionally, ovary may also possess Thyroid tissue or Adrenal tissues [29].

7 million primordial follicles are present at the time of fetal growth. But at birth, only 2 million ova are present and the remaining follicles undergo attrition. This 2 million ova enters 1st meiotic division but gets arrested at prophase. At the time of

puberty only 3, 00,000 ova are available and are called PRIMARY OOCYTES. Others get atrophied by apoptosis.

Throughout the reproductive period of every woman, approximately 500 ova are shed during ovulation. One ovum per menstrual cycle and the others are atrophied. At the time of ovulation they complete the 1st meiotic division. This ovum released during ovulation is the SECONDARY OOCYTE. If fertilisation occurs 2nd meiotic division proceeds otherwise they are arrested in metaphase and are released [30].

Functions of Ovary:

1) To ovulate single matured ovum during every menstrual cycle.
2) To secrete Sex hormones (Androgen and estrogen & Progesterone).

A normal menstrual cycle, starts from 1st day of the current cycle to the 1st day of the next cycle usually about 28 days, but varies (25-35 days). Menstruation lasts for 3-5 (range1-8) days. Average bleeding is about 30mL (upto 80 mL).

According to one theory, the onset of menstruation is due to lysosomal membrane leak in the endometrial tissues releases some enzymes. This helps in formation of Prostaglandin from cell membrane phospholipid. The Prostaglandins produced causes local inflammatory changes like vasospasm, endometrial necrosis, bleeding and shedding of endometrial mucosa. [31]

PHASES OF MENSTRUAL CYCLE:

1) MENSTRUAL PHASE:

The first phase in the cycle, during which shedding of mucosa & bleeding takes place. 1^{st} - 5^{th} day.

2) PROLIFERATIVE/FOLLICULAR/PRE-OVULATORY-PHASE:

The second phase starts from 5^{th} – 14^{th} day of the cycle. This is the estrogen predominant part. The endometrium proliferates, new blood vessels are formed, Follicles starts maturing.

OVULATION: occurs on 14^{th} day.

3) SECRETORY / LUTEAL:

The third phase of the cycle from 14^{th} day- 28^{th} day of the cycle. In this Post-ovulatory situation, Corpus luteum is formed which produces Progesterone.

OVARIAN CYCLE:

In the beginning of the cycle, several follicles on the ovarian surface start enlarging & forms sac filled with follicular fluid. In human, one of the follicles starts growing rapidly and by 6^{th} (5-7) day it forms the DOMINANT FOLLICLE. The dominant follicle selection seems to be related to the ability of the follicle to secrete sufficient estrogen, that is needed for final maturation.

A matured Graffian follicle has Theca externa & Theca interna just below its capsule. Under Theca interna is the granulose cell layer that has FSH receptor. It secretes estrogen, under the influence of FSH released from the Anterior pituitary. Theca Interna cells has LH-Receptor that secretes Androstenidione (Androgen). This

androgen from the Theca interna enters in to the granulose cells where it gets aromatised to Estradiol [32,].

REGULATION OF OVARIAN CYCLE:

Hypothalamus, the triggering centre for hormone synthesis, release Gonadotropin Releasing Hormone (GnRH) in pulse. Anterior pituitary has gonadotrophs in which receptor for GnRH is present. Luteinizing Hormone (LH) & Follicular Stimulating Hormone (FSH) are the stimulating hormones released from anterior pituitary. Granulosa cells of ovary has receptor for FSH, Theca interna has receptors for LH. FSH stimulates the growth & early maturation of graffian follicle which in turn secretes estrogen to the maximum level. LH surge takes place 36 hrs just before ovulation. Frequency of GnRH secretion is activated by Estrogens but inhibited by Testosterone & progesterone. GnRH stimulates the production of LH.

During the early part of follicular phase in menstrual cycle Estrogen is produced in moderate amount which acts on **negative feedback centre** situated in medial hypothalamus, LH is not produced now. But in late follicular phase estrogens are produced in excess and that stimulates the **positive feedback centre** in anterior hypothalamus inducing LH production. This production reaches the peak just 36-48hrs before ovulation called mid-cycle LH surge [28, 32].

After ovulation, corpus luteum is formed it secretes mainly progesterone and a very little amount of estrogen. Progesterone predominates estrogen in the luteal phase and plays a vital role in pregnancy, if fertilization takes place. If not, it favours the Prostaglandin formation and proceed towards menstruation.

FSH: (Follicular Stimulating Hormone)

It is a glycoprotein dimer, about 35.5KD. It has 2 polypeptide subunits α & β. Like the α subunits of other hormones (LH, TSH, hCG), it is not specific. The β subunit is more specific for its biological actions and binding with its (FSH) receptor. The t½ is 3-4 hrs [34]. Serum level varies to the phase of the cycle.

Reference Interval: [33]

Follicular: 1.4- 9.9 mIU/mL

Mid-cycle peak: 0.2-17.2 mIU/mL

Luteal: 1.1-9.2 mIU/mL.

LH (Luteinizing Hormone):

Also known as Lutropin/ Lutrophin. It is a heterodimeric glycoprotein. Each monomer has one α and one β subunit not covalently associated. The structure is similar to other glycoprotein hormones. The β subunit is specific for its biological function. Its t ½ is 20 min. Serum normal level for normal menstruating young women varies according to her phase of menstruation.[34]

Reference Interval [33]

Follicular : 1.7-15.0 mIU/mL

Mid-cycle peak: 21-56.6 mIU/mL

Luteal: 0.6-16.3 mIU/mL.

GnRH: (Gonadotropin Releasing Hormone)

Also known as Luteinizing hormone Releasing Hormone (**LHRH**), It is a trophic peptide hormone, synthesised and released from GnRH neurons within pre-optic area of the hypothalamus. It occupies the first step in maintaining hypothalamo-pituitary-gonadal axis. They act through GnRH Receptor, a seven transmembrane G protein coupled receptor that stimulates phosphoinositide phospholipase C which mobilizes calcium & protein kinase C, this activates protein in the synthesis of GnRH. It has 2 seperate feedback centres in hypothalamus as mentioned earlier, positive & negative feedback centre.

SYNTHESIS OF SEX STEROID HORMONES IN FEMALES:[35, 36, 37]

SEX HORMONES: ANDROGEN

ESTROGEN

PROGESTERONE

ANDROGEN: (DHEA, DHEAS, TESTOSTERONE)

Ovarian (Theca cells): Androstenidione, Testosterone.

Ovarian source contributes to 25% of circulating Testosterone, 50% of androstenedione, and 20%of DHEA. The **marker of ovarian androgen is Testosterone.**

Function : Undergoes Aromatisation & Estrogen production and its biological effect.

Adrenal (Zona reticularis): Dehydroepiandrosterone and its sulphated form.

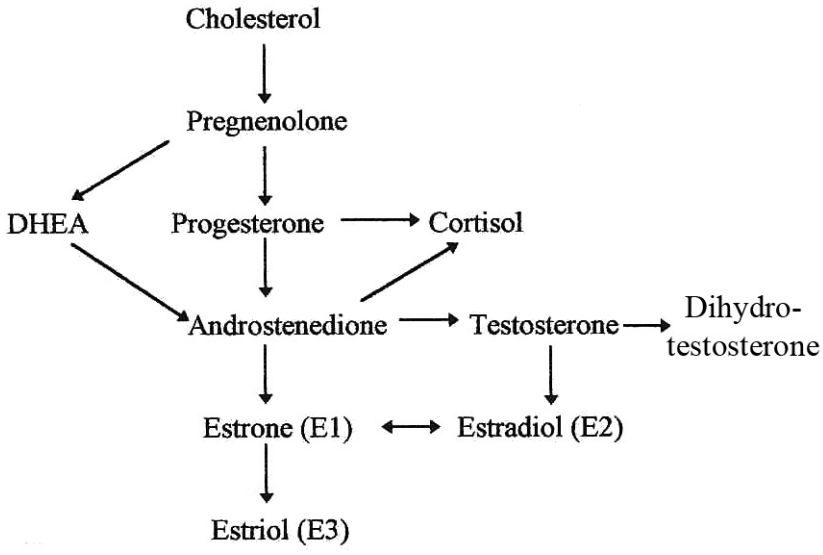
Adrenal gland contributes to 80% of DHEAS and DHEA, 50% of Androstenedione, and 25% of circulating Testosterone. It is correctly known as

didehydroepiandrosterone. It is the most abundant endogenous steroid hormone in human [30] produced by adrenal gland, gonad & brain. It is an intermediate in the biosynthesis of androgen and estrogen. It has a variety of biological effects like binding with its receptor, acting as neurosteroid [38] DHEA and DHEAS are not produced by the ovaries . The **marker of Adrenal androgen is DHEA**. Adrenal androgen is under the control of anterior pituitary (ACTH).

Function: Pubic hair & axillary hair growth (secondary sexual characters)

MOLECULAR SITE: MITOCHONDRIA

Cholesterol is the essential molecule for all steroidal compound synthesis and is supplied from (Lipoproteins) LDL Cholesterol or by de novo synthesis. This cholesterol which is in cytoplasm moves from the it and is transported to the inner mitochondrial membrane by a special transport protein called StAR –Steroidogenic Acute Regulatory Protein. The rate of steroid synthesis depends on the rate of entry of cholesterol into mitochondria rather than the Rate limiting enzyme (**11α OH lase**) [39,40]. Steroid synthesis takes place mainly in the gonads (Ovaries in female & testes in male) and to some extent in the adrenals.



TESTOSTERONE: (C19H28O2)

It is a C19 steroid is the principle androgen in men but also produced in female. Peak production is 4:00-8:00 am with a nadir between16:00-20:00hrs [41, 42]. Active form is Dihydrotestosterone (DHT) & its formation takes place in skin & prostate (male). Aromatisation of testosterone to estrogen takes place in many tissues particularly in liver and adipose tissues. Only 2-3% of Testosterone circulates freely in blood others are protein bound.[43] Sex Hormone Binding Globulin(SHBG) has high affinity but low capacity to bind testosterone (66-78%), and is the tightly bound form. Albumin has high capacity for binding Testosterone but with low affinity (20-30%) and is the weakly bound form [44, 45].

Reference Interval [51]:

Total Testosterone Adult (F): 15-70 ng/dL.

**Testosterone level > 60 ng/dL is more consistent with PCOS [47]

Free Testosterone Adult (F): 1.0- 8.5 ng/dL

Bioavailable Testosterone

It is the biologically available testosterone and includes both free and Albumin bound form and usually accounts for 35% of the Total Testosterone [41,42]. Albumin bound form easily gets disassociated from it and is available for tissue uptake. So, bioavailable testosterone correlates with free testosterone. [48, 49, 50]

Reference Interval: [51]

Bio available Testosterone Adult (F): 0.6-5.0 ng/dL.

The serum level of sex steroids shows a pattern of spiking i.e.

- It has a peak in utero by 7th week of gestation-comes to baseline at birth

- Shows again a peak by 2-3 months after birth
- Goes to very low level till puberty.

ESTROGEN [28,33]:

SOURCE: Granulosa cells of Ovary, Corpus luteum, and Placenta.

The parent compound for estrogen is Estrane: C18 compound with methyl group at C13 and aromatic ring A. All estrogen has a hydroxyl group attached at C3.

VARIOUS FORMS: E1, E2 & E3

E1: Estrone (Ketone group at C17)

E2: Estradiol (Hydroxy group at C17)

E3: Estriol.

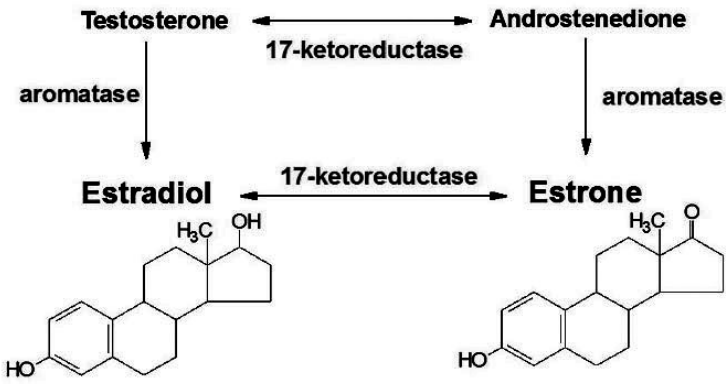
Functions:

a) Growth of graffian follicle, growth of female reproductive tract, breast.

b) Increases secretion of Thyroid binding globulin & Angiotensin,

c) It reduces cholesterol.

d) They are essential for female body configuration i.e. distribution and deposition of fat in female.



Theca Interna (ovary) is the site of androgen production and this androgen is transported down to granulose cells for aromatisation and gets converted to estrogen . Androstenedione is either directly converted into Estrone by aromatase or indirectly, gets converted into Testosterone by 17β-OH steroid dehydrogenase, then converted to 17-β Estradiol by aromatase. 17β Estrdiol is the most biologically active form of estrogen [40]. All sex steroids (progesterone, androgens & estrogens) are produced by ovary but because they lack in 21α-OH lase & 11β-OH lases, they do not produce other steroids like mineralocorticoids and glucocorticoids.

Reference Interval[51]:

12-17 yrs: 40- 410 pg/mL

Adult Female:

Early follicular phase: 20-150 pg/mL

Late follicular phase: 40-350 pg/mL

Mid-cycle: 150-750 pg/mL

Luteal phase: 30-450 pg/mL

In both sexes, sex steroids are transported through SHBG (α globulin) & Albumin. SHBG has high affinity to bind steroids; Albumin has high capacity to bind.

In female, SHBG bind 66-78% of testosterone & 40-60% of Estrogen. Albumin binds 20-30% of testosterone & 40-60% of Estrogen. A very minute amount 2-3%circulates freely (unbound).

SEX HORMONE BINDING GLOBULIN:[52, 53, 54, 55]

A homodimeric glycoprotein having 2 laminin G (LG) domain joined by a linker region. This LG domain serves like a pocket for steroid binding. The serine residue in the pocket binds with different steroid at different points androgen to A ring, estrogen to D ring. Sugar are attached at 2 different sites by N- glycosylation on Asparagine and 1 O-glycosylation on Threonine. SHBG is produced mainly by the liver other organs are brain, uterus, testes & placenta. Binding affinity for steroids are in the order of DHT> testosterone> androstenediol> estradiol> estrone. Bioavailability of sex hormones are influenced by SHBG. SHBG is influenced by factors like other plasma proteins. SHBG is increased in pregnancy, oral contraceptives, Hyperthyroidism.

SHBG is decreased in Hypothyroidism, obesity and liver diseases.

Causes of Increased SHBG

- Estrogens
- Thyroid hormone
- Pregnancy
- Estrogen-containing preparations

Causes of Decreased SHBG

- Androgens
- Synthetic progestins (norethindrone, norgestrel, desogestrel, norgestimate)

- Glucocorticoids
- Growth hormone
- Insulin
- Obesity
- Acromegaly
- Hypothyroidism
- Hyperinsulinemia

CHANGES IN HORMONES TOWARDS PUBERTY: [56, 57, 58]:

- Cortical androgen stimulating hormone starts elevating in both sexes.
- Hypothalamus and pituitary shows resistance to the negative feedback given by estrogen.
- GnRH is released in pulse in night.
- GnRH-stimulates LH, FSH production till it reaches adult level.
- Through FSH, it stimulates- ovaries to produce estrogen - stimulates – graffian follicle growth, uterus growth, breast development.
- These physiological & biochemical changes continues and accumulates in menarche –the beginning of **first Menstrual period.**

Usually adrenarche precedes puberty, adrenal androgen begins to increase by 6-7 yrs in girls[43].

HYPERANDROGENISM :

The sources of Androgen may be from Ovary, Adrenal gland, & Peripheral conversion.

CAUSES OF HYPERANDROGENISM [59]

LOCAL:

1. Adrenal gland related: Hypersecretion / Tumor.

    - Congenital Adrenal Hyperplasia (CAH)
    - Non-classical CAH
    - Tumor of Adrenal
    - Cushing's syndrome.

2. Ovary related:

    - **Polycystic Ovarian syndrome**
    - Tumor arising from Theca cells

SYSTEMIC CAUSE:

- **Hypothyroidism**
- Hyper-insulinemia. (Insulin Resistance)
- Hyperprolactinemia.

IN PCOS:

The proposed & accepted concept for ovarian hyper-androgenism is Cortical stromal Hyperplasia / Stromal hyper-thecosis (Hyper-cellular thecal layer) [60, 61, 62]. Theca cells of graffian follicle has receptor for LH and produces androgen. It not only produces andogen but also Activin & Inhibin-B. Activin, an activator or stimulator of

FSH. In PCOS, hyperplasia of thecal layer of ovary produces excess amount of androgen and Inhibin B.

Inhibin B [63]:

It is a non steroidal protein hormone having α & β subunits. It inhibits FSH production but stimulates LH from Anterior pituitary. The mechanism by which it is stimulates LH is not known. Inhibin is produced from ovary, pituitary gland, placenta, corpus luteum and other organs. Inhibin B is now used **as the marker of fertility in both male and female.**

Together with suppression of FSH by Inhibin, estrogen synthesised from excess androgen (by aromatization) also gives negative feedback inhibition to FSH. Stimulation of LH and inhibition of FSH collectively leads to increased LH/FSH ratio even in the follicular phase which is against the normal physiology. FSH hormone is the predominant one in the follicular phase and expected to be high when estimated during these days (usually D_2-D_3).

Low FSH from anterior pituitary is not sufficient enough for the growth of graffian follicle and there is no dominant follicle formation. Prolonged suppression of FSH & peaking of LH leads to chronic anovulation and formation of number of small Immature, unruptured follicle filled with fluid (follicular fluid). Multiple immature follicles found on the surface of the ovary give the picture and hence the terminology.

POLYCYSTIC OVARIAN DISEASE.

Hyperthecosis with multiple cysts causes enlargement of ovary, increases volume and stimulates fibrosis of the stromal layer and capsule. As there is no ovulation, corpus luteum is not formed and no Progesterone production. Occasionally,

prolonged exposure to high estrogen level causes hyperplasia of endometrium and shedding of them causing irregular menstruation.

There is a conflict over the concept that there exists or not in the defect of central ovarian axis (Pituitary-Ovarian axis). Most of the scholars do not accept this view because, many interventional studies have proved that Wedge resection of ovary has shown to restore the normal ovarian cycle. From this, it is also confirmed that it is only the ovarian androgen, the key cause for hyperandrogenism that has obstructed the normal ovarian cycle [46].

The overall effect is defect in development of ovarian follicle and induced premature ovarian atresia. Together with the above findings in the ovary, when patients have the below mentioned clinical features, then the complex disorder is called **POLY CYSTIC-OVARIAN SYNDROME.** This finding in ovaries of women having PCOS blends with the finding in ovaries of post menopausal Women where the ovarian reserve is lost [64].

MORPHOLOGY OF POLYCYSTIC OVARY:

The morphology of the ovary changes from smooth, soft pinkish structure to enlarged pearly white, firm encapsulated organ. The number of primordial follicles are found to be the same but the number of growing follicles are increased. Each ovary contains more than 50 cysts. On cut section, it shows increased cortical thickening with number of cystic follicles.

ANOVULATORY CYCLES

This is usually seen in the early months of onset of puberty & / few months prior to menopause where normal menstruation takes place without ovulation .

Prolonged an-ovulation alters the normal menstrual cycle. The length of the cycle prolongs more than 35 days. Secretary phase / Luteal phase is always constant of about 14 days but Proliferative phase may vary. Any change in the length of menstrual cycle is only because of the variation in the proliferative phase [65].

In anovulatory cycles, the graffian follicle does not attains maturity as expected and is not ruptured. This immature follicles formation if continues, then it results in multiple follicles on the surface of the ovary along with enlargement of the ovary volume called POLY CYSTIC OVARIAN DISEASE where chronic anovulation and multiple cysts in the ovary is seen. The anovulatory cycles alters the hormone levels particularly the estrogen and Androgens (Testosterone) which remains elevated and is the important cause of infertility.

CAUSES OF ANOVULATION [66-75]:

Hormone Imbalance(70%)

- Hypothalamus: Any disturbance in the Hypothalamo-pituitary-ovarian axis alters the regular menstrual cycle.
- Pituitary: Tumor / Dysfunction
- Ovarian: Polycystic ovarian syndrome.
- Systemic.

Ovarian:

Polycystic ovarian syndrome, the leading cause for anovulation in women going to infertility clinic. Up to 90% of cases of anovulation are by PCOS and is usually hereditary[72]. Anovulation in PCOS is disturbance in the normal ovulating menstrual cycle. The abnormal antral follicles are formed due to immature follicle not

able to rupture leading to anovulation. Elevated insulin also stimulates theca cells to increase production of androgen via Insulin like growth factors. This again arrest follicular development resulting in anovulation.

Systemic causes:

1) Hypothyroidism.

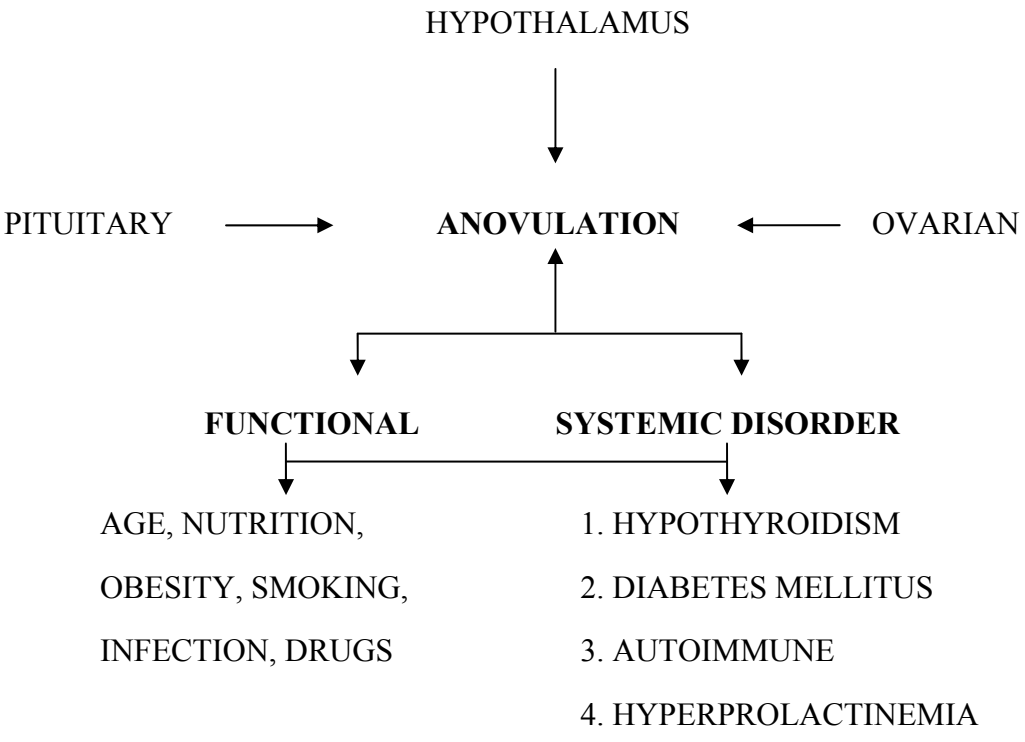
2) Hyperprolactinemia.

Functional cause :

1) Anorexia.

2) Stress.

3) Obesity .



HYPERINSULINEMIA[76-78] :

In 1921, Achard & Thiers first described the association of Carbohydrate metabolism with hyperandrogenism as "***the diabetes of bearded women***''. In 1947, Kierland et al, reported the skin lesions Acanthosis nigricans occurs frequently in hyperandrogenism & Diabetes mellitus [22].

Elevated Insulin level is a very common finding in PCOS patients though it is not included in diagnostic criteria. Insulin is not only a hormone of metabolic pathway but also has an important role in reproductive cycle. About 70-80% PCOS patients were found to have Insulin resistance. According to a study conducted by the Endocrine society of India, on PCOS women 23-35% were in Impaired Glucose Tolerance (IGT) state and 4-10% had Type2 DM which are seven fold higher than their age matched normal women. Indian women show difference in response to glucose tolerance than white women. Obese women particularly truncal obesity showed higher level of glucose intolerance than lean women but still lean PCOS women have higher intolerance when compared with their age matched Controls. Burghen et.al showed the association between Insulin resistance in PCOS women. He found a high positive Correlation among Insulin and sex steroids like androstenidione, testosterone in Polycystic ovarian syndrome patients [79] Ehrmann et.al, demonstrated that those patients having dysfunction of beta cells of pancrease are at risk of developing carbohydrate intolerance and Type 2 Diabetes mellitus among PCOS women.

Hyper-insulinemia may be due to increased production of insulin or reduced uptake of insulin by the peripheral tissues. The peripheral uptake is called **clearance** and is receptor mediated. Any defect in the number and or function of the receptor

causes decreased clearance of Insulin by the tissue. In PCOS, there is reduced clearance leading to Hyper-Insulinemia.[80, 81] but a little higher level of secretion by beta cells also happens.

Insulin Resistance [82, 83]:

Is the diminished biological response of the body below the normal level to the available insulin. This diminished response may be due to decreased sensitivity or decreased maximum responsiveness or both.

Peripheral resistance which is quantitated by **Euglycemic glucose clamp test**. In this test a known quantity of I.V glucose and I.V basal insulin is given simultaneously and serial arterial blood collection is done. The amount of glucose given by I.V equals the amount of glucose taken up by the peripheral tissue, . This phenomenon of peripheral uptake is called **Insulin Mediated Glucose Disposal (IMGD),** more essential in skeletal muscle uptake than in liver or adipose tissue. Euglycemic glucose clamp test is reduced in PCOS up to a level of Non-Insulin Dependent Diabetes Mellitus indicates that there is reduced peripheral uptake. Obesity in PCOS has synergistic negative effect in hepatic glucose production - an important factor for Insulin resistance. Body fat has higher negative effect in Insulin sensitivity in PCOS women [58,59].

Molecular view of Insulin resistance shows:

1) Pre-Receptor – Antibody mediated.
2) Receptor
3) Post receptor signalling pathway – PCOS level of resistance.

Insulin Receptor [84-89]:

It is a cell surface hetero-dimeric receptor with 2α and 2β subunits joined by disulphide bond. The 2α subunits has the N terminal ends, hanging outside of the cell surface and has the ligand binding domain. The 2β subunits spans on the cell membrane and extents intracellularly. It has the C terminal ends. The β subunit has intrinsic Tyrosine kinase activity and stimulate auto phosphorylation of its own certain tyrosine residue when the substrate binds.

Insulin Receptor belongs to a subfamily of protein tyrosin kinase receptor. Its phosphorylation converts it into an activated receptor. This activated (phosphorylated) receptor in turn phosphorylates intracellular substrate to initiate the signalling pathway. The first substrate identified was Insulin receptor substrate complex IRS-1, later IRS-2, IRS-3 were identified which binds to intracellular signalling substance phosphotidyl inositol3 phosphate kinase (PI3-K) leading to Insulin mediated glucose transport.

Insulin has a number of target tissue action like growth regulation, gene expression regulation, etc. Now it seems that Ras-Raf MEK pathway regulates growth PI3-K pathway in glucose uptake. But the termination of this signal is not completely known.It is seems to be the phosphorylation of serine residue instead of tyrosine that terminates the signal in EGF and other kinases, which may be the one that cause termination in Insulin Tyrosin kinase through Protein kinase C (PK-C). This is the step where the signalling pathway is disturbed and Insulin resistance is noted.

Insulin role in glucose metabolism is impaired in resistance but insulin induced androgen synthesis is intact which is paradoxical. The overall effect of hyper-insulinemia is Hyerandrogenism by decreasing hepatic Sex Hormone Binding

Globulin production . The protein bound androgen is reduced there by free androgen is elevated which causes the features of hyperandrogenism seen in PCOS women.

GENETIC FACTOR [90-92]:

It has a significant contribution to etiology as studies have prooved different levels of response to the same level of androgen level among different ethnic group. It runs in families siblings, mother, maternal aunt have the history of menstrual irregularities or treatment for Infertility have PCOS features on retrospective analysis. United Kingdom studies showed men with elevated LH/FSH ratio found to have 89% of their daughters with PCOS. The inheritance pattern is supposed to be Autosomal Dominant. In all the sub ship, 80% female were affected. Givens and associates from university of Tennessee have reported multiple PCOS kindreds, showing members in several generations

ENVIRONMENT [93]:

This factor also exists in the pathogenesis. Obesity alone plays an important role in etiology. Fatty diet, more intake of simple sugar or increased endogenous production of fat- all contributes to elevated fat in blood which is directed towards steroid genesis.

CRITERIA FOR PCOS [94, 95]:

In 1990, National Institute of Health (NIH) consensus workshop defined PCOS as chronic anovulation with clinical & / Biochemical hyperandrogenism, with exclusion of other mimicking etiologies like thyroid, Adrenal dysfunctions.

In 2003, the European Society for Human Reproduction / American Society of Reproductive Medicine (ESHRE/ASRM) conducted a workshop in Rotterdam

proposed that to diagnose a women with PCOS 2 out of 3 of the following should be satisfied 1. Oligomenorrhoea & / anovulation, 2. Clinical&/Biochemical hyperandrogenism and 3. Polycystic ovaries on Ultra sonogram.

Other etiologies should be excluded. Some argued that this expanded Rotterdam criteria may over diagnose or misdiagnose PCOS.

In 2009, the Androgen excess & PCOS (AE-PCOS) society published a report emphasizing that PCOS is primarily a hyperandrogenism disorder and proposed for revision in the definition of hyperandrogenism and ovarian dysfunction, there by encompassing the Rotterdam ultrasonogram criteria but requiring hyperandrogenism for diagnosis.

In all the above said societies, they don't separate the criteria for Adolescent girls. But some authors says that the criteria proposed for adults poses diagnostic problem as the characterestics of normal puberty overlaps with features of PCOS. So, some have proposed criteria for adolescent girls and are much stricter than for adult.

Sultan and Paris: Adolescent girls should meet 4/5 of following criteria [96]

1) Oligomenorrhoea or amenorrhoea > 2 years after menarchy.
2) Clinical hyperandrogenism.
3) Biochemical hyperandrogenism.
4) Insulin Resistance / hyperinsulinemia.
5) Polycystic ovaries in ultra sound.

Carmina & colleagues suggested applying Rotterdam criteria but limiting definitive diagnosis to adolescent girls only who meets all three criteria [97, 98]. But

adolescent girls PCOS criteria are not endorsed currently by the panel or society in the field. They too have limitation of inappropriate early diagnosis.

Even though there are a number of definitions, the Rotterdam consensus is the most widely accepted one across Asia, Europe and Australia and is used for guidelines. Recently, in October 2013, the Endocrine society released a practical guidelines to diagnose and treat PCOS which emphasise to use Rotterdam Criteria for diagnosis.

REVISED ROTTERDAM CRITERIA 2003[99]:

(DIAGNOSTIC CRITERIA FOR POLYCYSTIC OVARIAN SYNDROME)

Two of the following three criteria are required:

1. Oligomenorrhoea / Amenorrhoea.

   Oligomenorrhoea: Cycle >35 days / < 8 cycles per year.

   Amenorrhoea: Absence of cycle for 6 months who was menstruating regularly

2. Hyperandrogenism: Clinical / Biochemical

   Clinical: Acne / Hirsutism / Alopecia / Male pattern baldness.

   Biochemical : Serum Androgen (Testosterone) is elevated.

3. Polycystic ovaries on Ultrasound

   >12 cysts, each 1-2mm diameter in the periphery of the Ovary giving Pearl necklace appearance.

Other etiologies should be excluded such as Congenital Adrenal hyperplasia, Adrenal tumors, Cushing syndrome, thyroid dysfunction, hyperprolactinoma.

Some authors have given major and minor criteria for diagnosing PCOS in which USG finding of polycysts is considered only as minor criteria.

TABLE : II

MAJOR CRITERIA	MINOR CRITERIA
Chronic anovulation,Clinical signs of androgen excess Hirsutism Acne Alopecia Menstrual disturbance Infertility, Virilization, Exclusion of alternative causes of androgen excess. Exclusion of alternative causes of androgen excess	Onset at puberty Insulin Resistance, Elevated LH:FSH ratio (> 2.5–3) Ultrasonographic evidence of polycystic ovaries

CLINICAL PRESENTATIONS:

1) MENSTRUAL IRREGULARITY: Oligomenorrhoea / Amenorrhoea.
2) METABOLIC FEATURES: Obesity, Dyslipidemia, Diabetes mellitus.
3) ACNE.
4) HIRSUTISM.
5) ALOPECIA.
6) ACANTHOSIS NIGRICANS.
7) INFERTILITY.

MENSTRUAL IRREGULARITY [100, 101]:

The most common presentation is oligomenorrhoea & / amenorrhoea, usually secondary amenorrhoea but also present as primary amenorrhoea. Some may not have

menstrual irregularities at all. Prevalence of amenorrhoea is 5% in general population and 8.5% in post pubertal adolescent population.

Primary Amenorrhoea:

A condition in which a woman had never had menstruated so far.

Secondary Amenorrhoea:

Cessation of cycle at least for 6 months in a woman who had previously normal cycles (75%). After excluding pregnancy, secondary amenorrhoea may be caused by ovarian dysfunction, uterine dysfunction, adrenal, thyroid disorders, pituitary, Hypothalamus dysfunction, psychological stress. It is classified into 2 groups either with or without accompanied hyperandrogenism. PCOS is the ovarian cause for amenorrhoea with hyperandrogenism.

Oligomenorrhoea: Prolongation of menstrual cycle > 35 days / < 8 cycles per year.

HIRSUTISM [102-105] :

It is defined as excess growth of terminal hair on androgen sensitive area. It includes chin, upper lip, sternum, periareolar region, sideburns, umbilical, sacral and pubic region. Hirsutism is different from hypertrichosis / vellus in which thin hair is distributed throughout body & face & are depigmented. Hirsutism involves medulla of hair, thick and pigmented.

Mechanism of Hyperandrogenism causing Hirsutism:

In hirsutism, there is increased sensitivity of the hair follicle to normal level of circulating androgen and this causes excess hair growth. Androgen through androgen receptor in dermal papilla has strong effect in hair growth. Any defect of the androgen

receptor results in hirsutism. It has opposite effect in hair follicle of scalp and the follicle in body & face. Androgen stimulates the body & face hair but inhibit hair growth in the scalp causing alopecia (male pattern baldness).

Idiopathic hirsutism – accounts for 6-50% of hirsutism.

Non-Neoplastic hirsutism - occurs at puberty.

70-80% hirsutism were affliated with PCOS.

ALOPECIA [106]:

Hair loss in excess is a very rare presentation in PCOS women.

ACNE:[102, 106, 107] :

A common pre menstrual finding in most of young girls. Severe cystic comidones, painful and persisting is a typical in androgen dependent. Androgen have profound effect on skin, its appendages, sebaceous gland and hair follicle, strongly depends on biologically active androgen. In sebaceous gland, it stimulates sebocyte proliferation particularly in the face and leads to increased sebum secretion. Within the intra follicular duct of the pilo-sebaceous unit, androgen increases the rate of mitosis and epithelial proliferation, leading to hyperkeratosis.

4 key factors for Acne

- Increased sebum production.
- Follicular hyperkeratinisation.
- Colonisation of the pilosebaceous unit with propioni bacterium acnes.
- Production of inflammation.

OBESITY [108, 109, 110]:

It is increased deposition of adipocytes in the body.35-80% of PCOS women presents with obesity, particularly central / truncal obesity. Obesity assessment is by BMI & waist Hip ratio.

ACANTHOSIS NIGRICANS [22]:

A reddish black, thick, velvety lesion on the skin around the neck. It is an external **marker of Insulin Resistance**. Also found in other auto-immune disorders.

METABOLIC FEATURES:

Alteration in lipid profile, elevated cholesterol, LDL, Triglycerides are commonly found. In spite of increased level of Insulin, the tolerance to glucose is diminished to an extent that it may lead to NIDDM.[76,77] Incidence of Hypertension and Diabetes among postmenopausal women showed higher rate in those who had PCOS in their early age.

INFERTILITY [109-115]:

WHO defines infertility as inability of a woman to conceive after one year of unprotected intercourse (and there is no other reason, such as breast feeding or postpartum amenorrhoea)[109]. Infertility also refers to the state of a woman who is unable to carry a pregnancy to full term. The definition varies by different international organizations.

The consequences of infertility are manifold and includes societal repercussions and personal suffering. Infertility may have profound psychological effects. In many cultures, inability to conceive bears a stigma. More than 50% of

women visiting Infertility clinic were found to have PCOS. Chronic anovulation, the major cause for female infertility in PCOS women.

Primary Infertility:

Couple without any successful pregnancy so far.

Secondary Infertility:

A woman who had conceived previously but unable to conceive currently.

Subfertility:

Defined as less fertile than a typical couple.

The distribution of infertility [111]:

1/3 – Female infertility, 1/3 – Male Infertility, 1/3 – Unknown.

Female infertility is around 48 million women, and varies according to geographical location. In 2010, there was an estimate of 48.5 million infertile couples Worldwide, highest rate regions included Eastern Europe, North Africa, the middle East, oceania, and Sub-Saharan Africa [114].

According to the American Society for Reproductive Medicine (ASRM), Age, Smoking, Sexually Transmitted Infections, and being overweight or underweight can all affect fertility in women [108].

CAUSES OF FEMALE INFERTILITY:

Chromosomal disorders,

Anatomical & congenital defects in female reproductive system,

1. The most common cause is **ANOVULATION**

2. Functional disorders,

3. Genitic & Environmental factors

ANOVULATION :

CAUSES:

- Hormonal imbalance

  Hypothalamo-Pituitary-gonadal axis disturbance as mentioned early.

  Systemic disorders.

- Functional disorders.

OVARIAN CAUSE (Major) of anovulation: [116, 117]

Female infertility caused by anovulation is called "anovulatory infertility", as opposed to "ovulatory infertility" in which ovulation is present [91] and they will have normal menstruation. The most common ovarian cause for anovulation is PolyCysticOvarianSyndrome (PCOS).

SYSTEMIC CAUSES of anovulation:

1. Thyroid gland disorders:

Thyroid gland dysfunction both hyper and hypothyroidism causes menstrual problems. It alters the balance of reproductive hormones.

2. Diabetes mellitus

3. Auto-immune disorders.

FUNCTIONAL DISORDERS (10-15%):

AGE:

Woman's fertility is affected by her age. About 80% of the cycles are anovulatory in the first year of menarche, 50% up to three years and 10% till sixth year [83]. A woman's fertility peaks in early and mid 20s, after which it starts declining, and this declination accelerated after 35 yrs of age. For women aged 35, about 94 out of every 100 who have regular unprotected sexual intercourse get pregnant after three years of trying. For women aged 38, however, only 77 out of every 100 do so. This proved that the fertility of a woman decreases as her age increases.

SMOKING:

Cigarette smoking interferes with folliculogenesis, embryo transport, endometrial receptivity, endometrial angiogenesis, uterine blood flow and the uterine myometrium.

SEXUALLY TRANSMITTED DISEASES:

This one of the leading causes of infertility.

NUTRITION & OBESITY:

About 12% cause of infertility are due to nutrition a woman either being underweight or overweight. Fat cells also produces estrogens by aromatization, in addition to the primary sex organs. Too much body fat deposition causes production of excess estrogens (hyperestrogenism) and the body begins to react as if it

is on birth control (OCPills), limiting the odds of getting pregnant. As estrogen is a vital hormone in fertility, too little body fat causes insufficient production of estrogen and disruption of the menstrual cycle. Both under and overweight women have irregular cycles in which ovulation does not occur or in inadequate rate. Proper nutrition in early part of life is a major factor for later fertility. Dr Barbieri of Harvard Medical School has noted that cases of anovulation are quite frequent in women with a BMI (BODY MASS INDEX) over 27 $^{kg}/_{m}2$.

CHEMOTHERAPY [118]:

Therapuetic / stimulating drugs poses a high risk for infertility. Female infertility by chemotherapy may be secondary to premature ovarian failure by loss of primordial follicles.

HYPOTHYROIDISM

Hypothyroidism – a Greek word. Hypo-refers to "under" Thyroid refers to "Thyroid gland". It is also called as "Hypothyreosis". Hypothyroidism is one of the most common endocrine disorder seen among young women. It is defined as deficiency of thyroid hormones secretion & action that produces a variety of clinical signs & symptoms of hypometabolism [119,120]. In children, the hypofunctioning gland leads to under development of intellect and physical growth called "Cretinism". Hypothyroidism is more common in females than males.

Thyroid gland dysfunction (hypo-function) causes a variety of symptoms involving the entire body resulting in mental and physical sluggishness. The features may be due to decreased hormone synthesis or the effects of decreased thyroid hormone in target tissue. They have tiredness, cold intolerance, rapid weight gain,

menstrual irregularities, either in excess called menorrhagia or Amenorrhoea. They may also present with depression, anxiety. Hypothyroidism in early pregnancy increases the risk of pre-eclampsia, still birth, cretin child [121,122].

PREVALENCE:

TABLE : III

1. General population[123]	10-11%
2. Reproductive age women [124,128]	2-4%
3. Infertile women [125]	23-37%
4. Pregnant women [125]	0.3-0.5%
5. Western population [163]	0.3-0.4%
6. Subclinical Hypothyroidism [126,127]	3-15%

There are many temporary and permanent causes for reducing thyroid hormone secretion and there by leading to hypothyroidism. In most of the conditions the problem starts from the gland per se and sometimes from pituitary or hypothalamus.

TYPES OF HYPOTHYROIDISM:

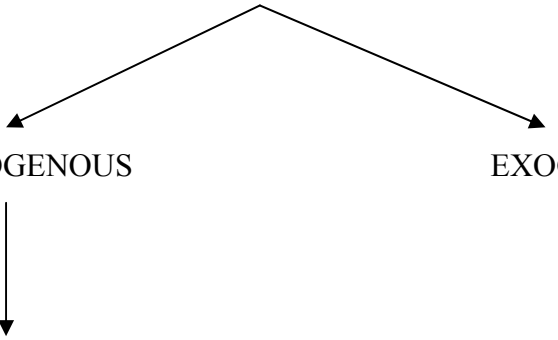
1. Primary hypothyroidism
2. Central hypothyroidism
3. Congenital hypothyroidism

PRIMARY HYPOTHYROIDISM: [129]

This is the most common type of hypothyroidism. The defect is within the gland, i.e. insufficient production of thyroid hormones. This may be due to nutritional

deficiency of Iodine, which is the most common cause for Primary hypothyroidism in Iodine deficient area. Autoimmune thyroiditis is the most common cause of hypothyroidism worldwide and in non-Iodine deficient area.

CAUSES OF PRIMARY HYPOTHYROIDISM: [129]



ENDOGENOUS

AUTOIMMUNE

INBORN ERRORS OF HORMON DYSGENESIS

DEVELOPMENTAL DISORDER OF GLAND

EXOGENOUS

IODINE DEFICIENCY

DRUGS

POST-SURGICAL

RADIOTHERAPY

Autoimmune Thyroiditis:

In autoimmune condition, the body's immune system is directed towards itself. This is the most common cause for primary hypothyroidism worldwide and the prevalence is higher in population taking iodine rich diet. Hashimoto thyroiditis, atrophic thyroiditis and post partum thyroiditis are the autoimmune thyroid diseases of the thyroid gland. In all these forms, the gland is diffusely infiltrated with lymphocytes. Both T cells and B cells are deposited in equal amount in the gland. The T cells are mistakenly tricked as invaders of its own cells. This stimulates B cells to produce antibodies called auto antibodies which destroys the thyroid gland. Autoimmune thyroiditis is usually associated with other autoimmune disorders like

vitiligo, Sjogren's syndrome, pernicious anaemia,etc. It occurs in syndrome of multiple Autoimmune Endocrinopathies like:

Type 1: Adrenal failure, hypoparathyroidism

Type2: Diabetes mellitus (type1), ovarian and adrenal failure.

Hashimoto Thyroiditis:

Most common thyroiditis found in United States. It is a genetic disorder named after a Japanese doctor who first described the inflammation of thyroid gland. It is 20 times more commonly found in female than males. Environmental factors also contribute to this condition. Goitre (enlargement of thyroid gland) is usually found in these patients. Hashimoto thyroiditis causes permanent damage to the gland and they need treatment throughout their life.

Atrophic Thyroiditis:

This type of thyroiditis is similar to Hashimoto but without goitre.

Riedel's Thyroiditis:

It is a rare form of thyroiditis in which the thyroid gland is filled with fibrous tissues and there by converting the consistency of the gland from soft to stony hard.

Post-partum Thyroiditis:

Hypothyroidism can also occur due to antibodies developed toward thyroid gland during pregnancy which causes inflammation of the gland after delivery halting the thyroid function.

Subacute thyroiditis:

About 10% of hypothyroid cases are due to sub-acute thyroiditis.

The patient may have silent / painless form or painful / granulomatus form.

Iodine abnormalities:

Both deficiency and excess of iodine causes hypothyroidism.

Drugs:

Amiodarone contains iodine which interfers with thyroid hormone synthesis, Lithium which inhibits thyroid hormone secretion. Drug induced hypothyroidism is reversible on discontinuation of the drug.

CENTRAL HYPOTHYROIDISM: [130]

Here the defect is at higher level involving pituitary or hypothalamus. Patients are symptomatic, with laboratory finding of low freeT4 but TSH not elevated as expected may be normal or even below the reference level. It may be **secondary hypothyroidism,** if it involves pituitary gland like surgery or irradiation, congenital disorders of pituitary, pituitary apoplexy, sub-arachnoid haemorrhage. **Tertiary hypothyroidism** if it involves the hypothalamus.

CONGENITAL HYPOTHYROIDISM: [131]

Thyroid gland dysgenesis, Thyroid dyshormonogenesis, mutation of gene for enzyme concerned with thyroid hormones synthesis, maternal antibodies, associated with congenital disorder.

BIOCHEMICAL CLASSIFICATION OF HYPOTHYROIDISM:

a) Subclinical Hypothyroidism [132, 133, 134]:

The prevalence of subclinical hypothyroidism is 3-15% [126,127] and in this condition patients have persistently elevated TSH (6-12 wks), but asymptomatic, serum free T4 within reference interval. The serum TSH being > 5μU/ mL but <10μU /mL. If untreated they may progress to overt hypothyroidism. Every year about 2%-4% of subclinical hypothyroid patients are progressing to overt hypothyroidism. Incidence of SCH increases with age, female gender and greater intake of dietary iodine intake. They are found among those who have antibodies and those who don't have it. Subclinical hypothyroidism can cause infertility and miscarriages [135]. They are more prone to develop cardio-vascular diseases [136]. There is controversy in treating the patient or not who have TSH <10μU/mL.

b) Overt Hypothyroidism [132, 133]:

A condition in which serum TSH is elevated more than10μU / mL, with symptoms of hypothyroidism. Free T3 and FreeT4 remains elevated only in borderline amount. Usually TSH rises only after the fall in T4 / T3.

TABLE : IV

TSH	T4	INTERPRETATION
Normal	Normal	Normal thyroid function
Elevated	Low	Overt hypothyroidism
Normal / Low	Low	Central hypothyroidism
Elevated	Normal	Subclinical hypothyroidism

THYROID GLAND & ITS HORMONES [137, 138, 139] :

Thyroid gland is a reddish brown butterfly shaped mass of glandular tissue present in front of the neck. It is a paired structure connected by an Isthmus. The gland extends from C5-T1 vertebra, and occupies tracheal rings T2, T3, T4. It is the first endocrinal gland to develop by about 24^{th} day of Gestation. It originates as a proliferation of endodermal cells of median surface of developing pharyngeal pouch (1 &2). The precursor of thyroid gland arises as mid line out pouching from foregut to form thyroid diverticulum. The thyroid gland descends from the diverticulum completely to its place by 7^{th} week. This later gets solidified and then divides into 2 lobes. From 10^{th} week onwards, fetal thyroid follicles and thyroid hormones are demonstrated. Thyroid hormone shows a trend in serum throughout the life. They rise from in utero till birth, within hours of birth they reach a peak (cold stress), by 2^{nd} -3^{rd} day it falls . TSH rises gradually as age increases.

Microscopically Thyroid gland consists of Thyroid follicle/ Acini covered by basement membrane, Para follicular cells. Each follicle is spherical shaped structure, lined by epithelium. These epithelial cells may be squamus or cuboidal and their height determine their activity. Centre of the acini is the lacuna that contains colloid composed of Thyroglobulin (Tg).

THYROID HORMONE SYNTHESIS [121, 140]:

Iodine (I_2) in the diet is converted into Iodide (I^-) in the intestinal lumen before it gets absorbed. 20%of absorbed iodide enters the circulation and 80% is excreted through urine. Iodide by circulation enters the thyroid follicular cells through Na / I^- symport, Na returns out through Na-K ATPase pump. Iodide, through Pendrin, a glycoprotein transporter reaches the lacuna where it is oxidised / organified back to

Iodine by Thyroperoxidise (TPO). Thyroperoxidase is code by TPO gene and requires iron for its action, glutathione is the cofactor for this enzyme.During this oxidation hydrogen peroxide is produced in the apical membrane by Dual Oxidase Enzymes (DUOX1 & DUOX2). Mutation in this enzyme leads to hypothyroidism [141]. Thyroglobulin in lacuna binds with iodine to form Moniodothyronine (MIT), Diiodothyronine(DIT), Triiodothyronine(TIT), Tetraiodothyronine(TIT) or Thyroxine (T4). Thyroglobulin with T3/T4 serves as reservoir of thyroid hormone within the colloid.

Thyronine is produced by substitution of 2nd phenol group for phenolic '**H'** in Tyrosine residue of Tg by ether linkage. 4 possible sites of iodine attachment are 3, 5, 3'& 5'. 3 & 5 are in the inner α ring 3'& 5' in the outer β ring.

T4 (thyroxine): 3, 5, 3' & 5' Tetra iodothyronine.

T3: 3, 5, 3' Tri iodothyronine. (80% formed by extra thyroidal conversion of T4-T3)

rT3: 3, 3'& 5' reverseTri iodothyronine, an isomer of T3 biologically inactive.

Thyroglobulin (Tg): [140]

It is a homodimer of 66KDa containing 134 tyrosine residue among which only 25-35 alone are iodinated. It has 2 subunits and has 10% carbohydrates. They are synthesised by the ribosomes of rough endoplasmic reticulum in follicular cells. 75% of glandular protein is thyroglobulin and a very little amount of Tg is released into circulation without iodination. TSH is the principle stimulator of Tg synthesis. Normal serum concentration is 3-40 ng/mL. When TSH is within normal range, each

gm of tissue produced 1ng/mL of Tg. **Thyroglobulin is the marker of thyroid cancer.**

Thyroid follicular cells have receptor for TSH, when TSH stimulates the gland Tg is taken up by the follicular cells by pinocytosis & forms vesicle. This vesicle fuses with primary lysosome and forms secondary lysosome. It digests the Tg and releases T3 & T4 into thyroid capillary. MIT, DIT within the cell in deiodinised by Dehalogenase (Dhal1& Dhal1b) to free iodine and recycled back to new hormone synthesis. There are 3 homodimeric deiodinase (selenium dependent) for the peripheral metabolism of Thyroxine (T4).

D1 - Outer & Inner ring deiodinase.

D2 - Outer ring deiodinase.

D3 - Inner ring deiodinase.

About 80% of T3 comes from 5' deiodination of T4 in the peripheral tissues & 20% from thyroid gland. T4 to T3 is regulated by D1/ D2/ D3. 40% of T4 is converted toT3 & 45% to rT3. Liver, kidney, brain, brown fat, anterior pituitary, pineal gland, heart, skeletal muscles are the organs involved in peripheral conversion. After sufficient amount of conversion, a small amount of T4 is conjugated with sulphate or glucouronic acid and excreted through bile which undergoes entero-hepatic circulation.

It is proved that T4/T3 enters the target cell through an organic anion transporter called Mono Carboxylate Transporter (MCT-8, 10) [141]. This mechanism was previously thought to be a passive diffusion. A special Organic anion transporting polypeptide, 1C (ONTP1C) is found in astrocytes. Within the target cells T3 (the

active form of thyroid hormone) binds with its receptor (intra-nuclear), act as a transcription factor and bind with DNA through Zinc finger motif.

Thyroxine (T4) [142]:

This is a tyrosine based hormone with 4 iodine groups at 3, 5, 3', 5' secreted by the follicular cells of the thyroid gland. The major form of thyroid hormone in blood is thyroxine. This hormone, undergoes deiodination in the peripheral tissues and thyroid gland and converted to T3, the biologically active form. The half life of thyroxine is longer than T3 . The ratio of T4:T3 is roughly 20:1 in the circulation. In blood, most of the hormone is protein bound and only a little part is free and is the active form freeT4. The major regulator of TSH and TRH release is freeT4.

Thyroid Hormone Binding Receptor :

It is a steroid hormone gene super family. Has 15 times more affinity for T3 than T4. It is of 2 types α and β. α has 3 subunits, β also has 3 subunits. α 1 is expressed throughout the body. Maximum TR is in anterior pituitary & adipose tissue which are mostly occupied by T3. T4/T3 binds with its receptor and regulates gene expression. They have ubiquitous effect in growth and development in all age regulates calorigenesis, BMR throughout the life. It also stimulates blood vessel.

MOLECULAR LEVEL ACTION OF THYROXINE[144]:

1) Increases O_2 consumption within tissues.
2) Enhances mitochondrial metabolism.
3) Increases sensitivity of catecholamine to heart tissue & rises heart rate and contractility.

4) Stimulates protein synthesis & carbohydrate metabolism.

5) Increases the synthesis & degradation of cholesterol and Triglycerides.

6) Increases vitamin requirement.

7) Regulates calcium & phosphate metabolism.

PHYSIOLOGICAL ROLE OF THYROXIN:

- **Metabolic rate**: Increases the oxygen consumption of the cell byincreasing 2,3 BisPhosphoGlycerate (2,3BPG). T3 binds with its Receptor (intranuclear) and this complex T-R binds with DNA through Zinc finger and increases the transcription of enzymes in the metabolic pathway.
- Carbohydrate: Increase intestinal absorption, Protein: Increase protein catabolism, Lipid: Decreases Cholesterol in blood byincreasing LDL-Receptor in liver.
- **Colorigenic action**: Mobilizes fat from adipose tissue and increases the NaK-ATPase activity.
- **Milksecretion**: stimulated.
- **CNS**: Increases Reticular activating system.
- **Skeletal muscle**: Weakness (protein metabolism).
- **Reproduction**: Normal ovulation by **menstruation and Fertility**.

In Circulation [140, 151]:

T3 & T4 are mostly bound with protein, and the unbound form (0.1%) is biologically active. So, only free Thyroid hormone correlates with the clinical condition of the patients. The concentration of free T4 is 10 times higher than free T3 in blood. T3 is ~ 15 times more active than T4. This narrows down the effectiveness between the two hormones. Free T4 is the major regulator of TSH secretion.

TABLE : V

THYROID BINDING PROTEIN (TBP)	THYROID BINDING GLOBULIN (TBG)	TRANSTHYRETIN (TTR)	ALBUMIN
Concentration	4-4.5 mg/dL	10-20 mg/dL	3.5-5g/dL
Affinity for T4	High	Modest	Low
T4 capacity (mcg/dL)	22	120	1000
T4 distribution	67%	20%	13%
T3 distribution	53%	1%	46%

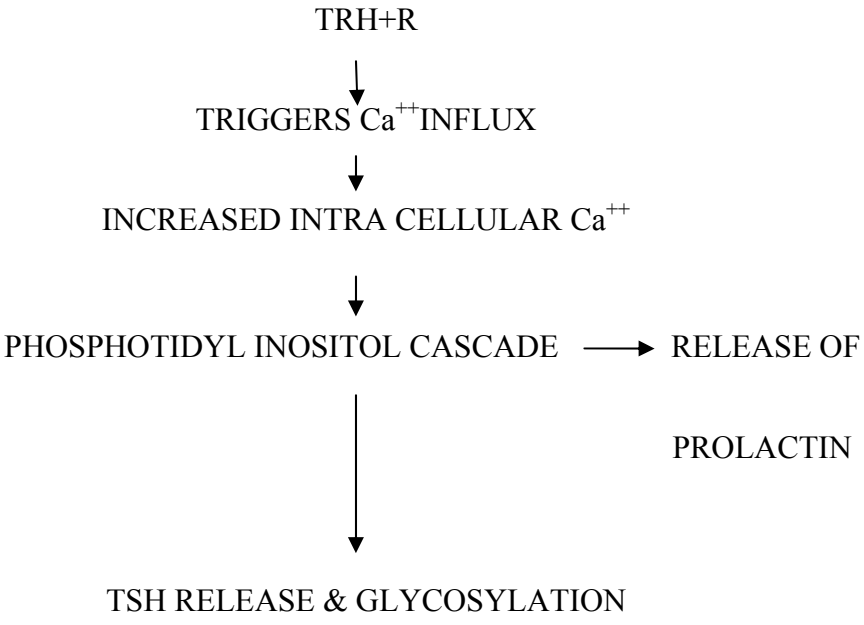
Albumin, Pre-albumin (TBPA), Transthyretin, Thyroid Binding Globulin (TBG) are the proteins involved in binding with T4 & T3. The capacity of Albumin for binding with Thyroid hormone is high when compared to TBG, but the affinity for Thyroid hormone to bind with TBG is higher than Albumin. Totally, TBG binds more T4 than albumin. Protein bound T4=99.98% and protein bounded T3 is 99.80%. i.e 0.02% of T4 is free and 0.2% of T3 is free in circulation [142]. The euthyroid status is maintained even when the TBG is elevated or decreased. Alteration in serum TBG profoundly affects total T3/T4 without affecting free hormone level.

REGULATON OF THYROID HORMONE SECRETION:

Hypothalamo - Pituitary - Thyroid gland axis has a negative feedback system in regulating thyroid hormone. The major feedback centre is Anterior Pituitary (thyrotrophs) and not the hypothalamus.

Thyrotropin Releasing Hormone (TRH):

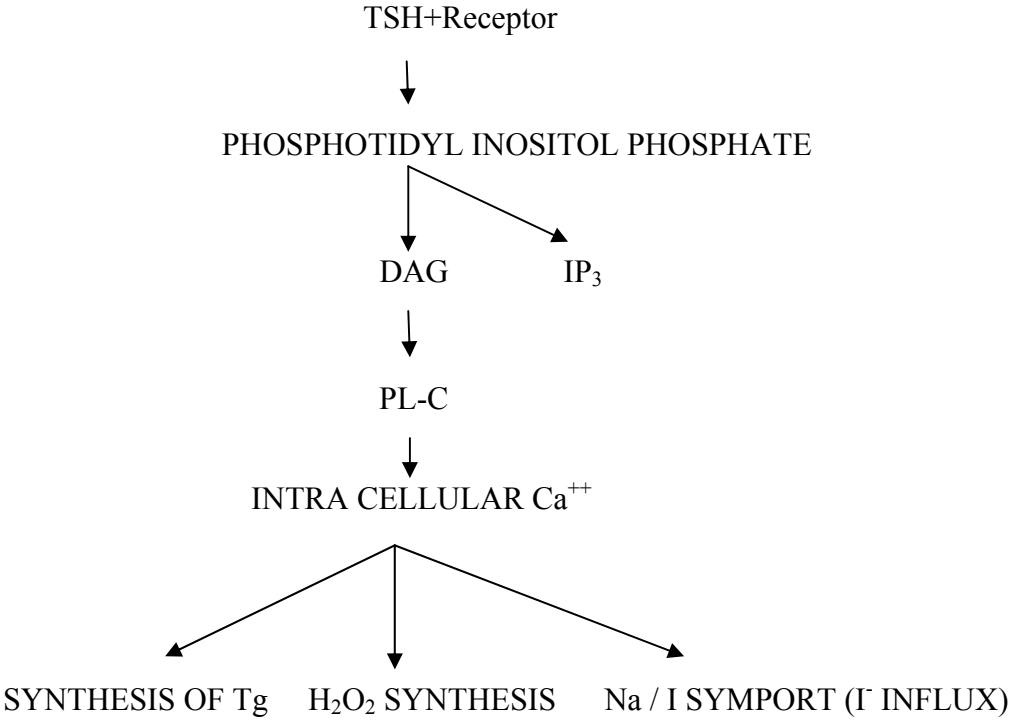
It is a tri-peptide (Proglutamate-Histidyl-Prolinamide) hormone produced by paraventricular nuclei of hypothalamus. TRH-Receptor (TRH-R) is a G protein coupled receptor in anterior pituitary thyrotroph cells. When TRH binds with its receptor, it stimulates the release of TSH from pituitary and also helps for its glycosylation. It modifies the sensitivity of thyrotrophs to negative feedback. When TRH is high, the sensitivity is decreased and when it is low, the sensitivity of thyrotrophs to negative feedback is increased. High TRH reduces the TR in thyrotrophs, so less sensitivity to negative feedback which stimulates TSH release in excess that is what happens in hypothyroidism.



Thyroid Stimulating Hormone [140]:

The primary regulator of Thyroid hormones is Thyroid Stimulating Hormone (TSH), also called as Thyrotropin. It is a 30KDa, heterodimeric glycoprotein having α & β subunit. It is secreted by thyrotroph cells of anterior pituitary. The α subunit is similar to α subunits of LH, FSH, hCG and has 2 oligosaccharides. The β subunit has 10 oligosaccharides & specific for TSH & its activities. Active form is glycosylated TSH, Non glycosylated form loss potency but retains immune reactivity. Free T4 & TSH has inverse relationship that 50% fall in T4 rises TSH by 100 fold. TSH secretion is lowest in the late afternoon and highest around hours of sleep.

Elevated TSH indicates that the gland is not functioning to produce adequate Thyroxine. The receptor for TSH is in thyroid follicular epithelial cells. **TSH Receptor (TR)** is a glycoprotein having 743 amino acids. Its molecular weight is 84.5 KDa and is a G protein coupled receptor. It acquires 2 mode of confirmation, **ON** (Receptor with TSH) and **OFF** (Receptor without TSH).



Only free T4/ freeT3 have feedback regulation and not the protein bound form. TSH in turn is regulated by the higher centre thyroid Releasing Hormone (TRH) produced by Hypothalamus.

Reference Interval: [148, 148, 150]

National Academy of Clinical Biochemistry (NACB) reference level for adult is 0.3-3μIU/mL. In United Kingdom, the reference range suggested is 0.4-4.5μIU/mL.

TSH: 21wk-20yrs: 0.7-6.4 μU/mL, 20-54yrs: 0.4-4.2 μU/mL

Free T4: 2wk-20yrs: 0.8-2.0 ng/dL, 21-87yrs: 0.8-2.7ng/dL.

The lower limit of TSH for diagnosing hypothyroidism is not exactly determined so far. It has ethnic variation, age variation. According to American Thyroid Association (ATA) – 2012 recommendation, serum thyrotropin (TSH) is a single and best test for screening primary hypothyroidism in adults for outpatient situations. Treating the patients having TSH level in subclinical states (<10μU/mL) is tailored according to the patients [149]. Worldwide accepted parameter for screening of neonatal hypothyroidism is TSH [147].

DYSFUNCTION OF THYROID HORMONE IN MENSTRUATION & FERTILITY [152-156]:

Thyroid hormones secretion is essential for growth & development of reproductive organs. It plays a vital role in maintaining fertility. The impact of hypothyroidism in menstruation has been reported from 1950s. Hypothyroidism in reproductive age group women cause anovulation or Luteal phase defect, which in turn causes menstrual cycle to occur less frequently (oligomenorrhoea) or even to stop (amenorrhoea). Rarely do they also result in premature ovarian failure. Animal

research has shown collagen deposition within the ovarian intracellular matrix [161]. In human hypothyroidism causes mucopolysaccharides (hyaluronic acid& chondroitin sulphate) deposition in various organs including ovaries and this increases the ovarian volume. Finally, results in dysfunctioning of ovary. Menstruation tends to be increased in hypothyroidism and decreased in hyperthyroidism but very often they present with oligo/amenorrhoea. Hypothyroidism induced oligo / amenorrhoea is either directly by increasing androgen or through another hormone Prolactin, a hormone for lactation usually after delivery.

MECHANISM OF ANOVULATION IN HYPOTHYROIDISM [157]:

There exists a common regulator at the level of Hypothalamo-Hypophysial axis for the secretion of Prolactin and Thyroid Stimulating Hormone. When Thyroxin level decreases in blood, it triggers Thyroid Releasing Hormone (TRH) from Hypothalamus, which not only stimulates the Thyroid Stimulating Hormone (TSH), It also stimulates prolactin release from anterior pituitary leading to **Hyperprolactinemia.** When Prolactin is increased, the body considers as if in lactation and birth control status and causes anovulation. In hypothyroidism the combined effect of hyperprolactinemia and hyperandrogenism leads to chronic anovulation, and infertility.

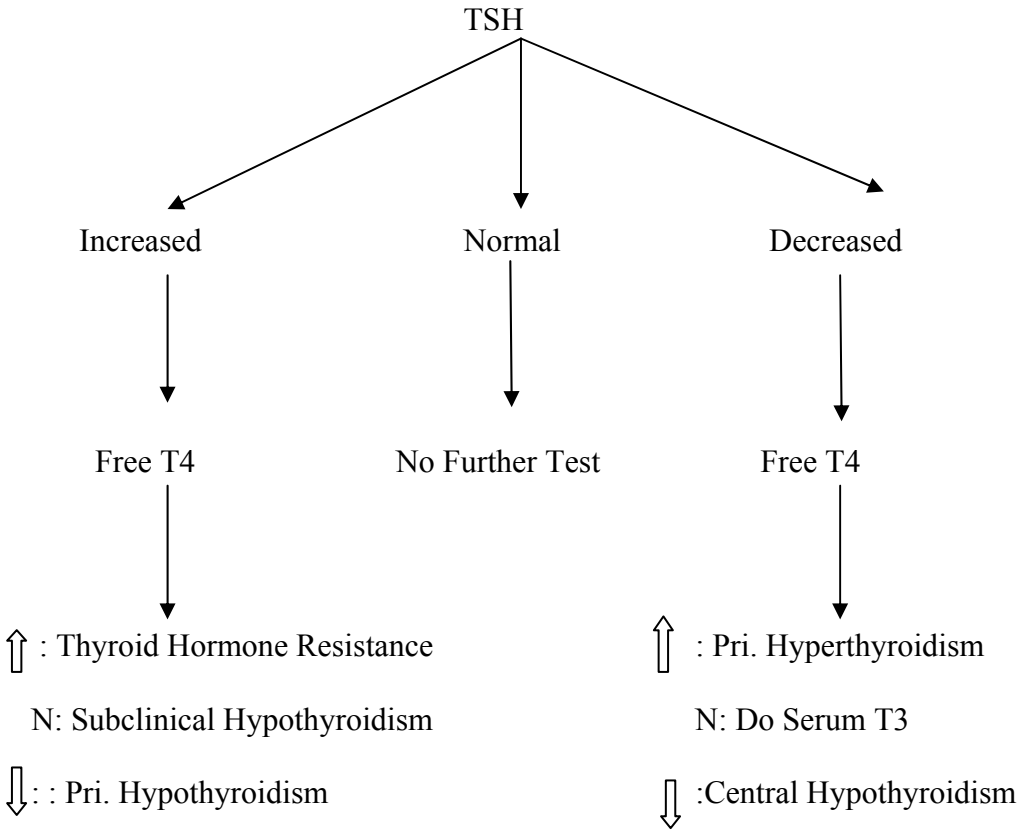
MECHANISM OF HYPERANDROGENISM IN HYPOTHYROIDISM: [158]

At the Hepatic level, Thyroid hormone influences the synthesis of Sex Hormone Binding Globulin (SHBG). Hyperthyroidism stimulates protein (SHBG) synthesis but Hypothyroidism inhibits SHBG synthesis. SHBG has high affinity toward sex hormones (Testosterone, Estrogen). So,reduced SHBG increases the free

androgen (**Hyperandrogenism**) i.e. free Testosterone, the biologically active form and the cause for all clinical features.

Ghosh S et al, interventional study conducted by Institute of Chemical Biology, Reproductive Biology Research division, Calcutta proved the hypothesis - hypothyroidism causes elevation of free Testosterone by reducing SHBG, there by producing multiple cysts in ovary due to hyperandrogenism. Sergei Avdiushko study in Oct 2012, have also shown that treating hypothyroidism in such patients have reduced their ovarian volume and the cysts have disappeared. So, infertile female with overt or subclinical hypothyroidism should not be ignored. Polycystic ovarian syndrome together with hypothyroidism either overt or subclinical have become fertile after correcting hypothyroid alone and this shows that reducing the serum TSH will reduce free testosterone, estrogen, ovarian volume, bring back the fertility. A woman diagnosed as PCOS should be evaluated for hypothyroidism without fail. The very first test to evaluate thyroid function status is estimating TSH level for women attending infertility / Gynaecology clinic. TSH was alone used for screening hypothyroidism in by Linclon et al.[142] and setting the upper physiological limit for TSH is still under investigation. This occult hypothyroidism which is a treatable cause for menstrual disorder and infertility if detected early could be treated. By an easy and simple tests like serum TSH the burden of the disease could be assessed. All the above factors and concepts made us to conduct this study with our population.

EVALUATION OF THYROID FUNCTION TEST



Evaluation of Thyroid function status begins with detailed history, physical examination, and biochemical investigation. In biochemical investigation, the very first test is estimation of serum TSH. If it is within reference limit, then this rules out thyroid disorder and they need not be subjected to further evaluation of thyroid function. If TSH elevated, then the next step is to estimate the T4, ideally free T4. The cause for hypothyroidism may be gland dysfunction or peripheral tissue uptake disorder (resistance) where thyroxine is in adequate amount without its physiological role. The overall effect is hypometabolism and other features of hypothyroidism. This stimulates the anterior pituitary to secrete TSH so, whatever the etiology may be the effect of reduced active thyroid hormone is elevated TSH.Thus, TSH is sufficient for screening hypothyroidism and we have followed this protocol in screening for hypothyroidism in our study population.

There are different assay for estimating TSH. Shifting towards more sensitive methods 2 decades ago prompted a change in strategy for laboratory investigation of thyroid dysfunction. Depending upon the analytical sensitivity, the assays are given generation numbers. First generation, second, third, fourth generations are available.

Generations and their analytical sensitivity:

1st generation: 1mU/L or 1(µU/mL)

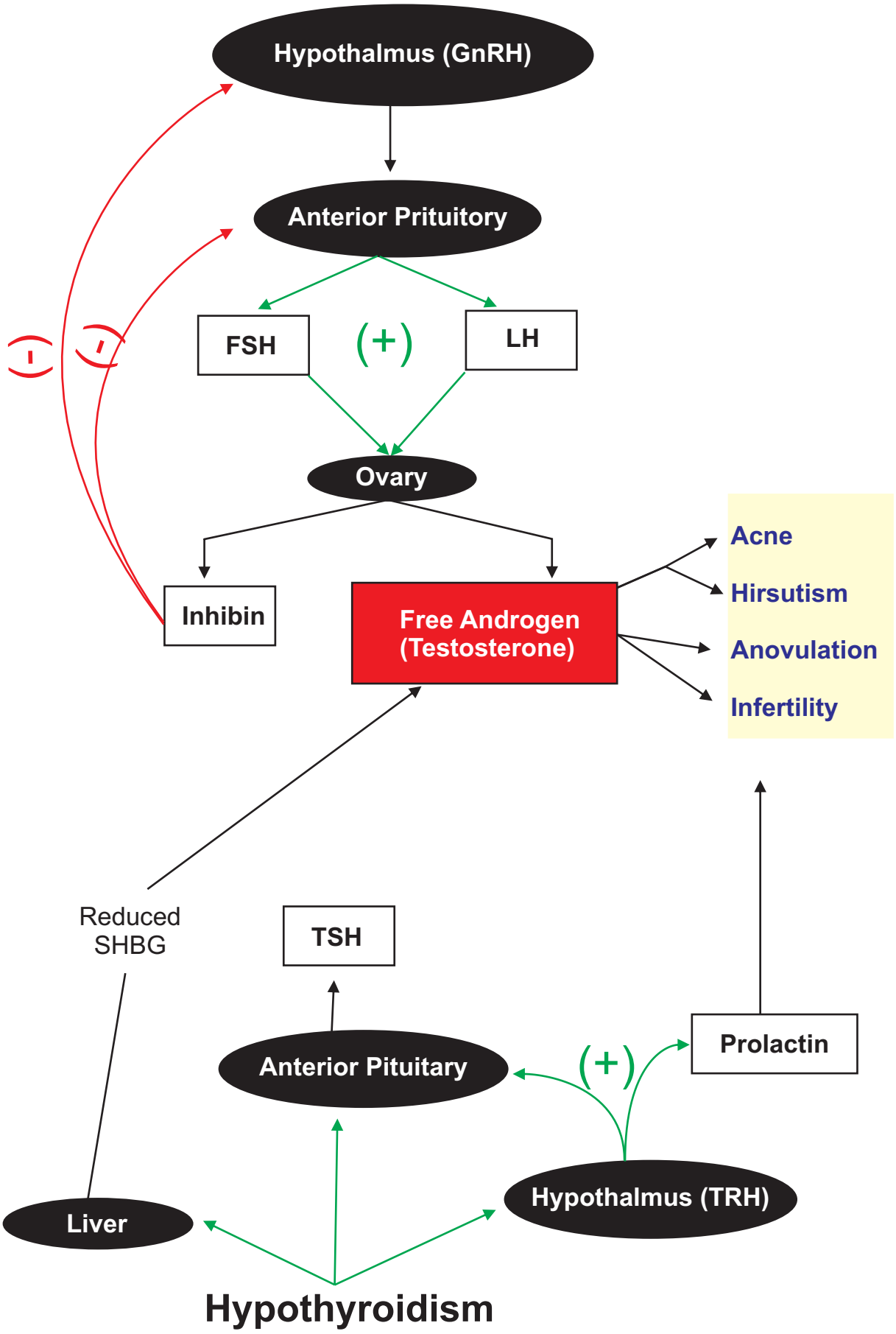
2nd generation: 0.1 mU/L

3rd generation: 0.01 mU/L

4th generation: 0.001 mU/L

Using highly analytical sensitive technique is gives the better resolution and perfect assessment.

HYPOTHESIS


Hypothalmus (GnRH)
Anterior Prituitory
FSH
(+)
LH
Ovary
Inhibin
Free Androgen (Testosterone)
Acne
Hirsutism
Anovulation
Infertility
Reduced SHBG
TSH
Anterior Pituitary
(+)
Prolactin
Liver
Hypothalmus (TRH)
Hypothyroidism

AIM AND OBJECTIVE:

1) To assess the Thyroid function status using serum TSH and estimate the prevalence of hypothyroidism in newly diagnosed PolyCystic Ovarian Syndrome patients.

2) To compare certain hormones and parameters between euthyroid and hypothyroid patients having Polycystic ovarian Syndrome.

MATERIALS AND METHODOLOGY:

In our study, we selected 73 post pubertal women who came with complaints of menstrual irregularities for a period of three months and /or infertility from Institute of Obstetrics & Gynaecology and medical Endocrinology clinic attached to Madras Medical College. With the ultrasound evidence of polycystic ovaries we proceeded the study. This study was approved by Institutional Ethic Committee.

Study design: A cross sectional, observation study.

Inclusion criteria :

1. Women aged between 15-35 yrs with menstrual irregularities or Infertility.
2. PCOS confirmation based on revised Rotterdam criteria 2003: (2 / 3 criteria should be satisfied)
    1. History of oligomenorrhoea (cycle lasting more than 35 days) or long cycles /Amenorrhoea (no cycles in the past 6 months).
    2. Clinical and / or Biochemical features of hyperandrogenism.
    3. Ultra sonogram finding : Multiple cysts (> 12 in number of 1-2mm) either one or both ovaries.

Other cause of hyperandrogenism like Congenital adrenal hyperplasia, Virilising tumor, Prolactinoma, Cushing syndrome should be ruled out.

Clinical hyperandrogensm is defined as to have acne and / or hirsutism and / or androgenic

pattern of alopecia. Biochemical hyperandrogenism was defined as elevated testosterone .

Exclusion criteria:

1. Patients on treatment for Hypothyroidism, Oral contraceptives, Anticonvulsants, Metformin.
2. Other conditions mimicking PCOS were ruled out by detailed history and complete physical examination.

PHYSICAL EXAMINATION:

1) Height (cms): Measured with Inch tape.

2) Weight (kg): Measured in weighing machine.

3) Waist circumference: According to WHO, the waist circumference should be measured at the midpoint between the lower margin of the last palpable rib and top of the iliac crest using a stretch resistant tape [159]. This point will be roughly 1 inch above the umbilicus.

   (Ethnic specific value for Asian Indian Female : 80 cm) [160]

4) Hip circumference: According to WHO, the Hip circumference should be measured around the widest portion of the buttock, with inch tape parallel to the floor.

BIOCHEMICAL INVESTIGATIONS:

1) Fasting Blood Glucose.
2) 2hrs post glucose.
3) Fasting (TSH) Thyroid Stimulating Hormone.

4) Fasting Insulin.

5) Total Testosterone.

CALCULATED PARAMETERS:

1) HOMEOSTATIC MODEL ASSESSMENT OF INSULIN RESISTANCE (HOMA-IR):

A valid method of assessing Insulin Resistance is HOMA-IR.

Homeostatic Assessment Model of Insulin Resistance (HOMA-IR)

$$= \frac{\text{Fasting plasma Insulin (μU/mL) * Fasting plasma glucose (mmol}}{22.5}$$

2) BODY MASS INDEX (BMI) / QUETLET INDEX:

It is defined as the individual's body mass divided by the square of their height, with the value universally being given in units of Kg/M^2

$$BMI = \frac{\text{Weight in Kg}}{\text{Height in Metre}^2}$$

WHO BMI CLASSIFICATION [162]

BMI RANGE	CATEGORY
Very severely underweight	<15
Severely underweight	15-16
Underweight	16-18.5
Normal	18.5-25
Overweight	25-30
Obese class 1 (moderately obese)	30-35
Obese class 2 (severely obese)	35-40
Obese class 3 (very severely obese)	>40

3) WAIST/ HIP RATIO: It is the ratio of Waist circumference to Hip circumference.It is an Index of central obesity.

Normal: <0.85.

WHO STEPS: states that abdominal obesity is defined as a waist/Hip ratio >0.9 for male and >0.85 for female, or Body Mass Index >30.[161]

Patient preparation:

Patient was advised to come in 8-10 hrs fasting. They were adviced to take their dinner by 8 PM on the previous day, then no food should (carbohydrate diet) be taken. Next day morning tea / coffee / any carbohydrate drink should avoided. Patient was to sit in a chair calmly and with stretched forearm. Blood was drawn by 7-8AM under aseptic precaution. Fasting sample was collected for Glucose, Insulin, Testosterone, TSH, then 75gm of glucose dissolved in 300ml of water was given to the patients and allowed to take for a period of 5 min. Post glucose sugar sample was taken 2 hrs after drinking glucose.In the mean time patients were instructed not to do exercise or walking for long distance.

BLOOD SAMPLING:

Fasting Glucose:

5mL of blood was drawn from cubital vein. The collected blood was transferred in to a plain test tube and kept aside without any disturbance. After the clot was formed completely (15-20 min) it was centrifuged for 15-20 min at 2000 rpm and the clear serum was separated and aliquoted for Glucose and hormone assay. Serum for hormone assay was stored in deep freezer at -20^0c until it was processed.

2 hrs Post Glucose:

Two hours after Glucose load, 1mL blood was drawn from the cubital vein, and then transferred to a plain clean test tube. The blood collected was kept undisturbed for few minutes. After separation of the clot, it was subjected to centrifugation for 15 min at 2000 rpm. Clear serum was taken for estimating Glucose level.

FASTING BLOOD GLUCOSE ESTIMATION:

METHOD: Trinder's GLUCOSE OXIDASE- PEROXIDASE-using ERBA Glucose reagent.

PRINCIPLE :

Glucose present in the sample is oxidised by (GOD) Glucose Oxidase in the reagent to Gluconic acid and hydrogen peroxide. This hydrogen peroxide produced is cleaved in to water and nasent oxygen by peroxidase in the reagent. The nasent oxygen binds with the substrate 4-Amino Anti pyrin (4-AAP) and phenol. After the Incubation period it gives pink colored complex Quinoneimine dye. The intensity of

the colour formed is directly propotional to the concentration of glucose present in the sample. The color formed is measured at 520 nm by Spectrophotometer.

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\textit{Glucose-oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4-AAP} \xrightarrow{\textit{Peroxidase}} \text{Quinoneimine dye} + H2O$$

(Pink Color)

ASSAY PARAMETERS:

Mode: End point

Wavelength: 505nm

Sample volume (µL): 10

Reagent volume (µL): 1000

Reaction time: 5 min

Linearity: 500 mg/dL

Standard concentration: 100 mg/dL

Blank absorption limit: 0.2

PROCEDURE:

1000µL of GOD-POD reagent (ERBA) was taken in a dry clean test tube, to that 10µL of the sample was added and mixed well. It was kept under incubation for 10 min. After 10 min, reading was taken in a spectrophotometer at 510nm. The concentration is directly propotional to the intensity of the color formed.

Reference Interval:

Fasting Blood Glucose = 70-110 mg/dL

Post Glucose = <140 mg/dL.

CHEMILUMINESCENCE[164,165]

Principle:

A chemiluminescence reaction is any chemical reaction in which one of the products of the reaction is light. The enzyme peroxidise can react with molecules such as luminal (5-amino-2,3-dihydro-1,4-phthalazinedione) to yield light as part of the reaction product. The luminol reaction results in photon emission in the range of 400-450nm. The low photon yield is enhanced by using enhancers (luziferin, 6-hydroxybenzothiazole), the reaction can be followed for many minutes with several thousand times increase in photon output.

The peroxidase is often part of an enzyme labelled immunoassay system in which peroxidase is the label. The reaction can be measured by very sensitive photomultiplier tube.

The major advantage of the technique is : High sensitivity.

The major disadvantage is : It needs separation step to remove matrices.

Other dye system that produces quantitative chemiluminescence reaction use aromatic acridinium esters and the dioxetanes. The acridinium esters are oxidised most often by hydrogen peroxide to yield light, whereas the dioxetanes are made into stable phosphate ester derivatives that, when hydrolysed, become spontaneously degraded, yielding light as one of the product. A light beam at 680nm is used to create singlet oxygen atom O^2*. These very reactive singlet oxygen atoms react with a dye to yield light, which in turn is transferred to a flurophore dye that emits fluorescence light.

Bio-luminescence: The naturally occurring chemiluminescence.

Electro-chemiluminescence: This is based on the formation of an exited state chemical intermediate that returns to the ground state by emitting a photon.

INSULIN ASSAY:

METHOD: AUTOMATED IMMUNOASSAY TECHNIQUE-CHEMILUMINESCENCE using Siemens ADVIA Centaur XP system.

PRINCIPLE:

Insulin assay, is a two site sandwich immune assay using direct chemiluminescent technology, which uses constant amount of two antibodies. The first antibody, in the LITE reagent, is a monoclonal Mouse anti-Insulin antibody labelled with acridinium ester. The second antibody, in the solid phase, is a monoclonal Mouse anti Insulin antibody, which is covalently bounded to paramagnetic particle.

PROCEDURE:

Before estimation, the sample was completely thawed, mixed then placed in the rack and it was ensured of no fibrin or any other particulate matters or bubbles in the sample.

1) The sample tube was labelled properly with the sample number and placed in the sample rack in queue provided in the system.
2) The reagent is also kept in the reagent tray/disc. The position for the reagent was fed into the system.
3) 25µL of the sample was dispensed into a cuvette.
4) 50µL of Lite reagent was added into the cuvette and incubated for 5 min at 37^0c

5) Now dispense 250µL of solid phase and incubate for 2.5 min at 37^0c.

6) After the incubation time, the magnetic particles were washed with reagent water.

7) Next, 300µL of each acid and base reagent were dispensed to initiate chemiluminescent reaction.

The chemical light reaction was measured by the Photo Multiplier Tube (PMT). A direct relationship exists between the Insulin present in the sample detected by the system and the amount of Relative Light Units (RLUs)

REAGENTS IN ADVIA CENTAUR:

1. LITE REAGENT:

5mL / reagent pack: monoclonal mouse anti insulin antibody (0.24µg/mL) labelled with Acridinium ester in buffered saline with bovine serum albumin, Sodium azide (<0.1%), and preservatives.

2. SOLID PHASE:

25mL/ reagent pack: monoclonal mouse anti Insulin antibody (6.0µg/mL) covalently coupled with paramagnetis particles in buffered saline with bovine serum albumin, sodium azide (<0.1%), and preservatives.

3. INSULIN DILUENT:

10.0mL / reagent pack: buffered saline with casein, potassium thiocyanate (3.89%), sodium azide (<0.1%), and preservatives.

CALIBRATION: **Two point calibration interval** Using IRI calibrator, two point calibrations was done using a **Calibrator Assigned Value card**.

QUALITY CONTROL:

Bio-Rad Immnuoassay plus control 1, 2 & 3 were used.

Each control was reconstituted with 5.0mL of CLRW (Clinical Laboratory Reagent Water).

The reportable range in ADVIA centaur Insulin assay is 1-1500 mU/L.

Reference Interval : 3.3-22.1 mU/L

Interferences: Hemolysis, Lipemia, Jaundice shows <6% changes in results.

TOTAL TESTOSTERONE ASSAY:

METHOD: AUTOMATED IMMUNOASSAY TECHNIQUE – CHEMILUMINESCENCE using seimen's ADVIA Centaur XP system.

PRINCIPLE:

Competitive Immunoassay using direct Chemiluminesence technology. Testosterone in the patient sample competes with Acridinium ester- labelled testosterone in the LITE reagent for a limited amount of Polyclonal rabbit anti testosterone antibody bounded to monoclonal mouse anti- rabbit antibody, which is coupled to paramagnetic particles in the solid phase. The assay uses Testosterone releasing agent to release bound testosterone from the endogenous binding protein in the sample.

PROCEDURE:

Samples were loaded in the entry queue and analysed as follows

1) 15µL of sample and 50µL of Releasing agent were dispensed into a cuvette.

2) After ensuring that the Probe wash is perfect, now dispense 50µL of LITE Reagent and 300µL of solid phase and incubate for 5 minutes at 37C.

3) It separates, aspirates and washes the cuvette with reagent water.

4) Dispenses 300µL each of Acid reagent and Base reagent to initiate the chemiluminescent reaction.

5) An inverse relationship exists between the amount of Testosterone present in the sample and the amount of Relative Light Units.(RLUs) detected by the system.

REAGENTS:

ADVIA Centaur TSTO Ready pack (Primary reagent pack).

1) **LITE REAGENT:** 2.5mL/ reagent pack has acridinium ester-labelled testosterone (~ 3.2 ng/mL) in buffered saline with preservatives. (maintained at 2-8^{0}C)

2) **SOLID PHASE:** 15mL/ reagent pack has polyclonal rabbit anti testosterone antibody (~0.03µg/mL) bound to monoclonal mouse anti-rabbit antibody (0.53µg/mL) covalently coupled to paramagnetic particles in buffered saline with sodium azide (0.1%) and preservatives. Stored at 2-8C.

3) **PROBE WASH:** 10mL/ reagent pack has buffered saline with sodium azide (0.1%) and preservatives.

ADVIA Centuar TSTO Ready Ancillary pack (Releasing agent pack).

It has 5.0mL/ reagent pack has steroid releasing agent (~0.1µg/mL) in buffered saline with Sodium azide (0.1%) and preservatives. It is also stored in 2-8^{0}C.

ADVIA Centuar : Ready pack Ancillary reagent pack:

Multi-Diluent 3 has 5.0mL/ reagent pack. It has human plasma with Sodium azide (0.1%) stored at 2-8^{0}C.

CALIBRATION**:** **Two point calibration**

QUALITY CONTROL:

Two levels of Quality control materials were used and treated along with all Sample. Bayer Health Care material were used. Expiry date was checked.

DILUTION : For serum having high value of Testosterone (>1500ng/dL) automatic dilution was programmed in the system using Multi-Diluent 3. Dilution factor 5 is applied.

Reference Interval:

Young Female: 14-76 ng/dL.

Interference: Hemolysed, Lipemia, Jaundice sample showed interference in the assay.

THYROID STIMULATING HORMONE (TSH) / THYROTROPIN ASSAY:

METHOD: AUTOMATED IMMUNOASSAY TECHNIQUE – CHEMILUMINESCENCE using Siemens ADVIA centaur XP system.

PRINCIPLE:

A 3rd generation TSH assay is a Non-Competitive two site Sandwich immunoassay using direct chemiluminometric technology. It uses constant amount of two antibodies. The first antibody, in the LITE reagent is a monoclonal mouse anti-TSH antibody labelled with acridinium Ester. The second antibody, in the solid phase, is a polyclonal sheep anti-TSH antibody which is covalently coupled to paramagnetic particles. These two antibodies holds the analyte of interest tightly. Seperation of magnetic field and washing removes materials unbound to the solid phase. Chemiluminescent substrate added to the reaction cuvette generates photons (light), light generated by the reaction is measured by the Luminometer. The photon production is propotional to the amount of conjugate bound to solid support. A direct relationship exists between the amount of TSH present in the patient sample and the amount of relative light units (RLUs) detected by the system.

PROCEDURE:

Serum was thawed completely and mixed well before keeping in the sample tray.

Fibrin, particulate matter and bubbles were checked before analysis.

1) The sample tube was labelled properly with the sample number and placed in the sample rack.
2) The reagent is also kept in the reagent tray/disc. The position for the reagent in fed in the system.

3) 200µL of the sample was dispensed into a cuvette.
4) 50µL of LITE reagent and 225µL of solid phase were dispensed into the cuvette and incubated for 7.5 min at 37 c.
5) After incubation, it separates, aspirates and washes the cuvette with reagent water.
6) Then, it dispenses 300µL each of acid and base reagent to initiate chemiluminescent reaction.
7) A direct relationship exists between the amount of human TSH present in the sample and the amount of Relative Light Units (RLUs) detected by the system.

REAGENTS :

TSH Ready packs Primary reagent pack:

LITE REAGENT:

5mL /Reagent pack has monoclonal mouse anti TSH antibody (~333 ng/mL) labeled with acridinium ester in phosphate buffered saline with sodium azide (<0.1%) and preservatives stored at 2-8^{0}C

SOLID PHASE:

22.5mL/ Reagent pack has polyclonal sheep anti TSH antibody (~43µg/mL) covalently coupled with paramagnetis particles in phosphate buffered saline with protein stablizers, sodium Azide (<0.11%) and preservatives stored at 2-8^{0}C.

Ready pack ancillary reagent pack:

MULTI-DILUENT 1:

25 mL / reagent pack has equine serum with sodium azide (0.1%), and preservatives stored at 2-8C. Dilution factor 2, 5. Dilution point : ≤ 150µIU/mL.

CALIBRATION**:** **Two point calibration** was done using a CALIBRATOR ASSIGNED VALUE CARD.

QUALITY CONTROL:

Bayer Health care two level quality control samples were run.

Each control was diluted with 5.0mL of CLRW (Clinical Laboratory Reagent Water)

Reference Interval: 3.5-5.5µU/mL

Interferences: Hemolysis, Lipemia, Jaundice shows <6% changes in results.

STATISTICAL ANALYSIS

Statistical analysis is done by SPSS software version 17.

1. Student t- test & Chi-square test were applied for comparing Age, BMI, WHR, FBS, PPBS, Ovarian volume, Testosterone, HOMA-IR between the two groups of PCOS patients (with Euthyroid and Hypothyroidism).
2. Pearson co-efficient correlation was applied for TSH & Testosterone, Total Testosterone & WHR.

Data of BMI W/H Ratio FBS, PPBS, TSH, Insulin, Testosterone for study Population

S.NO	AGE	C.CENTR	MAIN C/O	ASSO C/O	MEN.	MARI	FAM.	WAIST	HIP	W/H	HT(CM	WT(Kg)	BMI	NECI	UGS	OV.V	OV.V	FBS m	PPBS	F.IN(mIU	TSH(µIU	TESTO(r	FBS(mm	HOMA-IF
1	25	EGM	OLIGO,INFER	acne, hirs,	13	1	NO/0	86	107	0.80	140	62	31.63	N/0	B/L PCO	10	6	87	121	13.6	1.97	91.6	4.83	2.92
2	20	EGM	OLIGO,INFER		14	1	0	73	90	0.81	150	55	24.44	0	B/L PCO	11	11	86	104	12.6	4.33	26.2	4.78	2.68
3	21	EGM	OLIGO,INFER,W	ACne,	18	1	0	72	92	0.78	156	55	22.60	Goit1	B/L PCO	11	12	105	105	6.69	1.3	33	5.83	1.73
4	21	EGM	OLIGO,WT	HIRSUITISM	12	0	YES/1	95	117	0.81	155	63	26.22	1	B/L PCO	11	9	82	108	16	3.1	40	4.56	3.24
5	28	EGM	OLIGO, WT	ACNE,HIRSUITI	14	1	0	86	115	0.75	162	76	28.96	1	B/L PCO	10	10	96	126	16.6	2.49	43.03	5.33	3.93
6	23	EGM	OLIGO, WT	ACNE	14	0	1	82	98	0.84	154	50	21.08	0	B/L PCO	9	10	80	114	4.5	1.23	96.5	4.44	0.89
7	34	EGM	INFERTILITY	HIRSUITISM	14	1	0	85	103	0.83	170	75	25.95	0	B/L PCO	15	11	146	254	11.4	2.45	30	8.11	4.11
8	27	ENDO	OLIGO,INFER,W	ACNE,HIRSU,W	15	1	1	108	116	0.93	164	81	30.12	0	B/L PCO	9	12	115	123	29.73	2.03	141.7	6.39	8.44
9	28	EGM	OLIGO,INFER,W	ACNE	14	1	0	95	117	0.81	155	83	34.55	1	B/L PCO	10	9	89	126	23.68	11.2	89	4.94	5.20
10	23	ENDO	OLIGO,INFER,W	ACNE	12	1	0	92	107	0.86	155	100	41.62	1	B/L PCO	10	11	100	115	6.01	4.1	10.4	5.56	1.48
11	30	EGM	OLIGO,INFER,W	HIRSUITISM	13	1	0	84	92	0.91	149	59	26.58	0	B/L PCO	11	13	84	138	16	1	84.8	4.67	3.32
12	20	EGM	INFERTILITY	ACNE	13	0	0	70	84	0.83	162	48	18.29	0	B/L PCO	14	14	76	104	10	3	42	4.22	1.88
13	31	EGM	INFERTILITY	ACNE	16	1	1	75	83	0.90	156	39	16.03	0	B/L PCO	13	10	86	152	7.4	1	22.8	4.78	1.57
14	22	ENDO	OLIGOINFER,WT	ACNE	13	1	0	96	112	0.86	155	76	31.63	1	B/L PCO	9	9	80	112	23.6	10.3	40.1	4.44	4.66
15	23	EGM	OLIGO	ACNE	15	1	1	80	98	0.82	159	55	21.76	0	B/L PCO	12	13	96	115	14.9	4.8	44.5	5.33	3.53
16	33	EGM	OLIGO, INFER		15	1	0	92	114	0.81	160	76	29.69	0	B/L PCO	13	13	90	178	247	1.13	49.5	5.00	54.89
17	22	ENDO	AMENO,WT,INF	HIRSUITISM	14	0	0	94	99	0.95	170	73	25.26	0	B/L PCO	8	9	100	112	22	0.46	76.8	5.56	5.43
18	18	ENDO	AMENO,WT	HIRSUITISM	14	0	1	85	91	0.93	155	65	27.06	0	B/L PCO	14	9	72	89	6.1	1.3	17	4.00	1.08
19	30	EGM	AMENO		14	1	0	76	89	0.85	148	53	24.20	0	B/L PCO	13	19	97	140	6.9	2.4	48.3	5.39	1.65
20	23	ENDO	OLIGO,WT	ACNE	13	0	1	72	100	0.72	162	70	26.67	0	B/L PCO	8	11	79	115	10.5	0.46	93	4.39	2.05
21	28	ENDO	AMENO,WT		13	0	0	77	98	0.79	150	56	24.89	0	U/L PCO	12	9	72	126	9.26	4.2	56.3	4.00	1.65
22	32	EGM	OLIGO,		13	1	0	89	109	0.82	162	64	24.39	0	B/L PCO	10	12	108	163	31.4	2.32	26	6.00	8.37
23	28	EGM	INFERTILITY, W	HIRSUITISM	14	0	0	82	109	0.75	161	70	27.01	1	B/L PCO	12	11	107	128	11.1	8.29	17.1	5.94	2.93
24	30	EGM	OLIGO,		16	1	0	78	88	0.89	150	50	22.22	0	B/L PCO	11	13	68	143	18.7	1.34	67.7	3.78	3.14
25	28	EGM	OLIGO,WT		14	1	0	101	116	0.87	156	78	32.05	0	B/L PCO	12	14	79	129	10.5	1.95	43.1	4.39	2.05
26	23	EGM	INFERT, WT	ACNE	16	1	0	80	102	0.78	148	62	28.31	0	B/L PCO	10	11	53	116	10	1.27	38.9	2.94	1.31
27	24	ENDO	OLIGO,AMENO,W	HIRSUITISM	12	1	0	110	122	0.90	155	85	35.38	0	U/L PCO	30	16	74	119	7.6	2.12	67.9	4.11	1.39
28	30	ENDO	OLIGO,AMENO,W	ACNE,HIRSUITI	14	1	0	111	129	0.86	153	96	41.01	0	B/L PCO	10	8	78	144	13	3.5	41	4.33	2.50
29	30	EGM	OLIGO,INFER,WT		14	1	0	94	105	0.90	150	64	28.44	0	U/L PCO	9	14	66	155	28	3.5	10.9	3.67	4.56
30	33	ENDO	,AMENO,INFER,	HIRSUITISM	14	1	0	85	101	0.84	160	65	25.39	1	B/L PCO	13	13	72	318	30	8.6	11	4.00	5.33
31	17	EGM	OLIGO,WT	HIRSUITISM	12	0	0	84	91	0.92	156	55	22.60	0	U/L PCO	13	14	86	74	11.8	1.7	51.7	4.78	2.51
32	24	EGM	OLIGO,INFER,W	HIRSUITISM	15	1	0	115	125	0.92	150	90	40.00	1	B/L PCO	11	11	69	120	16.8	4.88	59.9	3.83	2.86
33	29	EGM	OLIGO,INFER	ACNE	12	1	0	97	113	0.86	150	65	28.89	0	B/L PCO	10	10	92	133	24	1.9	90	5.11	5.45
34	24	EGM	OLIGO,AMENO,W	RECUR-PREG L	12	1	0	100	103	0.97	161	75	28.93	0	B/L PCO	11	17	92	120	36	3.4	98.8	5.11	8.18
35	17	ENDO	OLIGO,WT	ACNE,HIRUITIS	11	0	0	71	90	0.79	161	57	21.99	0	B/L PCO	11	12	70	88	28	2.72	84.1	3.89	4.84
36	23	EGM	OLIGO,AMENO,W	ACNE	13	1	0	86	106	0.81	148	70	31.96	0	B/L PCO	10	11	96	116	52	25.4	69.4	5.33	12.33
37	20	EGM	OLIGO,AMENO,I	ACNE	12	1	0	81	94	0.86	140	57	29.08	1	B/L PCO	16	15	72	104	30.4	5.5	77.5	4.00	5.40
38	30	EGM	OLIGO,INFER	ACNE	11	1	0	78	93	0.84	150	54	24.00	0	B/L PCO	12	9	80	146	26.8	7.8	65.9	4.44	5.29
39	30	EGM	OLIGO,INFER,WT		18	1	0	88	95	0.93	150	50	22.22	0	B/L PCO	12	10	132	176	30	2.36	88.1	7.33	9.78
40	22	EGM	OLIGO,WT	HIRSUITISM	13	1	1	79	101	0.78	152	64	27.70	0	B/L PCO	9	10	84	112	20	6.6	22	4.67	4.15
41	27	EGM	OLIGO,WT	ACNE,GALAC	13	1	0	88	98	0.90	140	56	28.57	1	U/L PCO	11	11	88	124	22	2.55	96	4.89	4.78
42	32	EGM	WT	HIRSUITISM	12	1	0	105	123	0.85	159	95	37.58	0	B/L PCO	15	10	81	172	35	6.9	12.7	4.50	7.00

43	26	ENDO	OLIGO,AMENO,INFER		12	1	1	82	96	0.85	148	57	26.02	0	B/L PCO	10	9	72	122	24	28.5	90	4.00	4.27
44	19	EGM	AMENO,WT	ACNE,HIRSUITI	14	0	1	91	113	0.81	160	80	31.25	1	B/L PCO	15	16	129	140	11.34	1.31	60	7.17	3.61
45	27	ENDO	AMENO,INFER,WT		15	1	0	100	118	0.85	142	75	37.20	0	B/L PCO	12	13	91	124	18.9	3.17	56	5.06	4.25
46	21	EGM	OLIGO,WT	HIRSUITISM	13	0	0	104	117	0.89	163	85	31.99	0	U/L PCO	11	10	82	111	26.8	1.85	80.6	4.56	5.43
47	23	EGM	OLIGO,WT	ACNE	13	0	0	100	116	0.86	166	100	36.29	0	B/L PCO	12	14	72	117	12	5.11	88	4.00	2.13
48	22	EGM	OLIGO,INFER	ACNE	14	1	1	94	108	0.87	148	58	26.48	0	B/L PCO	10	12	105	114	24	4.46	98	5.83	6.22
49	21	ENDO	OLIGO	ACNE	16	1	0	90	112	0.80	153	58	24.78	0	B/L PCO	12	10	80	112	16	100	83	4.44	3.16
50	19	EGM	OLIGO,WT		14	0	1	70	91	0.77	145	51	24.26	1	U/L PCO	13	9	126	134	21	3.49	51.4	7.00	6.53
51	28	EGM	OLIGO,WT	ACNE	15	0	0	102	146	0.70	148	85	38.81	0	B/L PCO	12	not v	70	129	18	2.2	21	3.89	3.11
52	26	EGM	OLIGO,INFER,WT		13	1	0	99	135	0.73	161	70	27.01	0	B/L PCO	11	cyst	91	122	25	3.73	41.8	5.06	5.62
53	23	EGM	INFER	HIRSUITISM	14	1	0	94	132	0.71	152	76	32.89	0	B/L PCO	11	10	96	117	20	6.2	61	5.33	4.74
54	27	EGM	OLIGO,INFER	ACNE	13	1	0	90	130	0.69	155	59	24.56	0	B/L PCO	10	11	146	124	20	1	62.5	8.11	7.21
55	25	EGM	OLIGO,INFER,W	RECUR-PREG L	14	1	1	76	90	0.84	144	60	28.94	0	B/L PCO	11	9	83	122	13	6.9	71	4.61	2.66
56	22	EGM	OLIGO,INFER,W	ACNE	14	1	0	97	120	0.81	164	52	19.33	0	B/L PCO	9	11	78	114	34	1.1	22	4.33	6.55
57	28	EGM	OLIGO,WT		16	1	0	85	100	0.85	168	68	24.09	0	B/L PCO	10	8	96	130	10.9	4	98.1	5.33	2.58
58	32	EGM	OLIGO,INFER,W	HIRSUITISM	15	1	0	108	145	0.74	140	69	35.20	0	U/L PCO	12	13	91	175	13.24	1.2	29	5.06	2.97
59	20	EGM	OLIGO,AMENO,INFER		13	1	0	66	88	0.75	143	47	22.98	0	B/L PCO	15	13	74	104	7.7	2.7	36.3	4.11	1.41
60	28	EGM	OLIGO,INFER,W	ACNE	28	1	1	107	147	0.73	162	79	30.10	0	B/L PCO	11	12	87	133	31.6	4	31.9	4.83	6.79
61	17	ENDO	OLIGO,WT	ACNE,HIR	13	0	0	98	139	0.71	153	98	41.86	0	U/L PCO	10	13	97	89	74.7	5.5	94.3	5.39	17.89
62	18	ENDO	AMEN,WT	ACNE	13	0	0	93	123	0.76	154	76	32.05	1	B/L PCO	11	13	96	90	8.01	1.17	29	5.33	1.90
63	20	EGM	OLIGO,INFER	HIRSUITISM	24	1	0	88	121	0.73	162	67	25.53	0	B/L PCO	22	19	86	104	33.8	4	55.7	4.78	7.18
64	30	EGM	OLIGO,INFER,W	HIRSUITISM	13	1	0	84	92	0.91	149	59	26.58	0	B/L PCO	12	11	84	147	16	1	84.8	4.67	3.32
65	19	EGM	INFERTILITY	ACNE	13	0	0	70	84	0.83	162	48	18.29	0	B/L PCO	13	10	76	104	10	3	42	4.22	1.88
66	33	EGM	INFERTILITY	ACNE	16	1	1	75	83	0.90	156	39	16.03	0	B/L PCO	12	14	86	160	7.4	1	22.8	4.78	1.57
67	22	ENDO	OLIGOINFER,WT	ACNE	13	1	0	96	112	0.86	155	76	31.63	1	B/L PCO	10	11	80	114	23.6	10.3	40.1	4.44	4.66
68	23	EGM	OLIGO	ACNE	15	1	1	80	98	0.82	159	55	21.76	0	B/L PCO	12	15	96	118	14.9	4.8	44.5	5.33	3.53
69	33	EGM	OLIGO, INFER		15	1	0	92	114	0.81	160	76	29.69	0	B/L PCO	16	11	90	178	247	1.13	49.5	5.00	54.89
70	21	EGM	OLIGO, INFER		15	1	0	78	98	0.80	154	61	25.72	1	B/L PCO	10	8	94	112	17	4.8	38	5.22	3.95
71	22	ENDO	AMENO,WT,INF	HIRSUITISM	14	0	0	94	99	0.95	170	73	25.26	0	B/L PCO	12	14	100	114	22	0.46	76	5.56	5.43
72	18	ENDO	AMENO,WT	HIRSUITISM	14	0	1	85	91	0.93	155	65	27.06	0	B/L PCO	15	10	72	98	6.1	1.3	71	4.00	1.08
73	21	EGM	OLIGO, INFER		15	1	0	78	98	0.80	154	61	25.72	1	B/L PCO	12	14	94	112	17	4.8	38.9	5.22	3.95
MEAN	24.8							88.88	107.8	0.83	154.6	67	28.08			12.1	12	87.68	128.1	28.047	5.8225	55.8186	4.87	6.18
TD DE	4.7							11.28	15.7	0.07	7.185	14.09	5.78			3.08	2.5	17.39	34.93	39.346	12.204	27.837	0.97	8.82

Data of BMI W/H Ratio FBS, PPBS, TSH, Insulin, Testosterone for Hypothyroid PCOS Women

Sl.No	Waist	Hip	W/H rat	Ht	Wt	BMI	GOITRE	USG	LO	RO	FBS	PPBS	F.Ins	TSH	TESTO	FBS mmol	HOMA-IR	BIO-TEST
1	95	117	0.81	155	83	34.55	1	B/L PCO	13	14	89	114	23.68	11.2	89.8	4.94	5.20	31.43
2	96	112	0.86	155	76	31.63	1	B/L PCO	11	12	80	89	23.6	10.3	40.1	4.44	4.66	14.035
3	82	109	0.75	161	70	27.01	1	B/L PCO	14	9	107	129	11.1	8.29	71.9	5.94	2.93	25.165
4	85	101	0.84	160	65	25.39	1	B/L PCO	10	13	72	147	30	8.6	72	4.00	5.33	25.2
5	86	106	0.81	148	70	31.96	0	B/L PCO	11	13	96	172	52	25.4	69.4	5.33	12.33	24.29
6	81	94	0.86	140	57	29.08	1	B/L PCO	15	10	72	138	30.4	5.5	77.5	4.00	5.40	27.125
7	78	93	0.84	150	54	24.00	0	B/L PCO	15	16	80	118	26.8	7.8	65.9	4.44	5.29	23.065
8	79	101	0.78	152	64	27.70	0	B/L PCO	11	11	84	145	20	6.6	45.6	4.67	4.15	7.7
9	105	123	0.85	159	95	37.58	0	B/L PCO	16	15	81	126	35	6.9	67.9	4.50	7.00	23.765
10	82	96	0.85	148	57	26.02	0	B/L PCO	15	13	72	90	24	28.5	90	4.00	4.27	31.5
11	90	112	0.80	153	58	24.78	0	B/L PCO	14	14	80	88	16	100	76.8	4.44	3.16	26.88
12	94	132	0.71	152	76	32.89	0	B/L PCO	13	10	96	124	20	6.2	61	5.33	4.74	21.35
13	76	90	0.84	144	60	28.94	0	B/L PCO	22	19	83	105	13	6.9	71	4.61	2.66	24.85
14	98	139	0.71	153	98	41.86	0	U/L PCO	13	9	97	130	74.7	5.5	94.3	5.39	17.89	33.005
15	96	112	0.86	155	76	31.63	1	B/L PCO	11	12	80	89	23.6	10.3	40.1	4.44	4.66	14.035
16	100	116	0.86	166	100	36.29	0	B/L PCO	11	9	72	112	12	5.11	88	4.00	2.13	30.8
MEAN	88.938	109.6	0.82	153.2	72.44	30.71			13.4	12	84	119.8	27.24	15.82	70.08125	4.66	5.74	24.01219
STD DEV	8.642	13.48	0.05	6.327	14.56	4.90			2.85	2.7	10	23.44	15.65	22.72	16.40024	0.57	3.86	6.767065

Data of BMI W/H Ratio FBS, PPBS, TSH, Insulin, Testosterone for Euthyroid PCOS Women

Sl.No	WAIST	HIP	W/H	HT(CM)	WT(Kg	BMI	GOITR	UGS	OV.VOL (L	OV.VOL.(R	FBS mg%	PPBS mg%	F.IN(mIU/L	TSH(µIU/L)	TESTO(ng)	FBS(mmol/	HOMA-IR
1	86	107	0.80	140	62	31.63	0	B/L PCO	11	10	87	129	13.6	1.97	91.6	4.83	2.92
2	73	90	0.81	150	55	24.44	0	B/L PCO	12	10	56	128	12.6	4.33	26.2	3.11	1.74
3	72	92	0.78	156	55	22.60	1	B/L PCO	9	9	105	126	6.69	1.3	33	5.83	1.73
4	95	117	0.81	155	63	26.22	1	B/L PCO	9	10	62	74	16	3.1	40	3.44	2.45
5	86	115	0.75	162	76	28.96	1	B/L PCO	10	8	96	111	16.6	2.49	43.03	5.33	3.93
6	82	98	0.84	154	50	21.08	0	B/L PCO	12	14	80	115	4.5	1.23	96.5	4.44	0.89
7	85	103	0.83	170	75	25.95	0	B/L PCO	8	9	96	112	11.4	2.45	30	5.33	2.70
8	108	116	0.93	164	81	30.12	0	B/L PCO	9	11	115	175	29.73	2.03	141.7	6.39	8.44
9	92	107	0.86	155	100	41.62	1	B/L PCO	10	11	100	112	6.01	4.1	10.4	5.56	1.48
10	84	92	0.91	149	59	26.58	0	B/L PCO	9	10	84	124	16	1	84.8	4.67	3.32
11	70	84	0.83	162	48	18.29	0	B/L PCO	12	14	76	104	10	3	42	4.22	1.88
12	75	83	0.90	156	39	16.03	0	B/L PCO	10	12	86	104	7.4	1	22.8	4.78	1.57
13	80	98	0.82	159	55	21.76	0	B/L PCO	8	11	96	117	14.9	4.8	44.5	5.33	3.53
14	92	114	0.81	160	76	29.69	0	B/L PCO	12	13	90	114	247	1.13	49.5	5.00	54.89
15	94	99	0.95	170	73	25.26	0	B/L PCO	10	11	100	116	22	0.46	76.8	5.56	5.43
16	85	91	0.93	155	65	27.06	0	B/L PCO	10	11	72	104	6.1	1.3	17	4.00	1.08
17	76	89	0.85	148	53	24.20	0	B/L PCO	10	11	97	119	6.9	2.4	48.3	5.39	1.65
18	72	100	0.72	162	70	26.67	0	B/L PCO	12	14	79	108	10.5	0.46	13	4.39	2.05
19	77	98	0.79	150	56	24.89	0	U/L PCO	11	10	72	104	9.26	4.2	56.3	4.00	1.65
20	89	109	0.82	162	64	24.39	0	B/L PCO	12	15	168	318	31.4	2.32	26	9.33	13.03
21	78	88	0.89	150	50	22.22	0	B/L PCO	30	16	68	112	18.7	1.34	67.7	3.78	3.14
22	101	116	0.87	156	78	32.05	0	B/L PCO	11	11	79	114	10.5	1.95	43.1	4.39	2.05

23	80	102	0.78	148	62	28.31	0	B/L PCO	11	17	53	98	10	1.27	38.9	2.94	1.31
24	110	122	0.90	155	85	35.38	0	U/L PCO	10	6	74	152	7.6	2.12	67.9	4.11	1.39
25	111	129	0.86	153	96	41.01	0	B/L PCO	10	9	78	133	13	3.5	41	4.33	2.50
26	94	105	0.90	150	64	28.44	0	U/L PCO	11	9	66	144	28	3.5	10.9	3.67	4.56
27	84	91	0.92	156	55	22.60	0	U/L PCO	11	cyst	86	115	11.8	1.7	51.7	4.78	2.51
28	115	125	0.92	150	90	40.00	1	B/L PCO	9	12	69	112	16.8	4.88	59.9	3.83	2.86
29	97	113	0.86	150	65	28.89	0	B/L PCO	11	11	92	163	24	1.9	90	5.11	5.45
30	100	103	0.97	161	75	28.93	0	B/L PCO	12	13	92	133	36	3.4	98.8	5.11	8.18
31	71	90	0.79	161	57	21.99	0	B/L PCO	10	11	70	146	28	2.72	84.1	3.89	4.84
32	88	95	0.93	150	50	22.22	0	B/L PCO	12	9	132	178	30	2.36	88.1	7.33	9.78
33	88	98	0.90	140	56	28.57	1	U/L PCO	10	9	88	120	22	2.55	96	4.89	4.78
34	91	113	0.81	160	80	31.25	1	B/L PCO	10	10	156	178	11.34	1.31	60	8.67	4.37
35	100	118	0.85	142	75	37.20	0	B/L PCO	12	11	91	120	18.9	3.17	56	5.06	4.25
36	104	117	0.89	163	85	31.99	0	U/L PCO	12	not visible	82	122	26.8	1.85	80.6	4.56	5.43
37	94	108	0.87	148	58	26.48	0	B/L PCO	12	14	105	160	24	4.46	98	5.83	6.22
38	70	91	0.77	145	51	24.26	1	U/L PCO	10	8	126	140	21	3.49	51.4	7.00	6.53
39	102	146	0.70	148	85	38.81	0	B/L PCO	11	12	70	114	18	2.2	21	3.89	3.11
40	99	135	0.73	161	70	27.01	0	B/L PCO	10	10	91	143	25	3.73	41.8	5.06	5.62
41	90	130	0.69	155	59	24.56	0	B/L PCO	11	13	146	254	20	1	62.5	8.11	7.21
42	97	120	0.81	164	52	19.33	0	B/L PCO	13	19	78	102	34	1.1	22	4.33	6.55
43	85	100	0.85	168	68	24.09	0	B/L PCO	11	13	96	126	10.9	4	98.1	5.33	2.58
44	108	145	0.74	140	69	35.20	0	U/L PCO	10	8	91	121	13.24	1.2	29	5.06	2.97
45	66	88	0.75	143	47	22.98	0	B/L PCO	9	14	74	146	7.7	2.7	36.3	4.11	1.41

46	107	147	0.73	162	79	30.10	0	B/L PCO	12	9	87	123	31.6	4	31.9	4.83	6.79
47	93	123	0.76	154	76	32.05	1	B/L PCO	12	10	96	112	8.01	1.17	29	5.33	1.90
48	88	121	0.73	162	67	25.53	0	B/L PCO	12	11	86	115	33.8	4	55.7	4.78	7.18
49	84	92	0.91	149	59	26.58	0	B/L PCO	13	10	84	124	16	1	84.8	4.67	3.32
50	70	84	0.83	162	48	18.29	0	B/L PCO	12	14	76	104	10	3	42	4.22	1.88
51	75	83	0.90	156	39	16.03	0	B/L PCO	10	12	86	104	7.4	1	22.8	4.78	1.57
52	80	98	0.82	159	55	21.76	0	B/L PCO	15	10	96	117	14.9	4.8	44.5	5.33	3.53
53	92	114	0.81	160	76	29.69	0	B/L PCO	12	13	90	114	247	1.13	49.5	5.00	54.89
54	78	98	0.80	154	61	25.72	1	B/L PCO	13	13	94	122	17	4.8	38	5.22	3.95
55	94	99	0.95	170	73	25.26	0	B/L PCO	16	11	100	116	22	0.46	76	5.56	5.43
56	85	91	0.93	155	65	27.06	0	B/L PCO	15	11	72	104	6.1	1.3	17	4.00	1.08
57	78	98	0.80	154	61	25.72	1	B/L PCO	13	13	94	122	17	4.8	38.9	5.22	3.95
Mean	88.07	105.9	0.84	155.3	65.19	27.10			11.4035	11.80952	90.01754	128.7193	24.85404	2.454912	52.95316	5.00	5.64
Std Dev	11.91	16.07	0.07	7.453	13.39	5.70			2.95498	2.442073	21.62559	36.52673	43.17048	1.314178	28.0638	1.20	9.70

TABLE: 1

Shows the mean of all Physical & Biochemical parameters obtained for all participants.

TOTAL PARTICIPANTS IN THE STUDY= 73

PARAMETERS	**MEAN & STD-DEV FOR ALL PARTICIPANTS**	**Reference interval**
AGE	24.79(±4.64)	
BMI	28.08(±5.78)	18.5-24.9
WAIST/HIP RATIO	0.83(±0.07)	<0.85
FBS(mg/dL)	89.15(±19.75)	70-110 mg/dL
2 HRS POST GLUCOSE (mg/dL)	126.28(±34.65)	<140 mg/dL
FASTING INSULIN(mU/L)	28.04(±39.34)	2.6-37.6 mU/L
HOMA-IR	6.28(±8.8)	<2.5
TSH(mU/L)	5.82(±12.2)	0.3-5.0 mU/L
TOTAL TESTOSTERONE(ng/dL)	55.8(±27.8)	14-76 ng/dL

Table 1 shows the comparison of mean of age, BMI, W/H ratio, FBS, 2 hrs post glucose, Fasting Insulin, HOMA_IR, TSH, Total Testosterone between the study population and the expected reference interval.

TABLE: 2

Shows % presentation of criteria (ROTTERDAM CRITERIA)

Total subjects: 73

PCOS PATIENTS AS PER ROTTERDAM CRITERIA (NO. OF. CRITERIA SATISFIED)	PERCENTAGE (%)	NUMBER OF PATIENTS
Patients With Menstrual Irregularities & Usg-Pco (2/3)	88%	64
Patients Having Clinical F/O Hyperandrogenism, Usg- Pco (2/3 Criteria)	76.60%	53
Patients With Bio-Chemical Evidence Of Hyperandrogenism, Usg-Pco (2/3 Criteria)	26.02%	19
Patients Having Menstrual Irregularities, Clinical & / Biochemical F/O Hyperandrogenism, Usg- Pco (All 3 Criteria)	60%	44

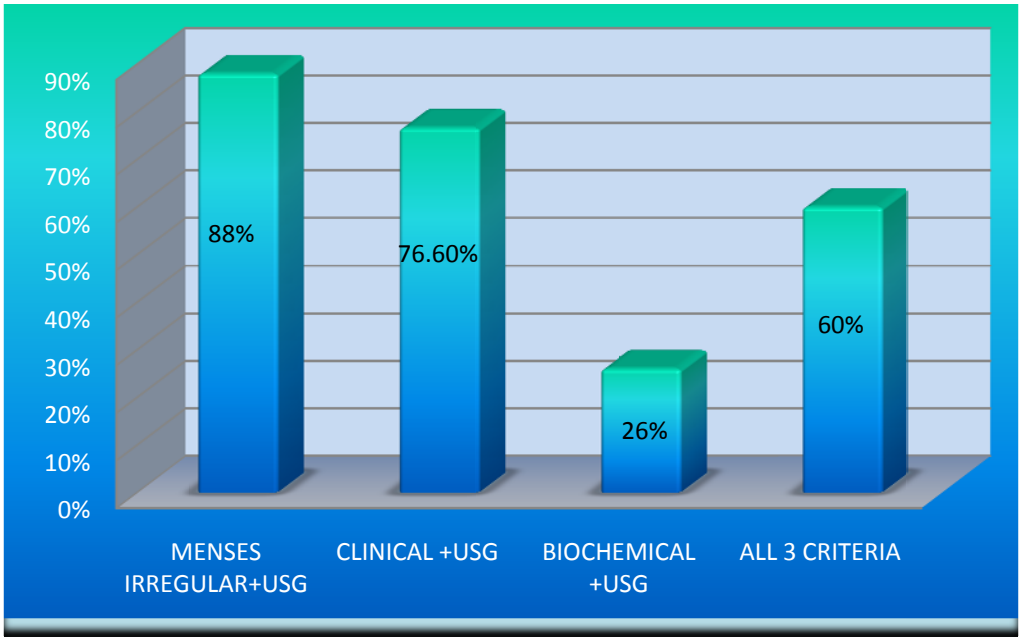


Fig:1 - Shows the percentage of presentation (ROTTERDAM CRITERIA). In our study, PCOS women with all three criteria were 60% (44), those with clinical F/O hyperandrogenism and Ultrasound finding of Polycystic ovaries were 76.6% (53), those with Biochemically hyperandrogenic and Ultra sound with polycystic ovaries were 26.02% (19), with menstrual irregularities and USG-PCO were 88%.

TABLE: 3

PERCENTAGE DISTRIBUTION OF PRESENTING COMPLAINTS FOR ALL PARTICIPANTS.

TOTAL PARTICIPANTS: 73

PRESENTING COMPLAINTS	NO.OF .INDIVIDUALS	PERCENTAGE
OLIGOMENORRHOEA / AMENORRHOEA	64	88%
ACNE / HIRSUTISM	54	74%
WEIGHT GAIN	47	56%
INFERTILITY	41	64%

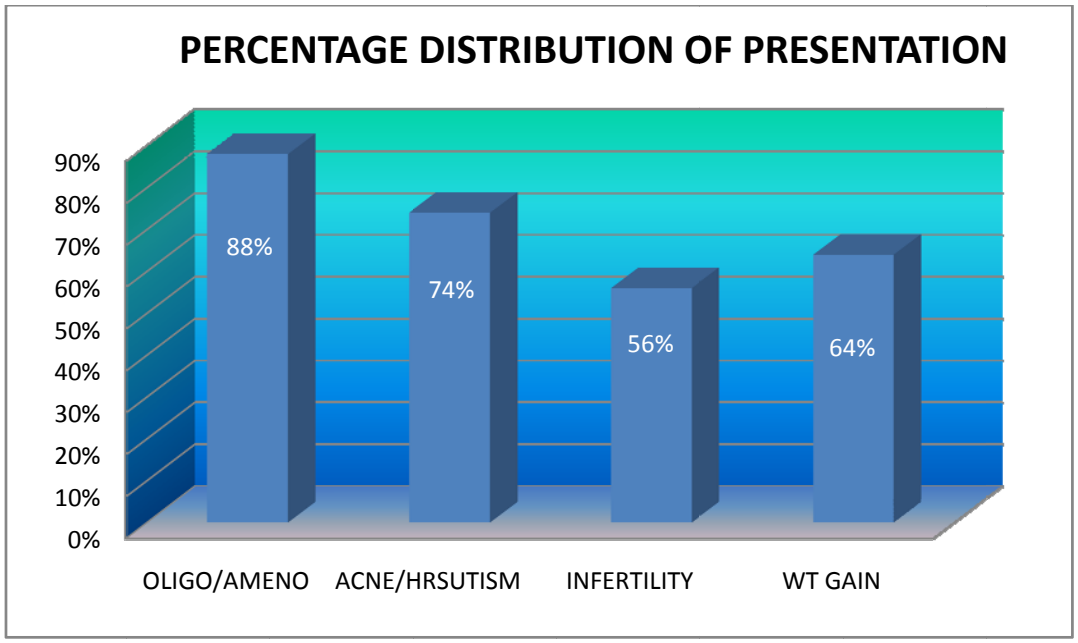


Fig : 2 - Shows the percentage distribution of different major & minor complaints. 88% of women had oligo/amenorrhoea. 74% of them had infertility, 56% had acne/ hirsutism (features of hyperandrogenism), 64% women came with complaints of weight gain.

TABLE: 4

AGE DISTRIBUTION IN PCOS (73 PARTICIPANTS)

AGE	NO.OF. INDIVIDUALS	PERCENTAGE
<19 yrs(Adolescence)	8	10.9%
≥20	65	89.04%

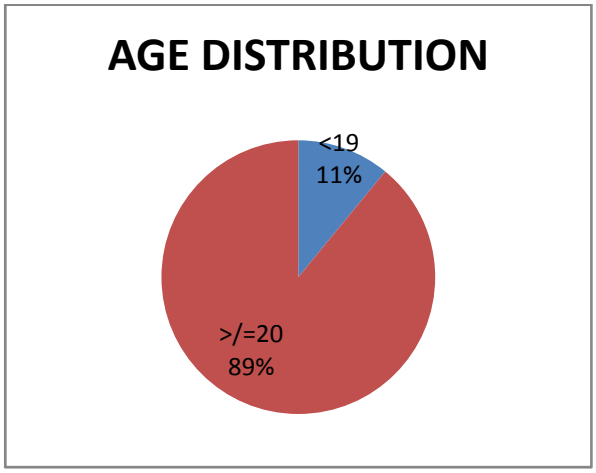


Fig:3 - Shows the distribution of age in 73 PCOS women. There were 8 girls in adolescent age (<19), and 65 women belonging to age ≥20 yrs.

TABLE: 5

BODY MASS INDEX (BMI) FOR 73 PARTICIPANTS

BODY MASS INDEX (BMI)	NO. OF. INDIVIDUALS	PERCENTAGE
UNDER WEIGHT <18.4	4	5%
NORMAL 18.5-24.9	19	26%
OVERWEIGHT 25-29.9	27	37%
OBESE ≥30	23	32%

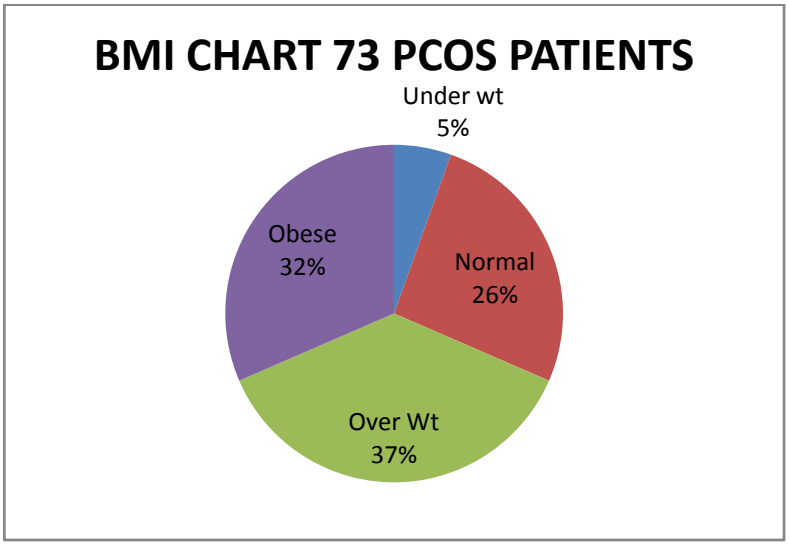


Fig:4 - Shows the BMI distribution among the study population. 5% of women (4) were under weight, 26% (19) were normal, 37% (21) were overweight, 32%(23) were obese.

TABLE: 6

DISTRIBUTION OF WAIST HIP RATIO FOR ALL PARTICIPANTS (73)

W/H RATIO	NO.OF INDIVIDUAL	PERCENTAGE
<0.8	23	32%
>0.8	50	68%

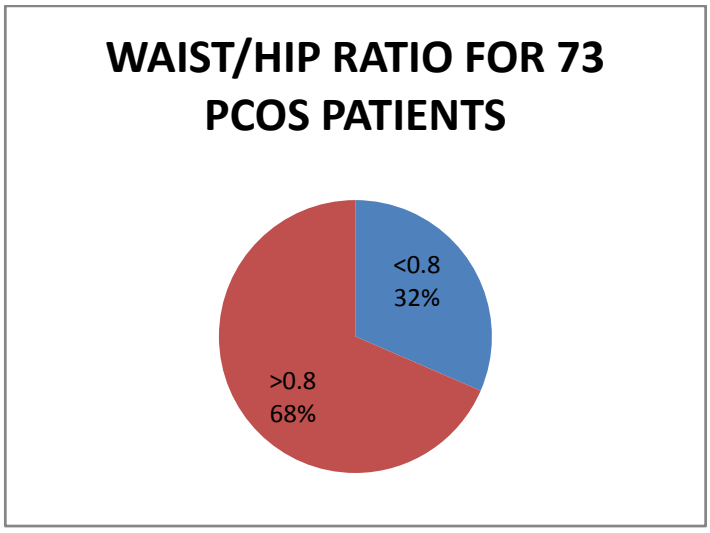


Fig:5 - Shows the distribution of Waist / Hip ratio for all participants. 32% of them were having W/H ratio less than 0.8 and 68% of them had W/H ratio more than 0.8.

TABLE : 7

THYROID FUNCTION STATUS IN ALL 73 PARTICIPANTS

ASSOCIATION OF HYPOTYROIDISM	NO.OF. INDIVIDUALS	PERCENTAGE
HYPOTHYROID PCOS (>5µU/mL)	16	21.91%
EUTHYROID PCOS (<5µU/mL)	57	78.08%

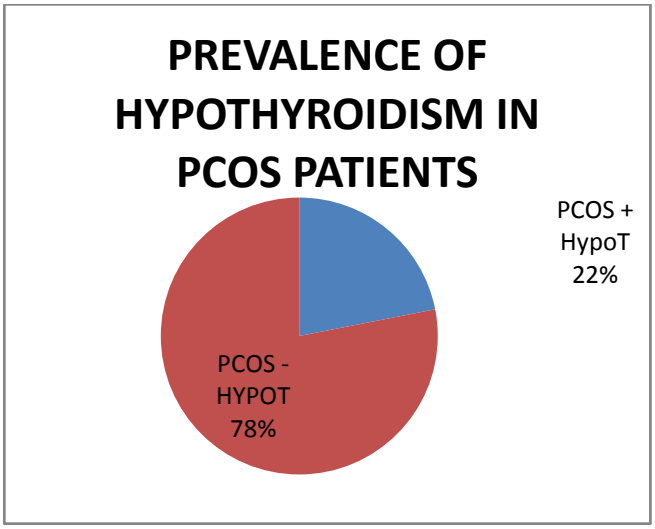


Fig:6 - Shows the prevalence of Hypothyroidism in the study population. ~22% (21.9%) of PCOS women (16) were found to have Hypothyroidism.

TABLE: 8

DISTRIBUTION OF SERUM TSH IN DIFFERENT AGE GROUP

AGE IN YRS	TSH<5mU/L (No. of. individuals)	TSH>5mU/L (No. of. individuals)	MEAN TSH
15-20	13	2	2.93
21-25	26	8	8.22
26-30	22	4	4.52
31-35	6	2	2.85

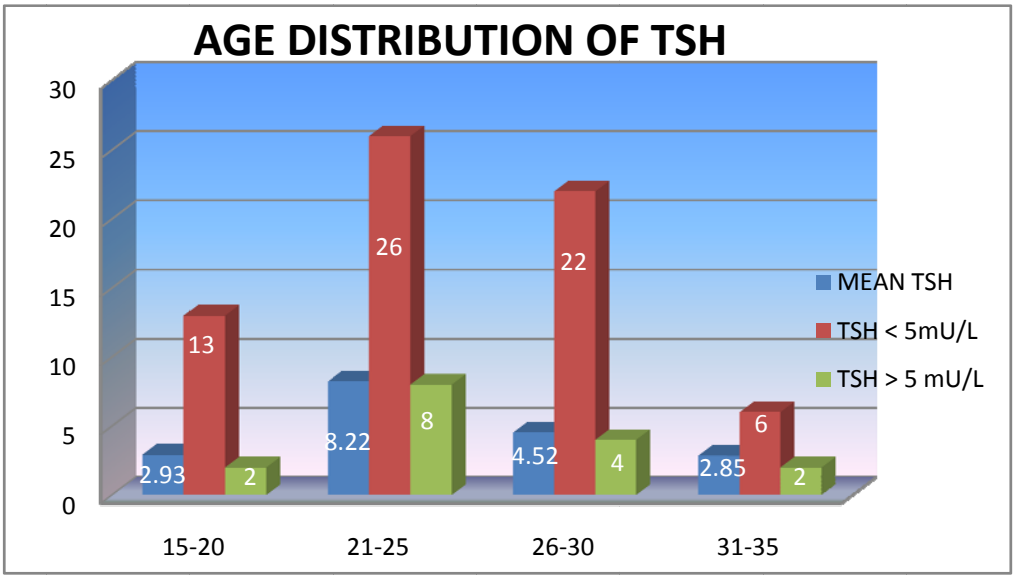


Fig:7 – Shows the Distribution of TSH in different age group.

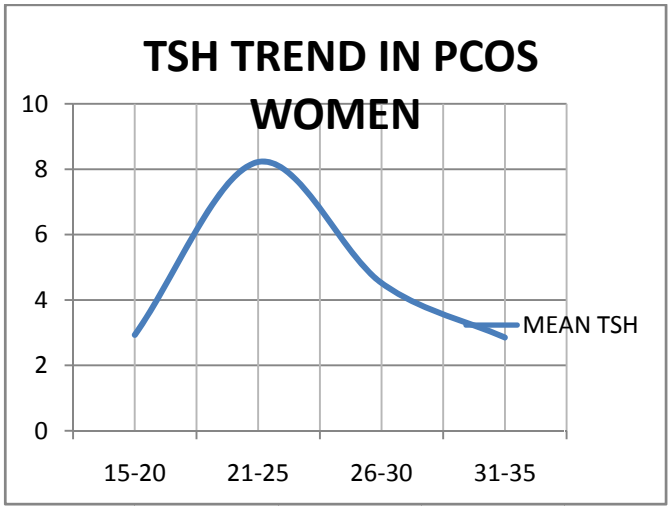


Fig:8 – Shows the Trent of TSH in PCOS Women

TABLE: 9

DISTRIBUTION OF FASTING GLUCOSE VALUES (mg/dL) IN ALL PARTICIPANTS

GLUCOSE LEVEL	NO.OF INDIVIDUALS	PERCENTAGE
70-110mg/dL	67	91%
110-126mg/dL	2	2%
>126mg/dL	4	5%

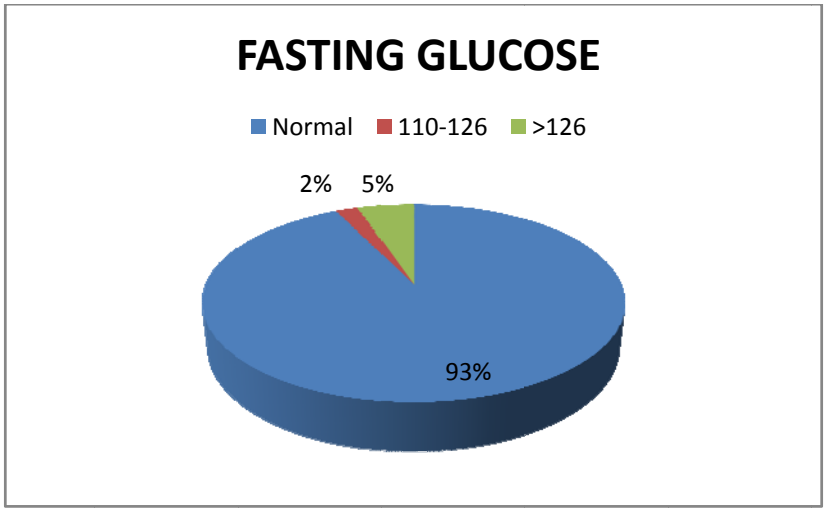


Fig:9 - Show the distribution of FBS among 73 participants. 93% of them were within normal limit (<110 mg/dL), 2% were in Impaired Fasting Glucose (110-126 mg/dL), & 5% of them had Diabetes mellitus (.126 mg/dL).

TABLE: 10

PERCENTAGE DISTRIBUTION OF 2 HRS POST GLUCOSE (mg/dL) FOR ALL 73 PARTICIPANTS.

GLUCOSE LEVEL	NO.OF. INDIVIDUALS	PERCENTAGE
<140	58	79%
141-200	13	17%
>200	2	2%

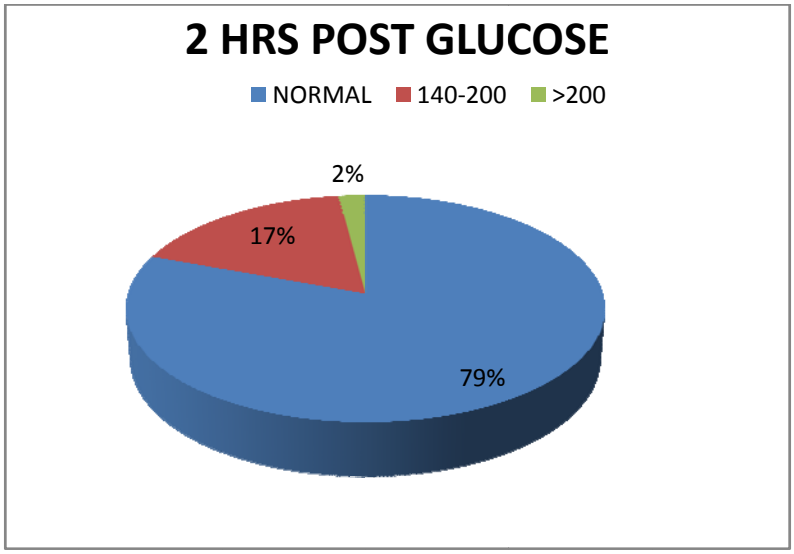


Fig:10 - Shows the percentage distribution of 2hrs post glucose for all the 73 participants. 79.4% of them (58) were within the reference limit (<140 mg/dL). 17.8% of them were in Impaired Glucose Tolerance state (13) and 2.7% of them(2) were diabetic(>200 mg/dL).

TABLE: 11

GLUCOSE STATUS OF 73 PCOS WOMEN	**FASTING GLUCOSE(mg/dL) (no. of individuals)**	**2 HRS POST GLUCOSE(mg/dL) (no. of individuals)**
Normoglycemic	67 (<110 mg/dL)	58
Impaired Fasting Glucose (110-126 mg/dL)	2	-
Impaired Glucose Tolerence (140-200 mg/dL)	-	13
Diabetes Mellitus	4 (>126 mg/dL)	2 (>200 mg/dL)

TABLE: 12

TOTAL PARTICIPANTS	73
EUGLYCEMIA (no)	59
IMPAIRED (no)	13
DIABETES MELLITUS (no)	1

TABLE: 13

DISTRIBUTION OF TESTOSTERONE LEVELS IN ALL 73 PARTICIPANTS

SERUM TESTOSTERONE (ng/dL)	NO.OF. INDIVIDUALS	PERCENTAGE
<76ng/dL	54	74%
>76ng/Dl	19	26%

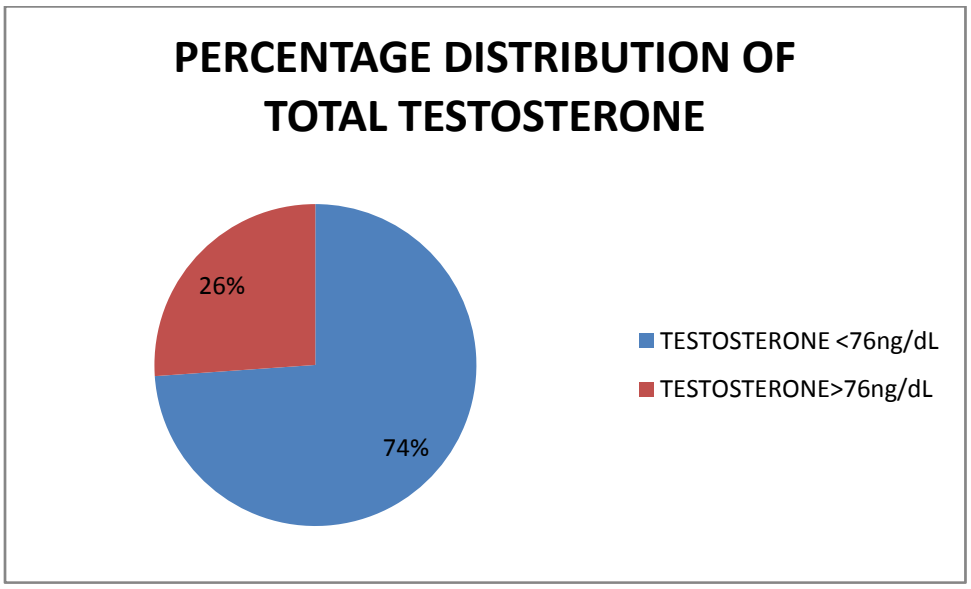


Fig:12 - Shows the percentage of patients within the reference range of testosterone (<76 ng/dL). 26%had hyperandrogenism, 74% of them had normal level of testosterone.

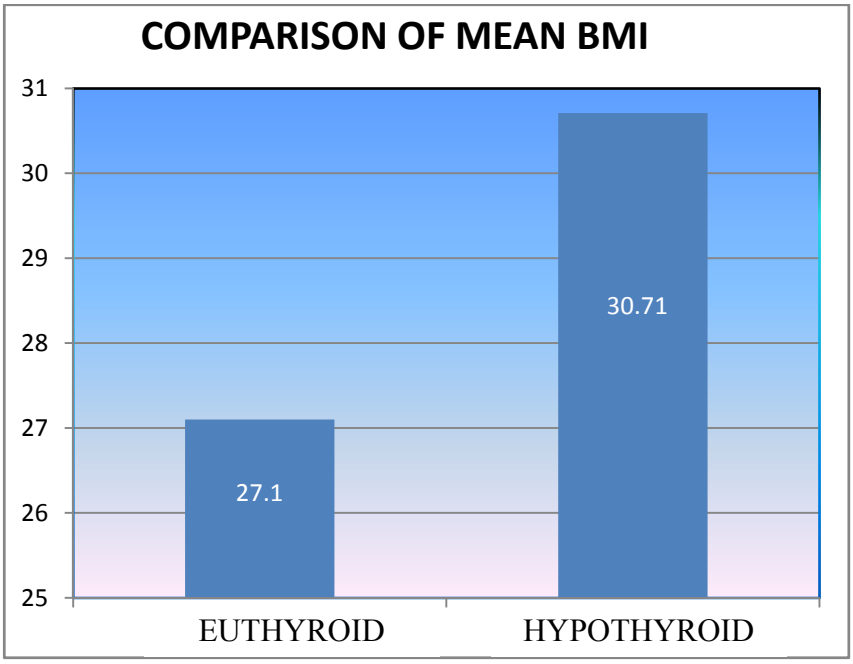
TABLE: 14

Group1: Euthyroid PCOS.

Group2: Hypothyroid PCOS

Fig:13

COMPARISON OF BMI BETWEEN TWO GROUPS OF PCOS WOMEN



This table shows the mean BMI between the two groups of PCOS women.

Mean BMI PCOS With Normal TSH	27.10
Mean BMI PCOS With Elevated TSH (Hypothyroidism)	30.71
P Value	0.026**

STATISTICALLY SIGNIFICANT**

TABLE : 15

COMPARISON OF WAIST HIP RATIO BETWEEN THE TWO GROUPS

Mean W/H Ratio For Euthyroid PCOS	0.84	Statistically Not Significant
Mean W/H Ratio For Hypothyroid PCOS	0.82	
P Value	0.28	

This Table shows the comparison of mean Waist/Hip ratio between the two groups of PCOS women.

TABLE: 16

Group1: PCOS women in Euthyroid state.

Group2: PCOS women with Hypothyroidism.

	MEAN FBS	MEAN 2 HRS POST GLUCOSE	
EUTHYROID -PCOS	90.01	128	Not Statistically Significant
HYPOTHYROID - PCOS	83.81	119.7	
P VALUE	0.27	0.36	

STATISTICALLY SIGNIFICANT P VALUE <0.05

This Table shows the comparison of mean FBS & 2hrs POST GLUCOSE between the two groups of PCOS patients and were not statistically significant.

Fig:14

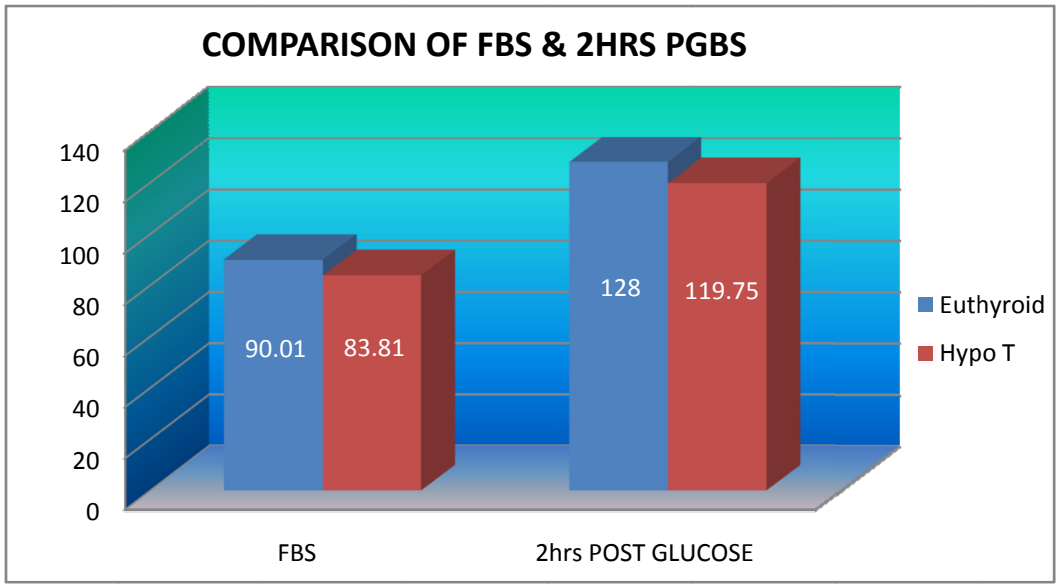


TABLE: 17

This Table shows the comparison of mean Fasting Insulin between the two groups of PCOS women.

	FASTING INSULIN	
EUTHYROID- PCOS	24.85	Not Statistically Significant
HYPOTHYROID- PCOS	27.24	
P-VALUE	0.83	

STATISTICALLY SIGNIFICANT P VALUE <0.05

TABLE: 18

COMAPRISON OF MEAN HOMA –IR BETWEEN EUTHYROID & HYPOTHYROID PCOS WOMEN

	MEAN HOMA-IR	
EUTHYROID-PCOS	5.64±9.79	Not Statistically Significant
HYPOTHYROID- PCOS	5.74±3.99	
P VALUE	0.96	

STATISTICALLY SIGNIFICANT P VALUE= <0.05

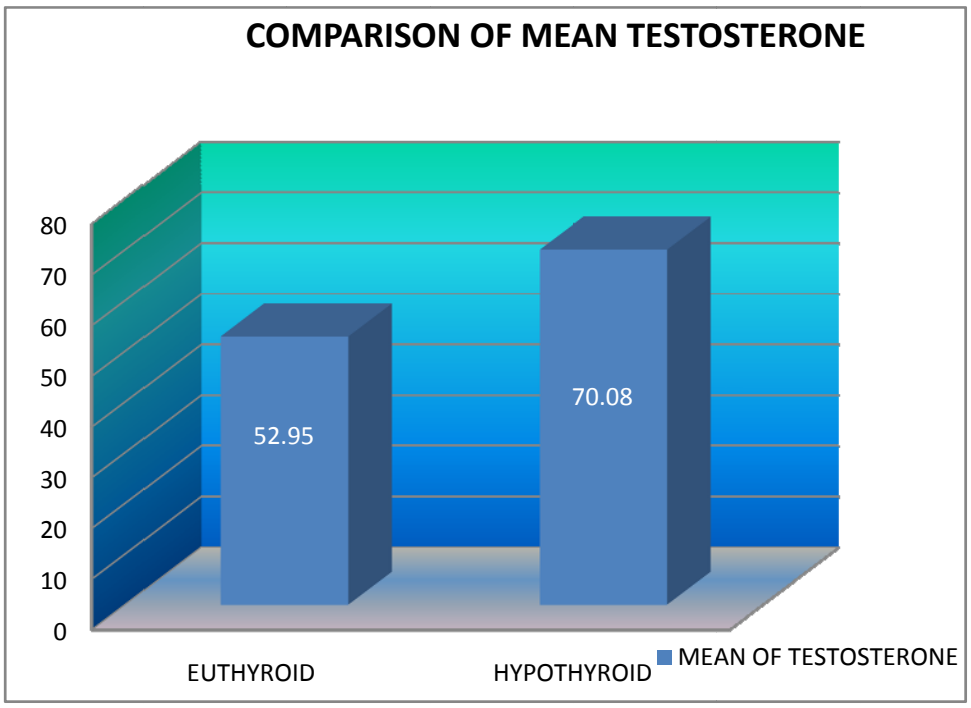
TABLE : 19

	MEAN TESTOSTERONE	
EUTHYROID- PCOS	52.95	Statistically Significant And Weak Positive Correlation
HYPOTHYROID-PCOS	70.08	
P VALUE (<0.05)	0.023**	
Correlation of Total Testosterone With Hypothyroidism In PCOS	0.14	

STATISTICALLY SIGNIFICANT P VALUE= <0.05**

This Table shows the comparison of mean Total Testosterone between the two groups.

Fig:15



CORRELATION OF TSH WITH TOTAL TESTOSTERONE in PCOS WOMEN WITH HYPOTHYROIDISM

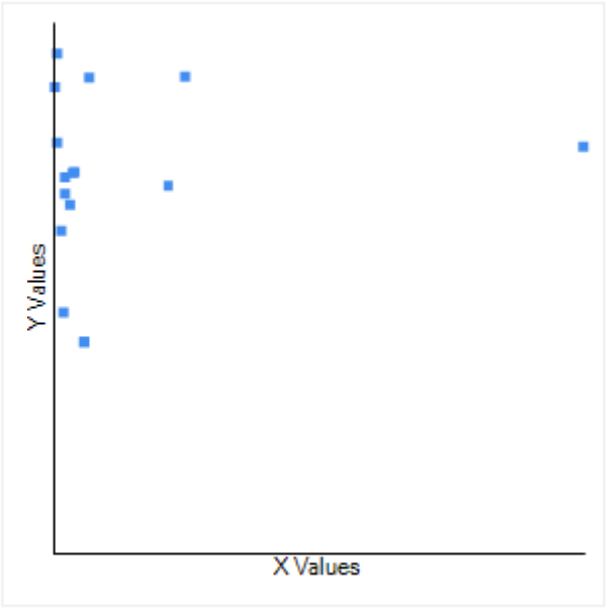


TABLE : 20

NUMBER OF PARTICIPANTS	73	
CORRELATION OF TESTOSTERONE WITH W/H RATIO IN PCOS+ HYPO T PATIENTS	0.38	POSITIVE AND WEAK CORRELATION.

SHOWS CORRELATION BETWEEN TESTOSTERONE & WAIST HIP RATIO IN PCOS PATIENTS WITH HYPOTHYROIDISM

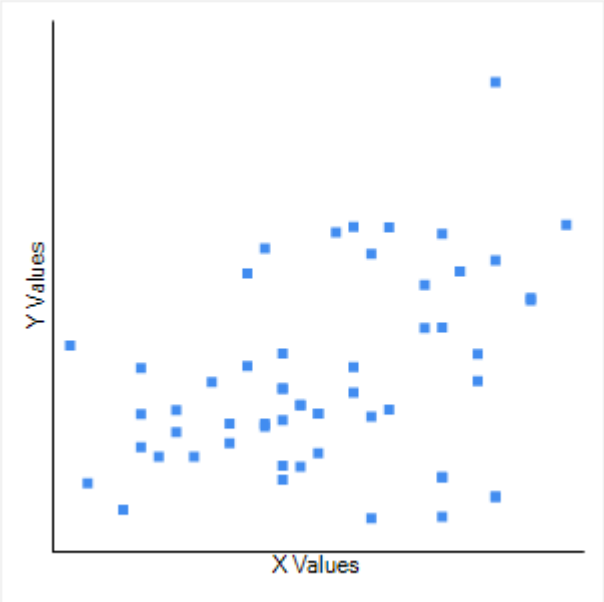


TABLE : 21

Parameters	Euthyroid-Pcos	Hypothyroid-Pcos	Pearson Coefficient Correlation Significant P Value(<0.05)
BMI	27.10+/- 5.75	30.71	0.026**
WAIST / HIP RATIO	0.84+/- 0.05	0.82+/- 0.07	0.28
OVARY VOLUME	11.39+/- 2.6	12.98+/- 2.8	0.03**
FASTING BLOOD SUGAR	90.01+/-21.81	83.81+/-10.5	0.27
2 HRS POST GLUCOSE	128.7+/- 36.85	119.75+/- 24.2	0.36
FASTING INSULIN	24.85+/-43.45	27.24+/- 16.16	0.83
HOMA-IR	5.64+/- 9.79	5.74+/-3.99	0.96
TESTOSTERONE	52.95+/-28.06	70.08+/- 16.93	0.023**

** SIGNIFICANT P VALUE

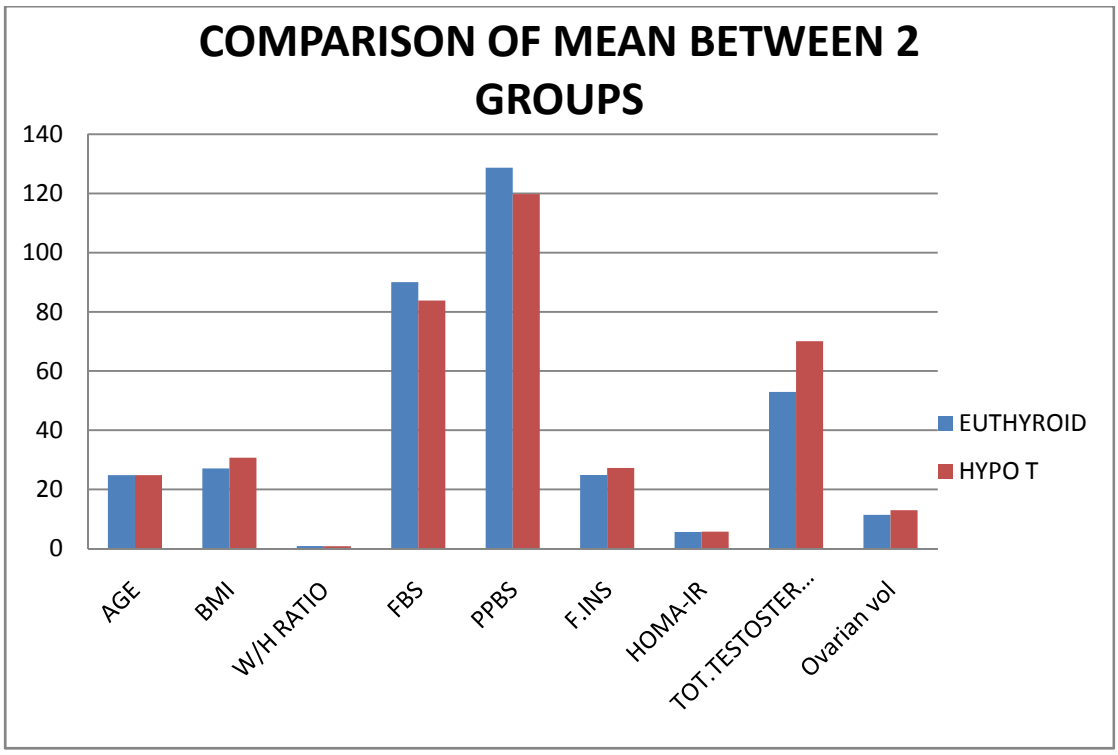


Fig. 16 shows different variable mean comparison between Euthyroid & hypothyroid PCOS women.

In our study population the mean age was 24.79 (±4.64), 88% of 73 PCOS patients had 2/3 criteria (menstrual irregularity+ USG finding) while 76.6% had clinical features of hyperandrogenism & USG –PCO finding, Biochemical features of hyperandrogenism & USG-PCO were observed in 26% & 60% women presented with all 3 criteria (Rotterdam), Table: 2.

Table:3 shows the percentage distribution of different presenting complaints 88% women presented with menstrual disorders, 74% came for infertility, 56% came with acne / hirsutism, 64% came with rapid weight gain. Table: 4 gives the prevalence of PCOS among adolescence (teenage) and adults(≥ 20yrs). In our study we had 8 girls aged ≤19yrs, 65 women ≥ 20yrs. In our study, most of the participants were obese. Table: 5 show the prevalence percentage of obesity based on BMI was 5% under

weight, 26% were normal, 37% were in overweight category & 32% obese (clinical & morbid). Table: 6 gives the Waist/Hip ratio distribution for the entire study group. 32% (23) had their W/H ratio < 0.8 & 68% (50) had >0.8. Table:7, Shows the thyroid function status for all the participants based on TSH with cut-off level of about 5µU/mL. Table:7 gives the serum TSH distribution in various age group. Age between 15 to 20 showed the mean TSH of about 2.93, 20 to 25yrs age group showed the mean of 8.22, 25-30 yrs groups showed 4.52 as their mean TSH, and 30-35 yrs group women showed 2.85 as their mean. The trend of TSH shows that the peak is in between 20s to 30s. Table:9 Gives the distribution of Fasting blood sugar in the study population. 67 of the participants had normoglycemia (70-110 mg/dL), 4 of them had FBS more than 126mg/dL, 2 of them had impaired fasting glucose. Table: 10, shows the distribution of 2 hrs post glucose level in all the 73 participants. 59 people showed normoglycemia, 13 were in impaired glucose Tolerance, and one of them was frank diabetic. Table: 11, gives the overall distribution of FBS and 2hrs PP for all the participants and the following table (12) gives the full statistics. Table: 13, shows the serum testosterone level among the PCOS women. Table: 20, showed the difference between the mean of BMI, Waist/Hip ratio, FBS, 2 hrs PP, Fasting Insulin, Testosterone, Ovarian volume, HOMA-IR between the two groups. BMI, Testosterone, Ovarian volume, showed statistical significance between them. There was no statistical difference in Waist/Hip ratio, Fasting Insulin, HOMA-IR between the two groups.

DISCUSSION

As the incidence of infertility and its morbidity are increasing in the recent days, the root cause should be identified and treated accordingly. The leading cause for female infertility is menstrual irregularities apart from anatomical and genetic defects. Major contributing factors for this irregular menstruation are from local (ovarian) cause like Polycystic ovarian Syndrome and /or the systemic cause like Hypothyroidism, Hyperprolactinemia, Hyperinsulinemia. All the above said factors pose individual risk for anovulation. Often they present in combination. PCOS and hypothyroidism are the most common endocrine disorders found in young reproductive age group women. A common finding in gynaecology outpatient department is that while treating individuals for PCOS for a period of few months, in some patient menstruation and ovulation is restored while it remains uncorrected in some individuals. On further evaluation of these non-responding patients for the other causes of infertility / menstrual disorder they were found to have co-existing Hypothyroidism which was mostly undiagnosed and /or untreated. These individuals were found to be mostly in subclinical status and some in overt hypothyroid state. Screening studies helps to assess the prevalence of hypothyroidism and gives an insight about the epidemiology of this disorder in the population. However studies comparing thyroid status in PCOS women is very minimal in all regions of India. With this background we have undertaken this study with the aim to estimate the thyroid function status in newly diagnosed PCOS patients

A cross sectional study was conducted among 73 newly diagnosed PCOS women attending the Gynaecology & Endocrinology outpatient department for menstrual irregularity and infertility. They were diagnosed based on revised

Rotterdam criteria, 2003. With serum TSH level of 5µU/L as the cut-off point to diagnose hypothyroidism we have identified the prevalence of hypothyroidism among 73 newly diagnosed PCOS women to be about 22% (no:16). In a case control study conducted by Maryam et al. a significant prevalence of auto immune thyroiditis and goiter in PCOS [166] was reported. Sridhar et al reported a prevalence of 1.04% (2/13) of polycystic ovaries among hypothyroid patients. In our study, 22% of our patients had hypothyroidism. This shows that there is a correlation between thyroid dysfunction and PCOS which is also supported by the findings of Maryam et al & Sridhar et al.[175] A similar finding of hypothyroidism among PCOS women was observed by Onno E Janssen et al study (20.6%)[167].

PREVALENCE OF HYPOTHYROIDISM & SUBCLINICAL HYPOTHYROIDISM IN VARIOUS CONDITIONS[168,169]

TABLE:VI

	GENERAL POPULATION	**INFERTILITY**	**PEER GROUP PCOS STUDY [163]**	**THIS STUDY**
HYPO-THYROIDISM	10-11% 2-4% (20-40 yrs women)	23.9%	2.5%	22% (21.91%)

The age distribution of TSH table and trend of TSH in PCOS shows that serum TSH level was at maximum between 20-30yrs. This peaking may be because the requirement of thyroxin is high in this age for all organs including reproductive system, which can be considered as an extension of physiological hypothyroidism. If physiological demand for thyroxin is not quenched adequately it might be a reason for developing subfertility later and ultimately leading to infertility.

Table: 1 shows the comparison of mean of all parameters for the study population against the reference interval. From this it is understood that BMI, Fasting Insulin, HOMA-IR, TSH & Testosterone were higher among PCOS patients than the reference values. This infers that obesity, hyperinsulinemia, hypothyroidism, hyperandrogenism are all the major contributing factors for PCOS.

Based on TSH cut-off value of 5µU/mL, the entire study population was divided into 2 groups. Group1: PCOS with Euthyroid & Group2: PCOS with Hypothyroid. Euthyroid PCOS women comprised 78% while hypothyroid PCOS women 22%.

Testosterone was significantly elevated in hypothyroid patients when compared with the euthyroid group. (Table:19) Mean testosterone of group 1 was 52.95(±28.06) while in group 2 it was 70.08(±16.93) and the difference was statistically significant (P value 0.023). On comparing the testosterone values with that of TSH for the two groups we found observed a positive but weak correlation for hypothyroid group (r = 0.14). There was no correlation between Testosterone and TSH for the euthyroid group. This infers that as TSH rises Testosterone also increases and indicates that Hypothyroidism is a contributing factor for hyperandrogenism and its sequale (polycystic ovarian syndrome). This also infers that the existing hyperandrogen status is worsened by the co existence of hypothyroidism. This may be the cause for persistence of complaints like menstrual disorders, infertility, USG – finding of cysts in some patients having treated for PCOS. This reinforces the importance of evaluating serum TSH in a patient diagnosed as PCOS.

Table 14: Gives the comparison of mean BMI between Group 1=27.1(±5.75) Group2 =30.71(±5.06) with statistically significant P value of 0.026. As thyroid hormones are essential for lipid and protein metabolism, in hypothyroidism the

function is slowed down (hypometabolism) leading to fat and extracellular matrix deposition leading to obesity.

Table: 15, Shows the comparison of mean Waist / Hip ratio between Group1=0.84(±0.07) & Group2 =0.82(± 0.05). We have not observed any significant variation between the groups. This shows that the distribution of fat is not different in 2 groups as all of them are having PCOS. The overall mean W/H ratio was 0.83 slightly in the upper expected limit (0.85) indicating that PCOS women are basically towards developing truncal obesity.

In table:16, comparison of mean FBS & 2hrs Post glucose between the 2 groups were shown and was not significant. Table:17 Comparison of fasting Insulin between Group1=24.85(±43.45) and Group 2=27.24(±16.16) showed P value 0.83 and mean HOMA-IR of Group1=5.64(±9.79) and Group 2=5.74(±3.99) with P value 0.96. This implies that there is no difference in Blood sugar and Insulin levels between those who have euthyroid and hypothyroid status. This may be because the serum TSH for most of the patients diagnosed as hypothyroid were possibly in subclinical state and the metabolism might not have been significantly affected. But the mean HOMA-IR of the study group (6.28±8.8) when compared with the reference range (<2.5) is very much high indicating that the study population have insulin resistance universally [170]. From our study, we have found that the 59 patients had normoglycemia, 13 had impaired glucose level and one patient had both FBS and 2hrs PP in diabetic range.

The mean ovarian volume between the 2 groups showed a significant difference. Group 1had a mean value of 11.39cc (±2.6) & Group2 had a mean value of 12.98cc (±2.8) with P value=0.03. PCOS women with hypothyroidism had higher ovarian volume than PCOS with euthyroid state. In hypothyroidism, there is collagen/

cellular matrix deposition in most parts of the body including the ovaries increasing the ovarian volume and impairs their function. Also the cyst number is expected to be higher with thyroid dysfunction further contributing to increase in ovarian volume[171,172]. This again emphasises the importance of screening for hypothyroidism in newly diagnosed PCOS women and as per the recommendation of American Thyroid Association 2012, serum TSH alone can be used to rule out thyroid disorders.

SUMMARY

A cross sectional study was conducted in 73 newly diagnosed Polycystic Ovarian Syndrome patients. Among 73 participants, 16 were diagnosed to have hypothyroidism based on serum TSH (Thyroid Stimulating hormone) level with the cut-off value of 5µU/mL. They were divided into 2 groups.

Group: 1 PCOS women with euthyroidism.

Group: 2 PCOS women with hypothyroidism.

From our study, we found that:

1) Mean BMI, Waist / Hip ratio, Fasting Insulin, HOMA-IR, Testosterone among PCOS patients were found to be higher than the recommended reference range.
2) The prevalence of hypothyroidism in PCOS patients was (22%), which is higher than the prevalence of hypothyroidism in general reproductive age women (2-4%). Thus, prevalence studies helps to bring many submerged / occult disorders to light there by aiding in correct diagnosis and proper treatment.
3) Our study followed the American Thyroid guidelines recommendation of using serum TSH alone instead of the entire thyroid profile for screening hypothyroidism in adults outpatient department which may be cost effective and economical for the patients and the health care providers.
4) Comparison of mean Testosterone between euthyroid and hypothyroid patients shows that hypothyroidism poses an additional contributing factor in developing features of hyperandrogenism, subfertility, infertility and even ovarian failure.

CONCLUSION

Polycystic Ovarian Syndrome, an ill defined complex disorder and needs a keen attention while treating. As ethnicity plays a vital role in this disorder, the characteristics of this disorder in different population should be known. There exists a cycle in the pathogenesis of PCOS from hypothalamus to ovary and vise-versa. The ultimate finding is hyperandrogenism. Systemic disorders like Hypothyroidism, Hyperprolactinemia, Hyperinsuliemia also contributes to hyperandrogenism by reducing the hepatic SHBG production. All these factors lead to hormonal imbalance affecting ovarian homeostasis resulting in anovulation. The final outcome may be single or multiple,from simple acne to complicated infertility. Very often these PCOS women were found to have hypothyroidism remaining submerged / undiagnosed which augments the existing hyperandrogenism. Timely and appropriate investigations can halt this cyclical event and its progression by early diagnosis and treatment.

From this study, it is concluded that :

Hypothyroidism is definitely an important contributing factor for hyperandrogenism, hence screening for hypothyroidism along with reproductive hormone profile should be evaluated in PCOS / infertile women for early diagnosis and management.

LIMITATIONS OF THE STUDY

1. This is a cross sectional and observational study conducted on a small group comprising of 73 subjects diagnosed as PCOS, who were further grouped based on their thyroid function status into euthyroid and hypothyroid. All data were compared between the two groups only. Hence some of the parameters did not show statistical significance as PCOS was a common factor for the two groups. A case- control study would have provided more information on the hormonal status of healthy women in reproductive age group (controls) and the comparison with PCOS individuals(cases) would have provided a better statistical significance.

2. The hyperandrogenic status of the patients in this study was biochemically confirmed by measuring only Total testosterone rather than both total and free hormone due to cost limitations. Although all patients in this study had clinical features of hyperandrogensm, we found that 74% of the patients had normal testosterone, and only 26% of the patients were biochemically hyperandrogenic (as per the reference range of the kit used). Perhaps the free testosterone levels (which was not measured) of these patients was possibly higher contributing to the clinical features of hyperandrogenism.

SCOPE FOR FURTHER STUDY

1. The reference range of various hormones for our ethnic population in different age group can be worked out in future studies.

2. As obesity is a very common finding in PCOS and Hypothyroidism, association of certain serum adipokines with PCOS and infertility can be considered for further studies.

3. A case-control study with more number of subjects can be done which will be useful for statistical analysis and provide more information on the mechanism of interplay of the various hormones.

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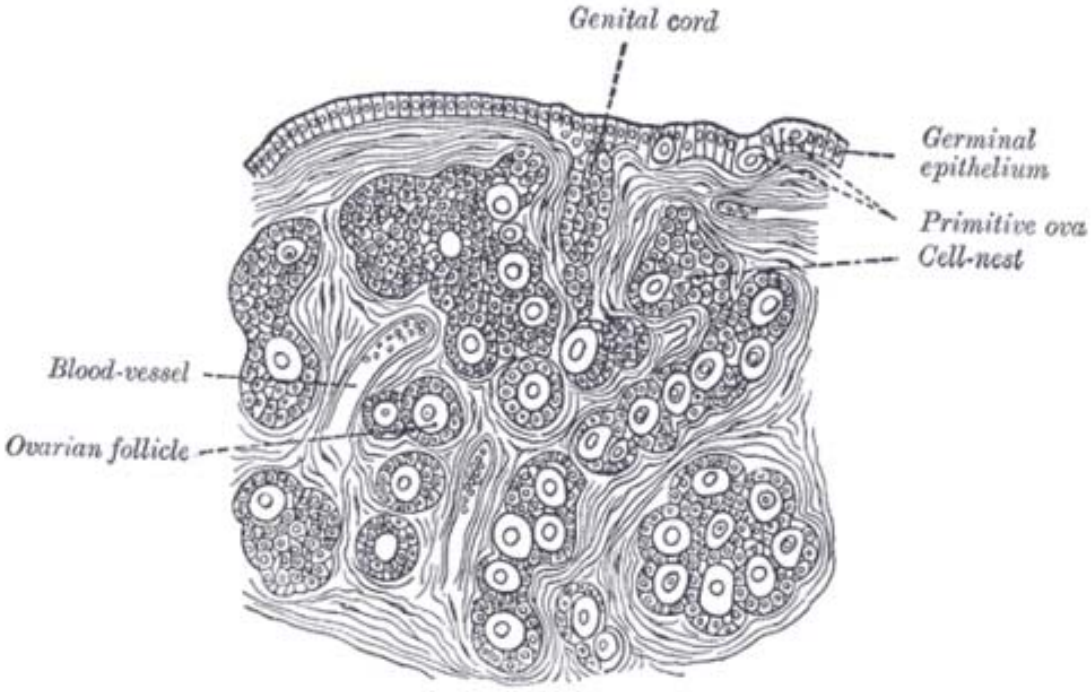
DEVELOPMENT OF OVARY



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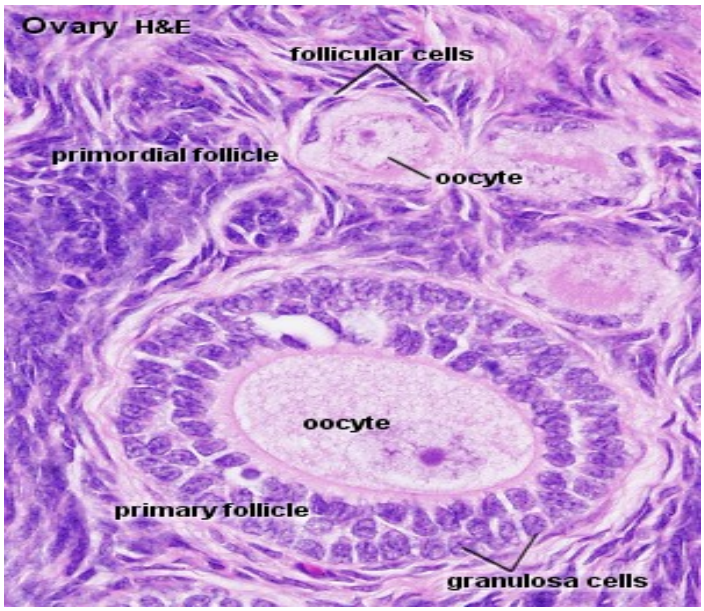

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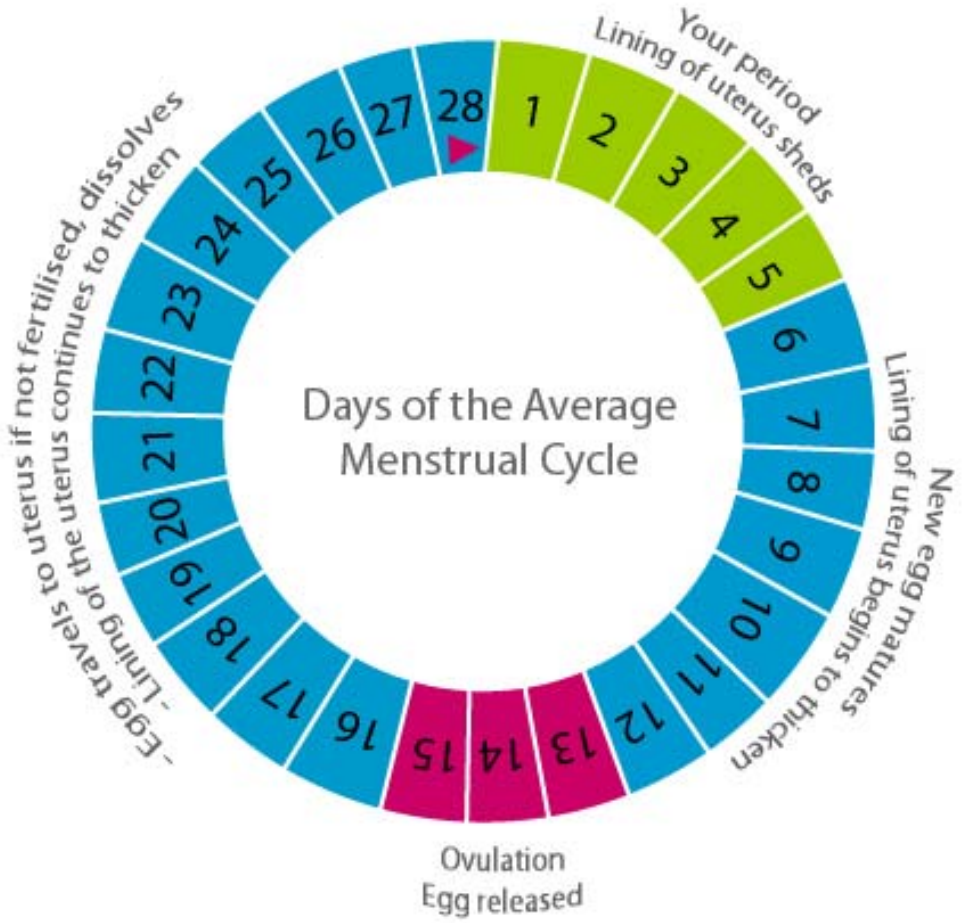

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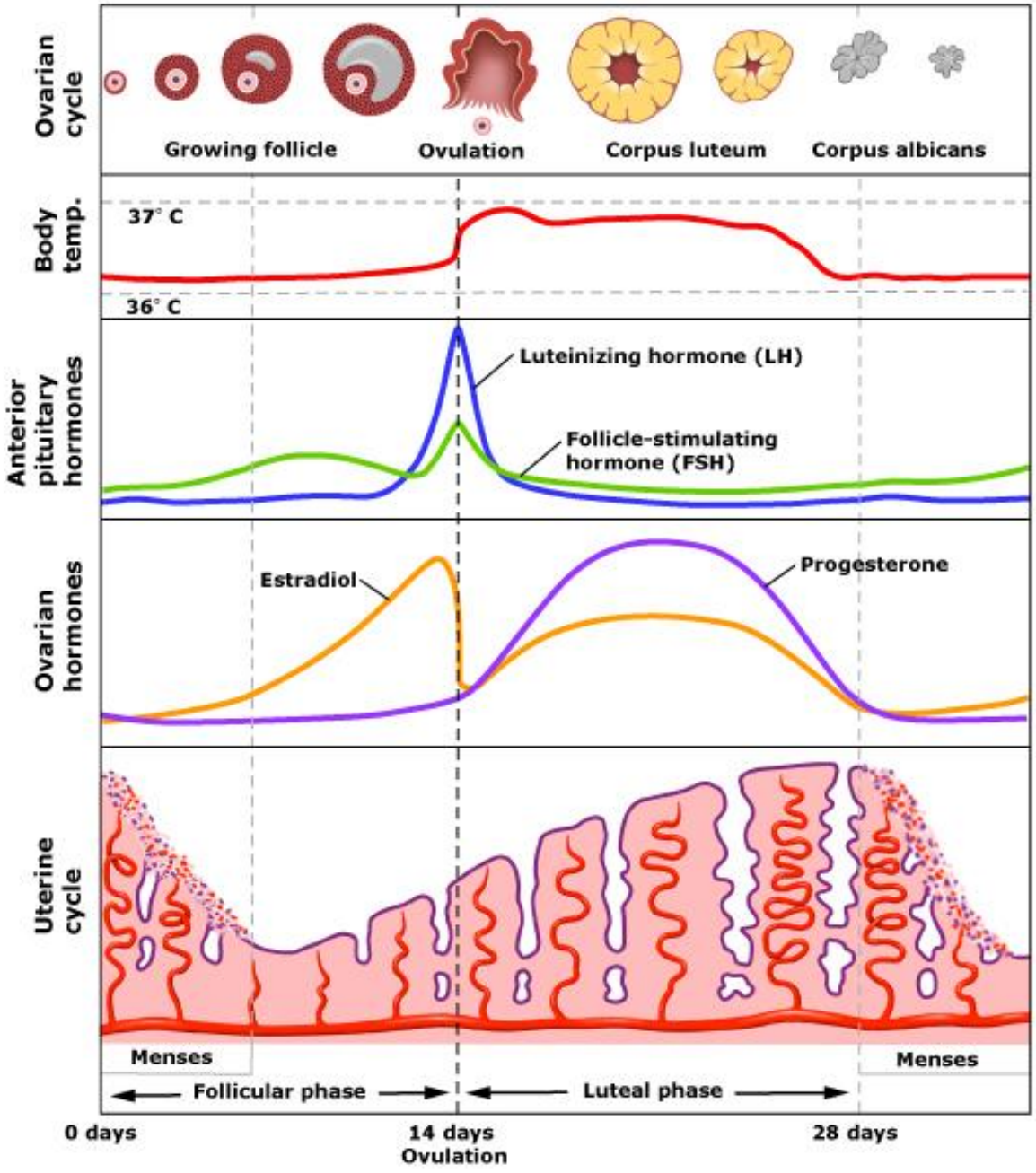

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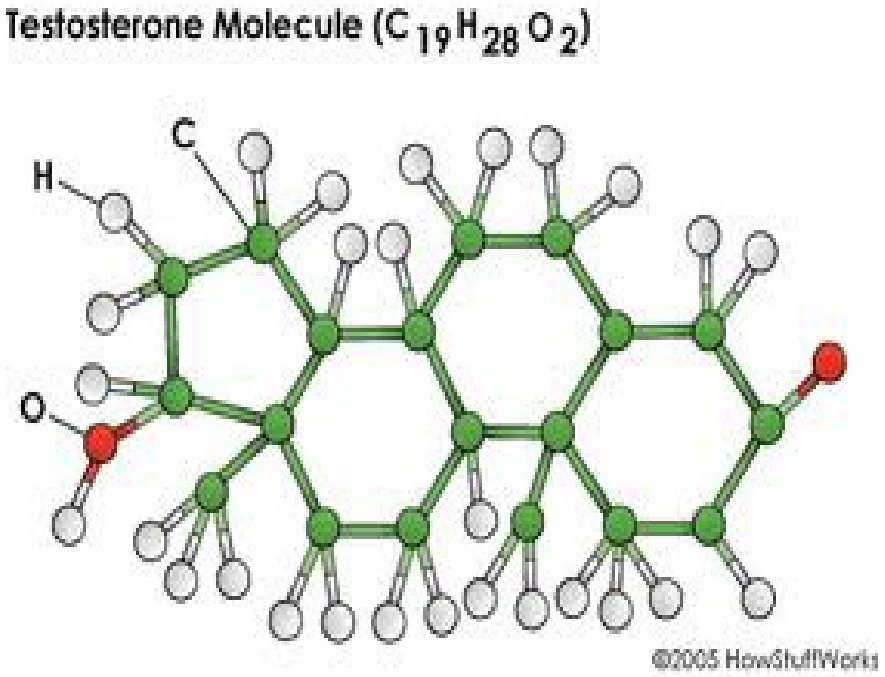

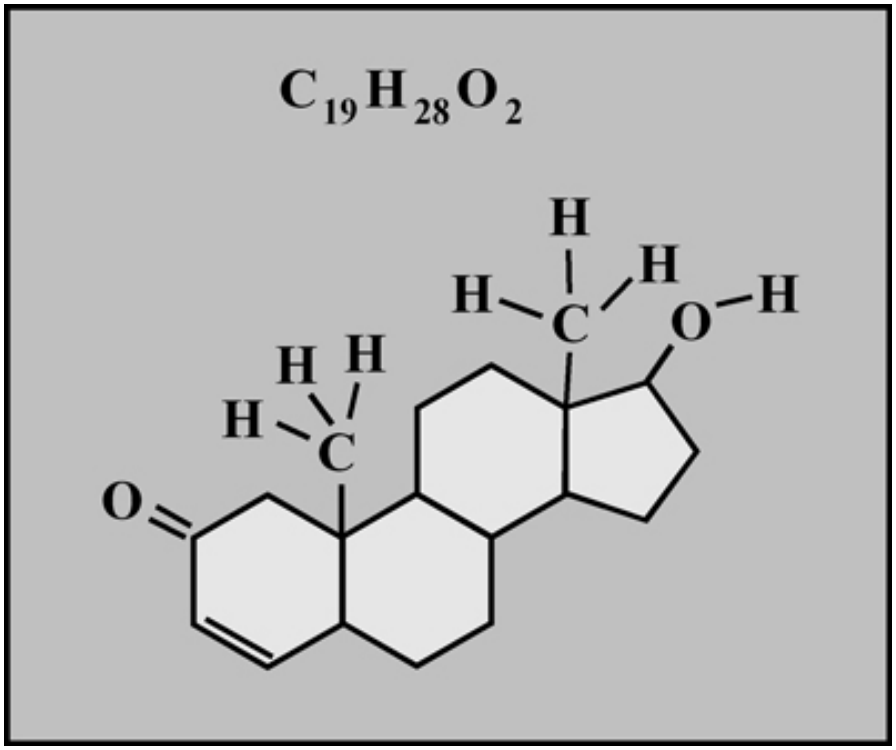

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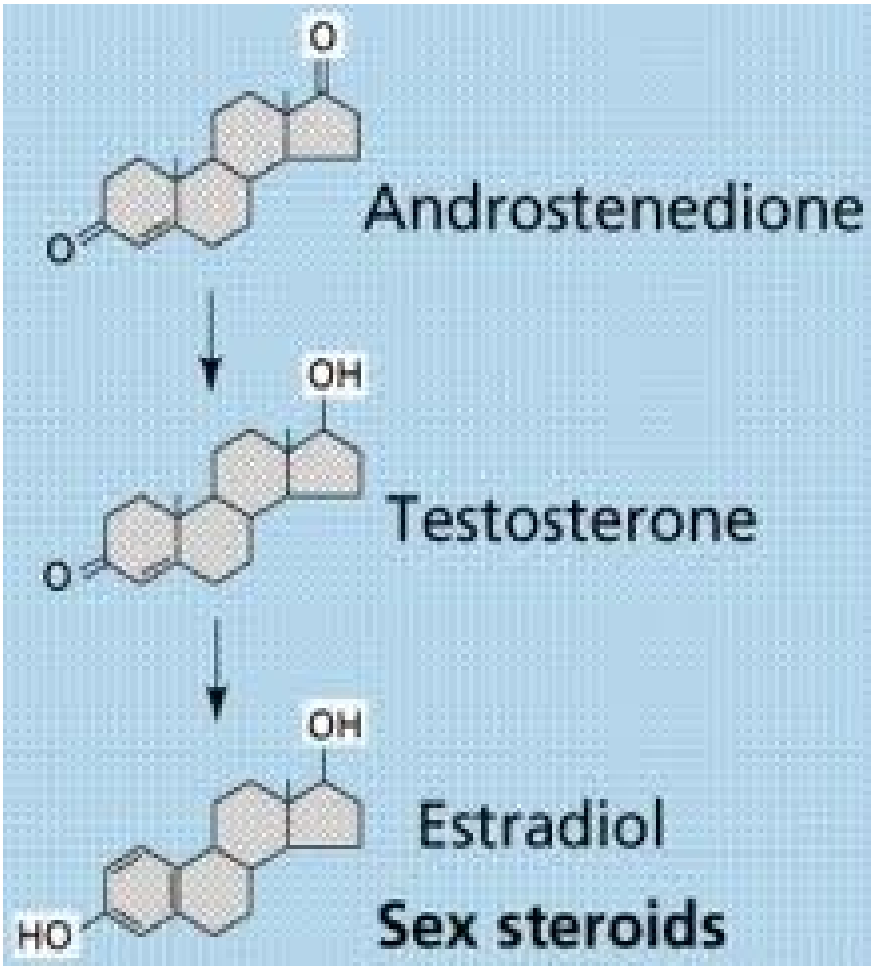

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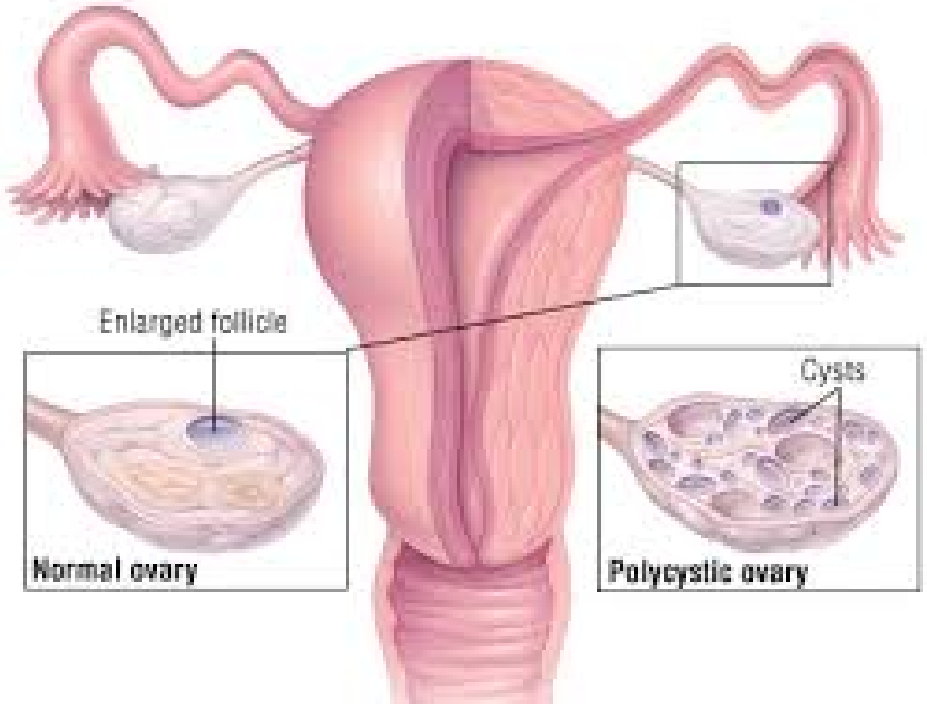

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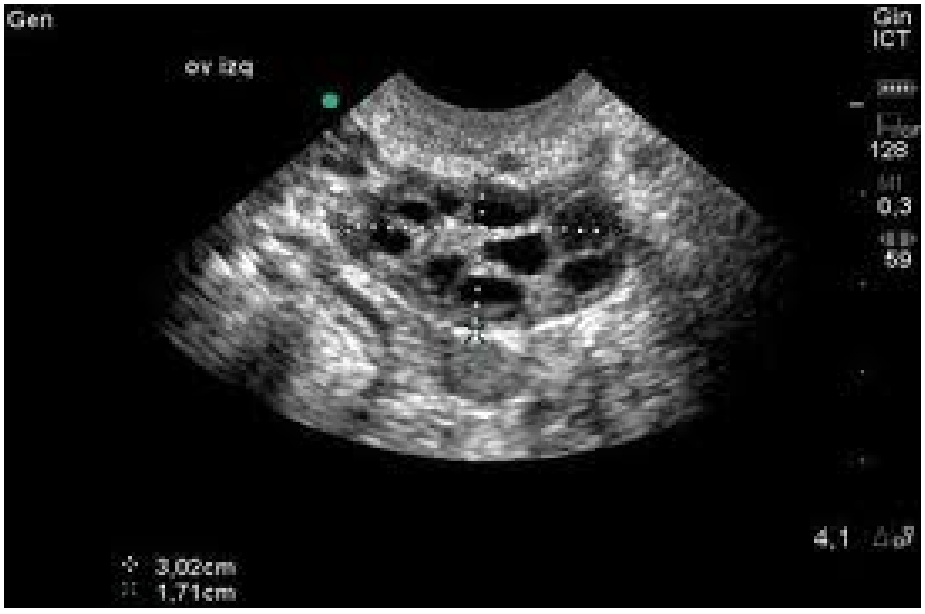

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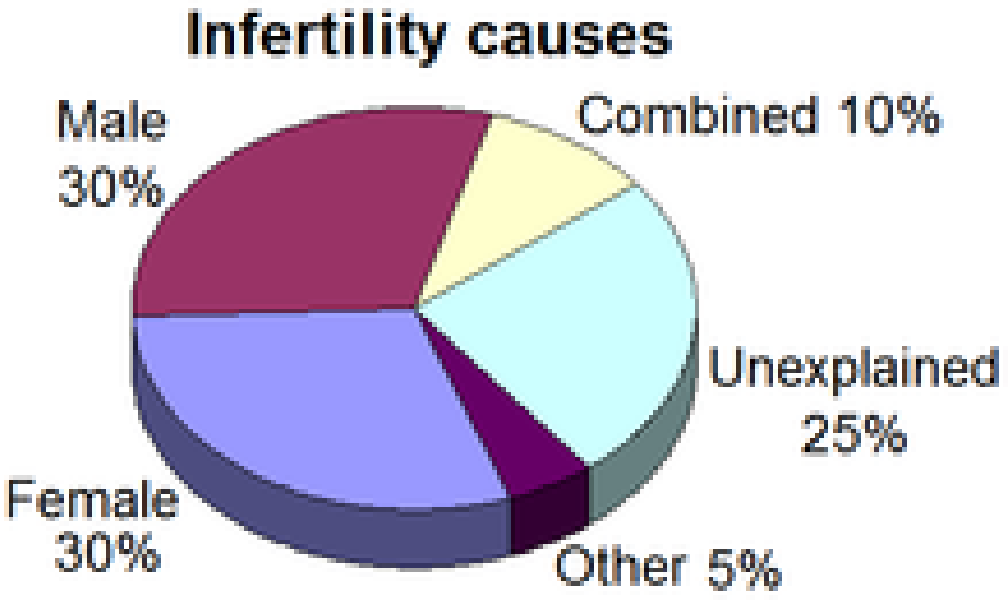

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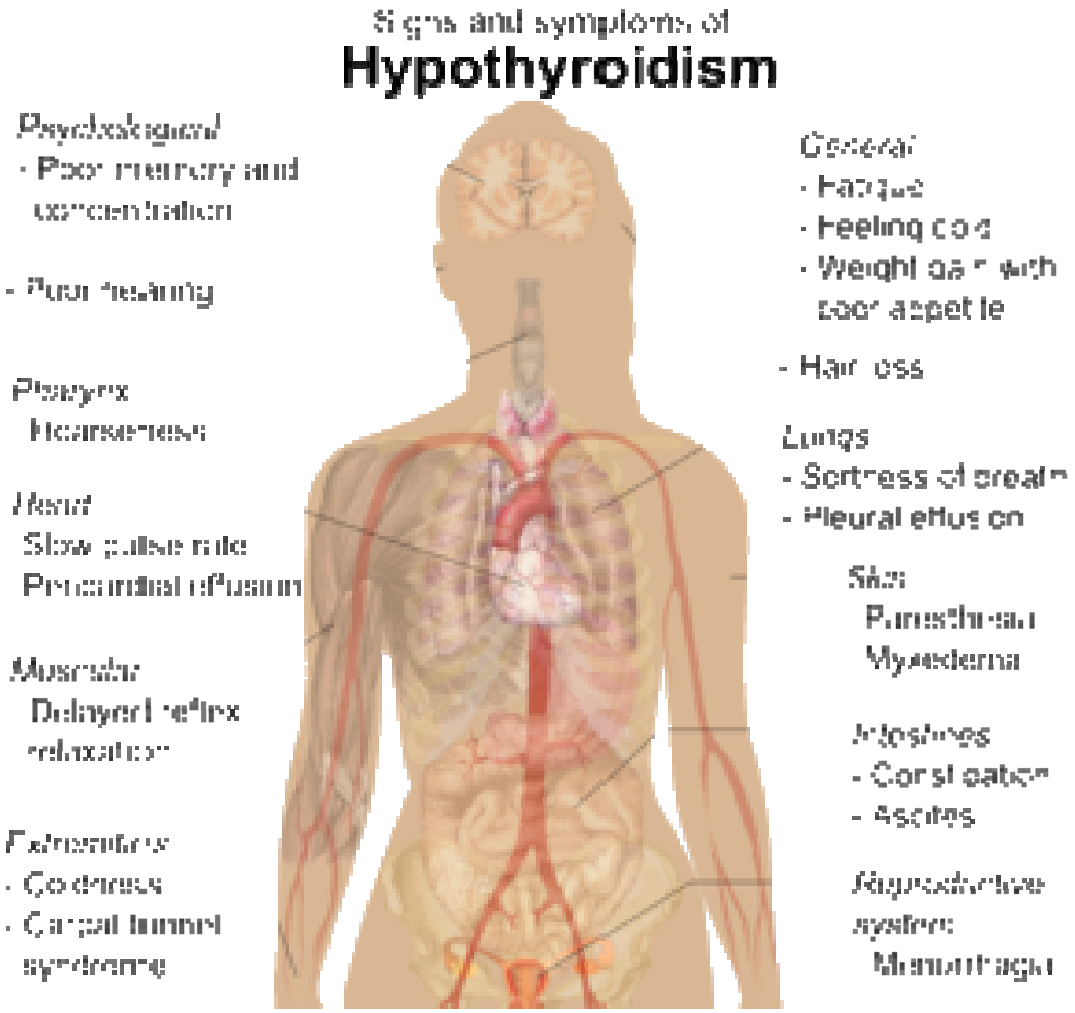
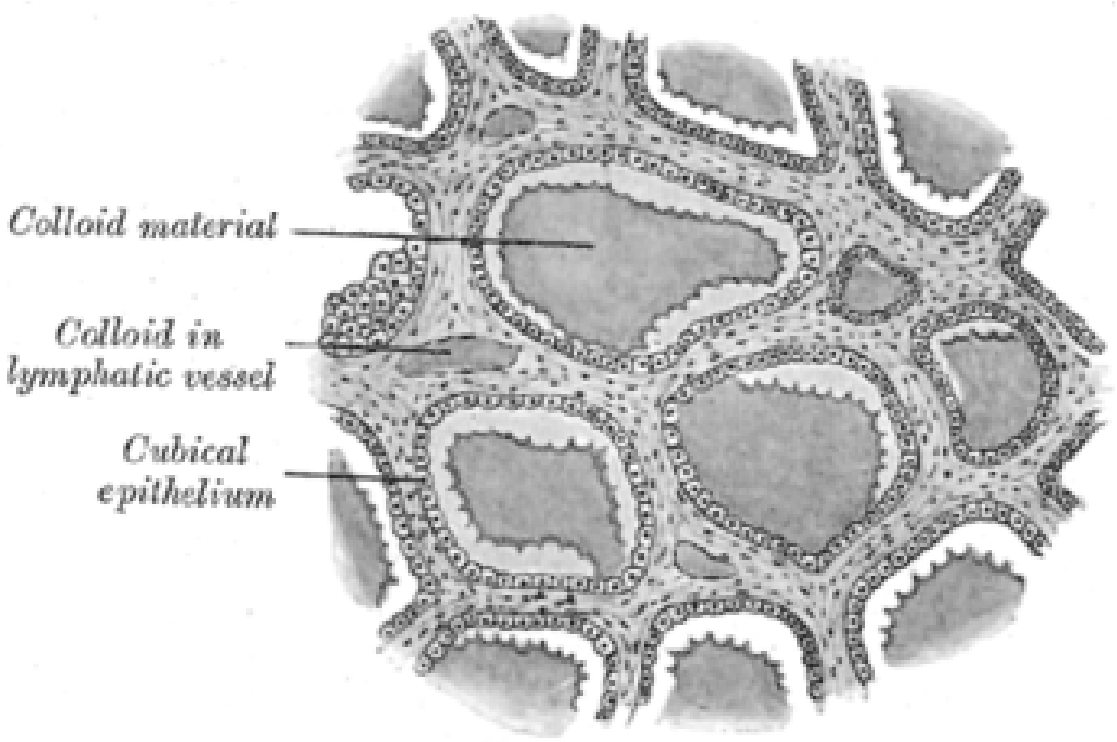
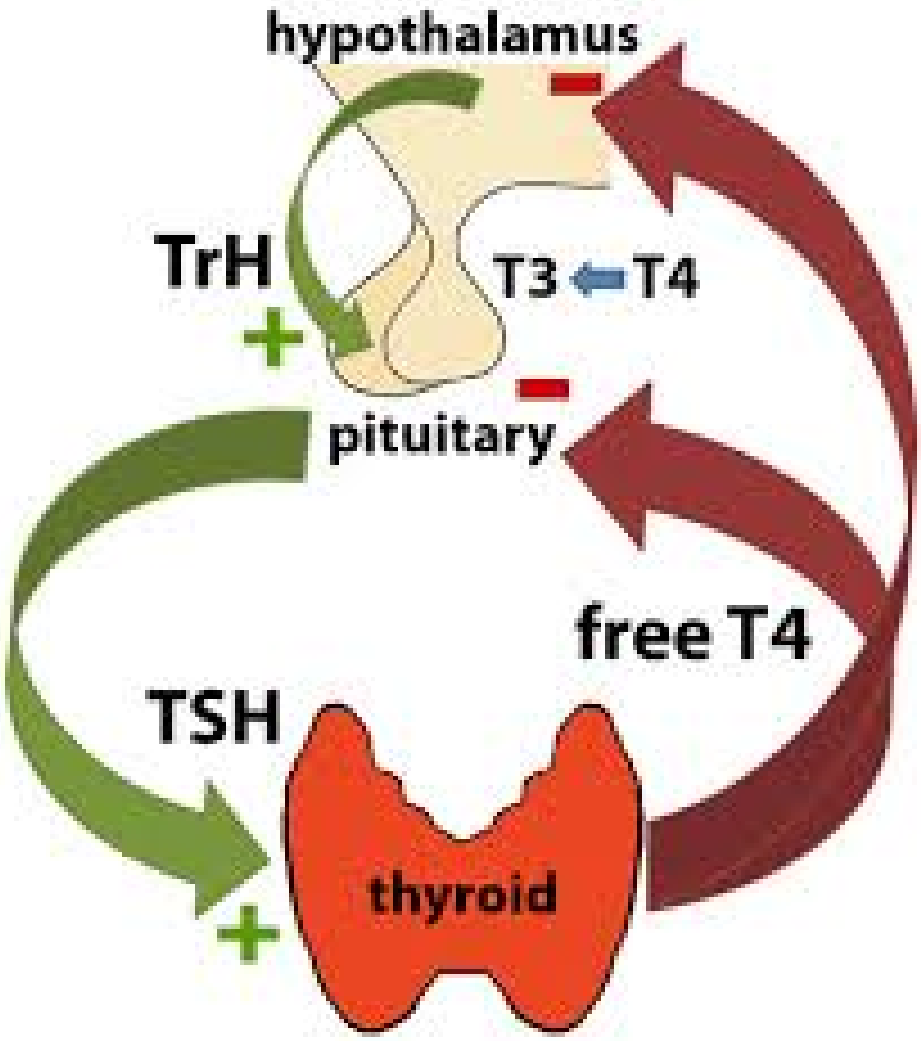


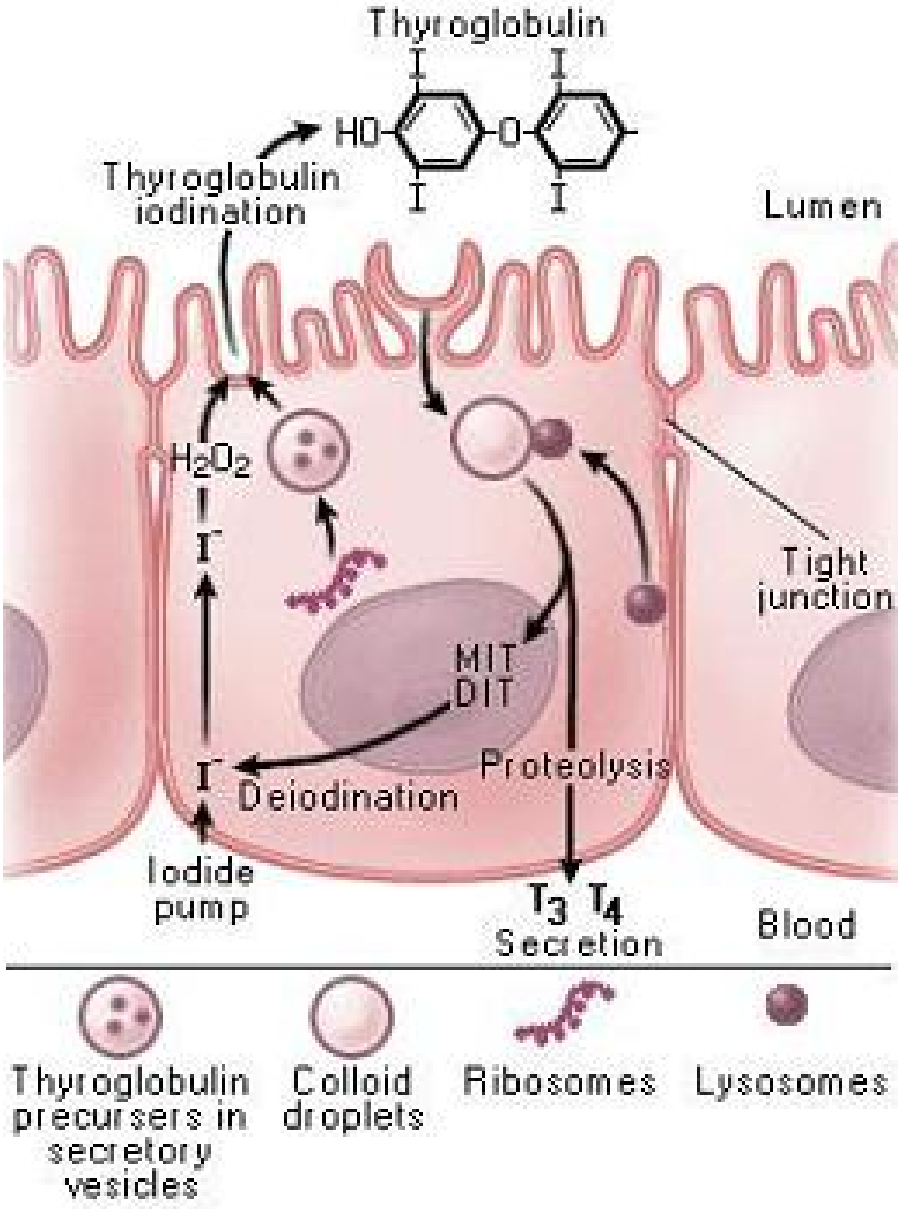
HISTOLOGY OF THYROID GLAND



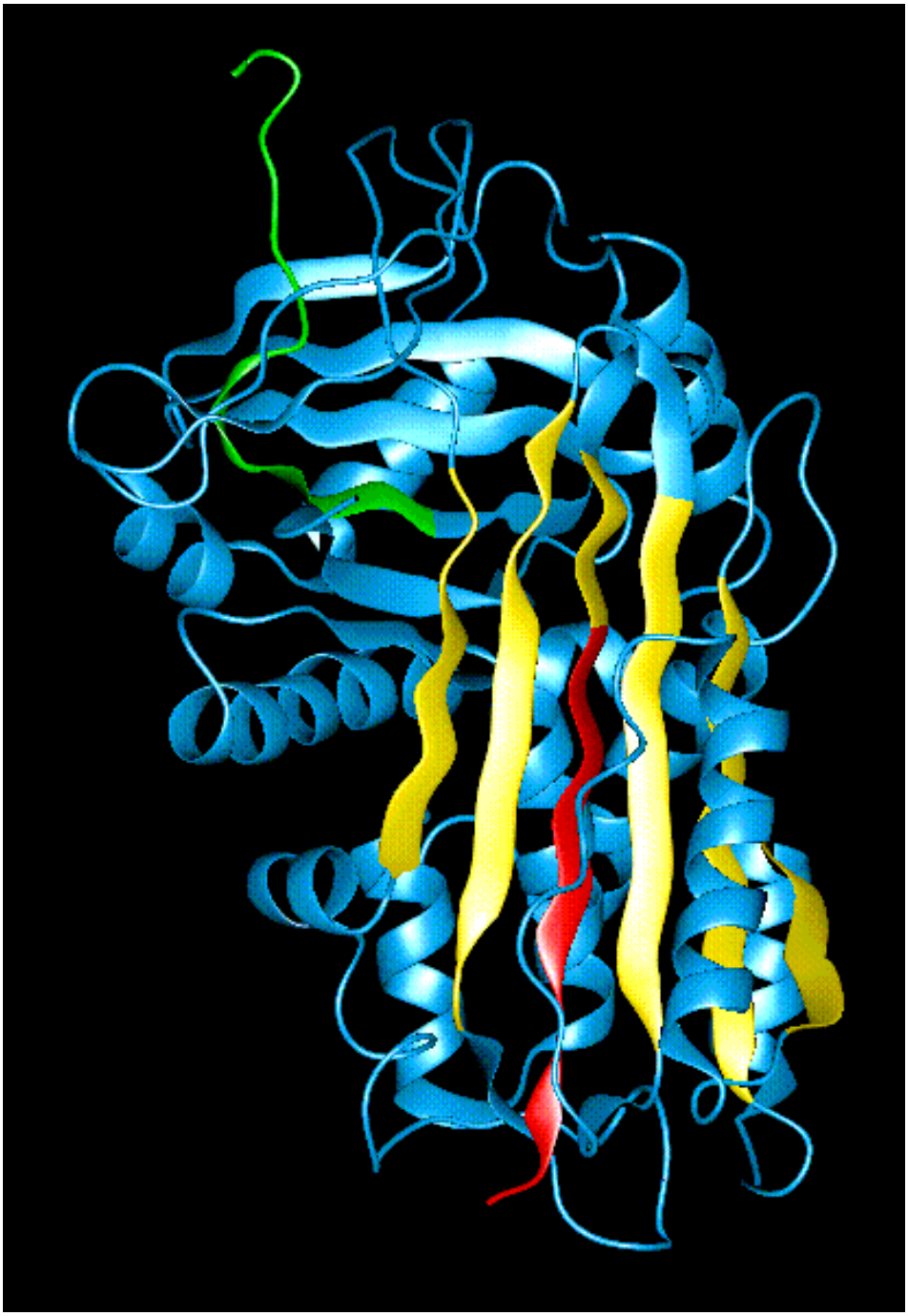
REGULATION OF THYROID HORMONE



SYNTHESIS OF THYROID HORMONE



THYROID STIMULATING HORMONE



INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No : 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. R. Amirtha Jansi Rani,
PG in MD Biochemistry,
Institute of Biochemistry,
Madras Medical College, Chennai-3.

Dear Dr. R. Amirtha Jansi Rani,

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"Prevalence of hypothyroidism in newly diagnosed polycystic ovarian syndrome patients"** No.17022014

The following members of Ethics Committee were present in the meeting held on 04.02.2014 conducted at Madras Medical College, Chennai-3.

1. Dr. G. Sivakumar, MS FICS FAIS -- Chairperson
2. Dr. Kalai Selvi, MD -- Member Secretary
   Prof. of Pharmacology, MMC, Ch-3
3. Prof. Dr. K.Ramadevi, MD -- Member
   Director i/c, Instt. of Biochemistry, Chennai.
4. Dr. Geetha Devadoss, -- Member
   Associate Professor of Pathology, MMC,Ch-3.
5. Prof. Dr. Sivasubramanian, -- Member
   I/c Director, Institute of Internal Medicine, MMC, Ch-3.
6. Thiru. S. Govindasamy, BABL -- Lawyer
7. Tmt. Arnold Saulina, MA MSW -- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

13/2/14

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

PATIENT CONSENT FORM

PREVALENCE OF HYPOTHYROIDISM IN NEWLY DIAGNOSED POLYCYSTIC OVARIAN SYNDROME PATIENTS

Name :

Date :

Age :

OP No :

Sex :

Project Patient No :

Address:

Phone NO:

The details of the study have been provided to me in writing and explained to me in my own language.

I confirm that I have understood the above study and had the opportunity to ask questions.

I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected.

I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).

I have been given an information sheet giving details of the study.

I fully consent to participate in the above study conducted by Dr.Amirtha jansiRani.

Signature of the investigator.

Signature of the Participant

Date:

ஆராய்ச்சிட ஒப்புதல் கடிதம்

ஆராய்ச்சி தலைப்பு : **அண்ட பல்நீர்கட்டிநோய் கண்டறியப்பட்ட நோயாளிகளில் தைராய்டு சுரப்பு குறைபாடு பரவியுள்ளமையை கண்டறிதல்.**

ஆராய்ச்சியாளர் : மரு. இரா. அமிர்தா ஜான்சிராணி,
2ம் ஆண்டு (முதுநிலை பட்டதாரி)
உயிர்வேதியியல் துறை,
சென்னை மருத்துவக் கல்லூரி,
சென்னை - 600003.

ஆராய்ச்சி நடைபெறும் இடம் : சென்னை மருத்துவக் கல்லூரி மற்றும்
இராஜீவ் காந்தி அரசு பொது மருத்துவமனை,
சென்னை - 600003.

பெயர் /வயது :

பால் :

தேதி :

புறநோயாளி எண் :

ஆராய்ச்சி சேர்க்கை எண் :

இந்த ஆராய்ச்சியை பற்றிய விவரங்கள் அதன் நோக்கங்களும் முழுமையாகவும் தொளிவாகவும் விளக்கப்பட்டது.

அதனை புரிந்து கொண்டு நான் என் சம்மதத்தை தெரிவிக்கிறேன். எனக்கு இரத்தப் பரிசோதனை செய்து கொள்ள சம்மதம்.

அண்ட நீர்கட்டி நோய் கண்டறியப்பட்ட பெண்களில் தைராய்டு சுரப்பி குறைபாடும் பரவி உள்ளதா என்ற இந்த ஆராய்ச்சியின் விவரங்கள் கொண்ட தகவல் தாளை பெற்றுக் கொண்டேன்.

இந்த ஆராய்ச்சியில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில்தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் என்பதையும், அதனால் என் சிகிச்சைக்கு எந்த பாதிப்பும் ஏற்படாது என்பதையும் புரிந்து கொண்டேன். மேலும் இதன் மூலம் எந்த பின்விளைவும் வராது என மருத்துவர் மூலம் தெரிந்துகொண்டு என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் என்னையும் சேர்த்துக் கொள்ள நான் சம்மதிக்கிறேன்.

ஆராய்ச்சியாளர் கையொப்பம்

தேதி :

பங்கேற்பாளர் கையொப்பம்

PROFORMA

NAME : **DATE:**

AGE : **SEX :** **PROJECT SERIAL NO:**

IP/OP NO : **CASE/CONTROL:**

ADDRESS :

CONTACT NO:

SAMPLE COLLECTION CENTRE :

MAIN COMPLAINTS : MENSTRUAL IRREGULARITY ☐ OLIGO/AMENNORRHOEA INFERTILITY ☐
WEIGHT GAIN ☐ OTHERS ☐ LMP
DURATION _____

ASSOCIATED COMPLAINTS : ACNE ☐ HIRSUITISM ☐ HYPERTRICHOSIS ☐
RECURRENT PREGNANCY LOSS ☐ GALACTORRHOEA ☐ OTHERS ☐

MENSTRUAL HISTORY : MENARCHY AGE ☐ CYCLE : REGULAR/IRREGULAR DURATION_____
ASSOCIATED WITH ABDOMINAL PAIN ☐

MARRIED SINCE : CYCLE: REG/IRREGULAR DURATION _____
ABORTION ☐ NO ☐ GESTATIONAL AGE ☐

FAMILY HISTORY : MOTHER CYCLE: ☐ SISTER: ☐ AUNTY: ☐

TREATMENT HISTORY : OC.PILLS ☐ THYROXINE ☐ CORTICOSTEROIDS ☐ ANTIEPILEPTICS ☐
AMIADARONE ☐ OTHERS ☐ DURATIION_____

CLINICAL EXAMINATION : HEIGHT: _____ WEIGHT: _____ BMI: _____
Waist: Hip:

CLINICAL EXAMINATION OF THE : NECK:

BREAST:

INVESTIGATIONS:

FASTING GLUCOSE :

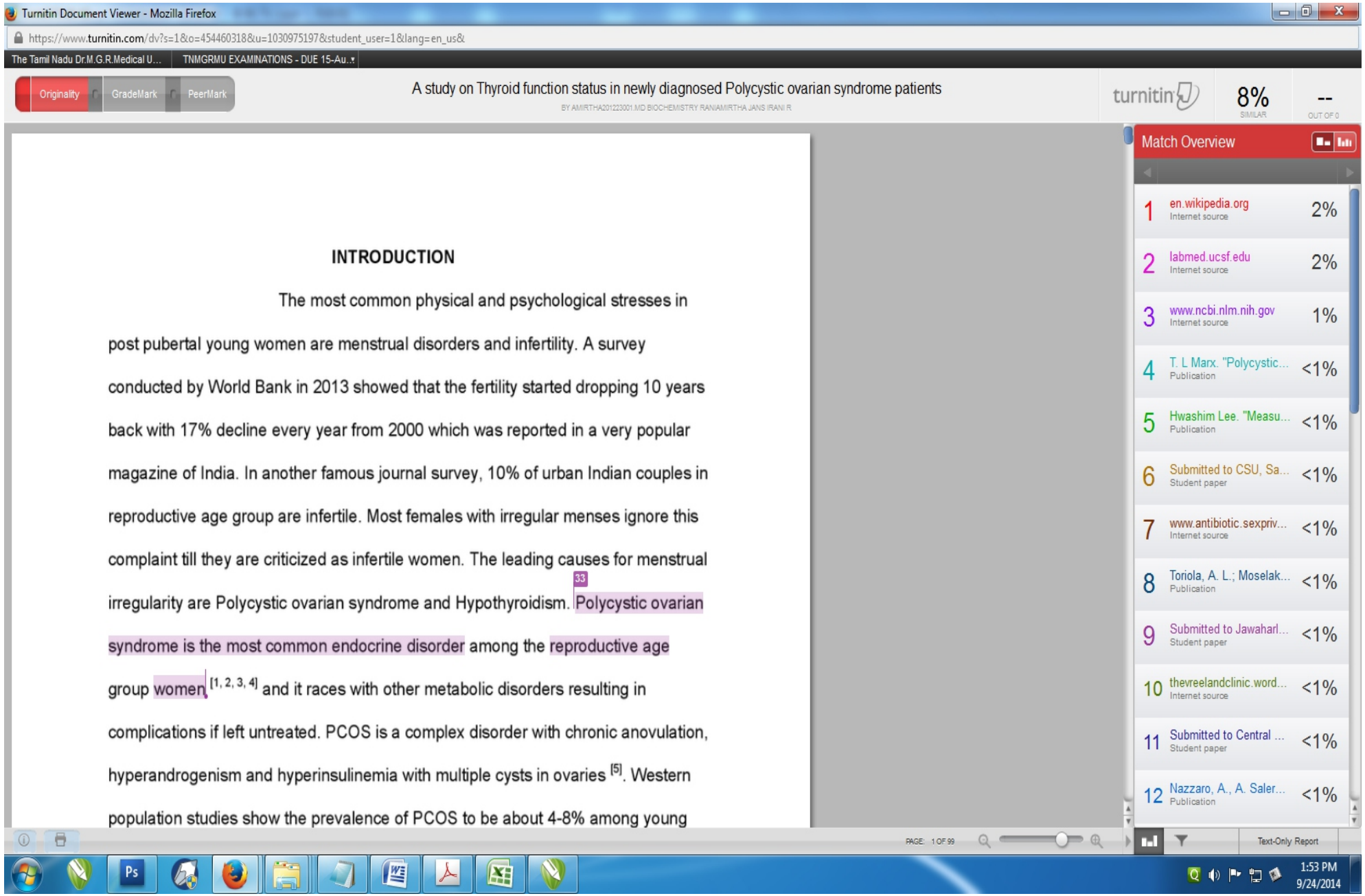
2HRS POST GLUCOSE :

FASTING INSULIN :

TSH :

TESTOSTERONE :

ULTRASOUND PELVIS & OVARIES: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A study on Thyroid function status in newly diagnosed Polycystic ovarian syndrome patients
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INTRODUCTION
The most common physical and psychological stresses in post pubertal young women are menstrual disorders and infertility. A survey conducted by World Bank in 2013 showed that the fertility started dropping 10 years back with 17% decline every year from 2000 which was reported in a very popular magazine of India. In another famous journal survey, 10% of urban Indian couples in reproductive age group are infertile. Most females with irregular menses ignore this complaint till they are criticized as infertile women. The leading causes for menstrual irregularity are Polycystic ovarian syndrome and Hypothyroidism. 33 Polycystic ovarian syndrome is the most common endocrine disorder among the reproductive age group women [1, 2, 3, 4] and it races with other metabolic disorders resulting in complications if left untreated. PCOS is a complex disorder with chronic anovulation, hyperandrogenism and hyperinsulinemia with multiple cysts in ovaries [5]. Western population studies show the prevalence of PCOS to be about 4-8% among young
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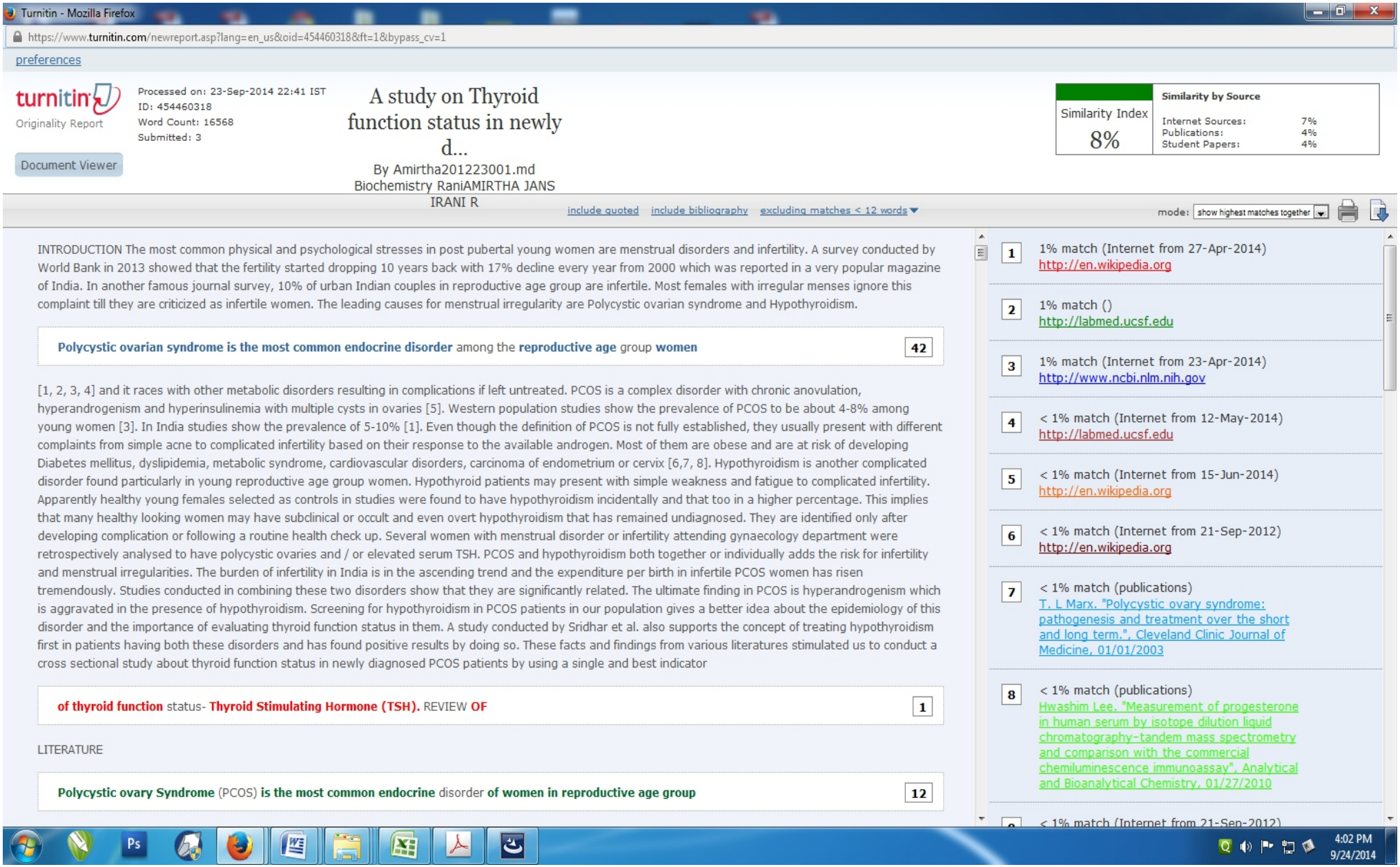
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INTRODUCTION The most common physical and psychological stresses in post pubertal young women are menstrual disorders and infertility. A survey conducted by World Bank in 2013 showed that the fertility started dropping 10 years back with 17% decline every year from 2000 which was reported in a very popular magazine of India. In another famous journal survey, 10% of urban Indian couples in reproductive age group are infertile. Most females with irregular menses ignore this complaint till they are criticized as infertile women. The leading causes for menstrual irregularity are Polycystic ovarian syndrome and Hypothyroidism.
Polycystic ovarian syndrome is the most common endocrine disorder among the reproductive age group women
42
[1, 2, 3, 4] and it races with other metabolic disorders resulting in complications if left untreated. PCOS is a complex disorder with chronic anovulation, hyperandrogenism and hyperinsulinemia with multiple cysts in ovaries [5]. Western population studies show the prevalence of PCOS to be about 4-8% among young women [3]. In India studies show the prevalence of 5-10% [1]. Even though the definition of PCOS is not fully established, they usually present with different complaints from simple acne to complicated infertility based on their response to the available androgen. Most of them are obese and are at risk of developing Diabetes mellitus, dyslipidemia, metabolic syndrome, cardiovascular disorders, carcinoma of endometrium or cervix [6,7, 8]. Hypothyroidism is another complicated disorder found particularly in young reproductive age group women. Hypothyroid patients may present with simple weakness and fatigue to complicated infertility. Apparently healthy young females selected as controls in studies were found to have hypothyroidism incidentally and that too in a higher percentage. This implies that many healthy looking women may have subclinical or occult and even overt hypothyroidism that has remained undiagnosed. They are identified only after developing complication or following a routine health check up. Several women with menstrual disorder or infertility attending gynaecology department were retrospectively analysed to have polycystic ovaries and / or elevated serum TSH. PCOS and hypothyroidism both together or individually adds the risk for infertility and menstrual irregularities. The burden of infertility in India is in the ascending trend and the expenditure per birth in infertile PCOS women has risen tremendously. Studies conducted in combining these two disorders show that they are significantly related. The ultimate finding in PCOS is hyperandrogenism which is aggravated in the presence of hypothyroidism. Screening for hypothyroidism in PCOS patients in our population gives a better idea about the epidemiology of this disorder and the importance of evaluating thyroid function status in them. A study conducted by Sridhar et al. also supports the concept of treating hypothyroidism first in patients having both these disorders and has found positive results by doing so. These facts and findings from various literatures stimulated us to conduct a cross sectional study about thyroid function status in newly diagnosed PCOS patients by using a single and best indicator
of thyroid function status- Thyroid Stimulating Hormone (TSH). REVIEW OF
1
LITERATURE
Polycystic ovary Syndrome (PCOS) is the most common endocrine disorder of women in reproductive age group
12
1 1% match (Internet from 27-Apr-2014) http://en.wikipedia.org
2 1% match () http://labmed.ucsf.edu
3 1% match (Internet from 23-Apr-2014) http://www.ncbi.nlm.nih.gov
4 < 1% match (Internet from 12-May-2014) http://labmed.ucsf.edu
5 < 1% match (Internet from 15-Jun-2014) http://en.wikipedia.org
6 < 1% match (Internet from 21-Sep-2012) http://en.wikipedia.org
7 < 1% match (publications) T. L Marx. "Polycystic ovary syndrome: pathogenesis and treatment over the short and long term.", Cleveland Clinic Journal of Medicine, 01/01/2003
8 < 1% match (publications) Hwashim Lee. "Measurement of progesterone in human serum by isotope dilution liquid chromatography–tandem mass spectrometry and comparison with the commercial chemiluminescence immunoassay", Analytical and Bioanalytical Chemistry, 01/27/2010
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INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No : 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. R. Amirtha Jansi Rani,
PG in MD Biochemistry,
Institute of Biochemistry,
Madras Medical College, Chennai-3.

Dear Dr. R. Amirtha Jansi Rani,

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"Prevalence of hypothyroidism in newly diagnosed polycystic ovarian syndrome patients"** No.17022014

The following members of Ethics Committee were present in the meeting held on 04.02.2014 conducted at Madras Medical College, Chennai-3.

1. Dr. G. Sivakumar, MS FICS FAIS -- Chairperson
2. Dr. Kalai Selvi, MD -- Member Secretary
   Prof. of Pharmacology, MMC, Ch-3
3. Prof. Dr. K.Ramadevi, MD -- Member
   Director i/c, Instt. of Biochemistry, Chennai.
4. Dr. Geetha Devadoss, -- Member
   Associate Professor of Pathology, MMC,Ch-3.
5. Prof. Dr. Sivasubramanian, -- Member
   I/c Director, Institute of Internal Medicine, MMC, Ch-3.
6. Thiru. S. Govindasamy, BABL -- Lawyer
7. Tmt. Arnold Saulina, MA MSW -- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

13/2/14

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003

PATIENT CONSENT FORM

PREVALENCE OF HYPOTHYROIDISM IN NEWLY DIAGNOSED POLYCYSTIC OVARIAN SYNDROME PATIENTS

Name :

Date :

Age :

OP No :

Sex :

Project Patient No :

Address:

Phone NO:

The details of the study have been provided to me in writing and explained to me in my own language.

I confirm that I have understood the above study and had the opportunity to ask questions.

I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected.

I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s).

I have been given an information sheet giving details of the study.

I fully consent to participate in the above study conducted by Dr.Amirtha jansiRani.

Signature of the investigator.

Signature of the Participant

Date:

ஆராய்ச்சிட ஒப்புதல் கடிதம்

ஆராய்ச்சி தலைப்பு : **அண்ட பல்நீர்கட்டிநோய் கண்டறியப்பட்ட நோயாளிகளில் தைராய்டு சுரப்பு குறைபாடு பரவியுள்ளமையை கண்டறிதல்.**

ஆராய்ச்சியாளர் : மரு. இரா. அமிர்தா ஜான்சிராணி,
2ம் ஆண்டு (முதுநிலை பட்டதாரி)
உயிர்வேதியியல் துறை,
சென்னை மருத்துவக் கல்லூரி,
சென்னை - 600003.

ஆராய்ச்சி நடைபெறும் இடம் : சென்னை மருத்துவக் கல்லூரி மற்றும்
இராஜீவ் காந்தி அரசு பொது மருத்துவமனை,
சென்னை - 600003.

பெயர் /வயது :

பால் :

தேதி :

புறநோயாளி எண் :

ஆராய்ச்சி சேர்க்கை எண் :

இந்த ஆராய்ச்சியை பற்றிய விவரங்கள் அதன் நோக்கங்களும் முழுமையாகவும் தொளிவாகவும் விளக்கப்பட்டது.

அதனை புரிந்து கொண்டு நான் என் சம்மதத்தை தெரிவிக்கிறேன். எனக்கு இரத்தப் பரிசோதனை செய்து கொள்ள சம்மதம்.

அண்ட நீர்கட்டி நோய் கண்டறியப்பட்ட பெண்களில் தைராய்டு சுரப்பி குறைபாடும் பரவி உள்ளதா என்ற இந்த ஆராய்ச்சியின் விவரங்கள் கொண்ட தகவல் தாளை பெற்றுக் கொண்டேன்.

இந்த ஆராய்ச்சியில் பிறரின் நிர்பந்தமின்றி என் சொந்த விருப்பத்தின் பேரில்தான் பங்கு பெறுகிறேன் மற்றும் நான் இந்த ஆராய்ச்சியிலிருந்து எந்நேரமும் பின்வாங்கலாம் என்பதையும், அதனால் என் சிகிச்சைக்கு எந்த பாதிப்பும் ஏற்படாது என்பதையும் புரிந்து கொண்டேன். மேலும் இதன் மூலம் எந்த பின்விளைவும் வராது என மருத்துவர் மூலம் தெரிந்துகொண்டு என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் என்னையும் சேர்த்துக் கொள்ள நான் சம்மதிக்கிறேன்.

ஆராய்ச்சியாளர் கையொப்பம்

தேதி :

பங்கேற்பாளர் கையொப்பம்