“EVALUATION OF THYROID DYSFUNCTION IN DIFFERENT STAGES OF FEMALES”

FULL TERM TRAINING REPORT SUBMITTED TO
LOVELY PROFESSIONAL UNIVERSITY, PUNJAB
IN THE PARTIALFULFILMENT OF REQUIREMENTS
FOR THE DEGREE OF
MASTER OF SCIENCE
IN
CLINICAL BIOCHEMISTRY
SUBMITTED BY

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CERTIFICATE

This is to certify that the present full term training report entitled **"Evaluation of thyroid dysfunction in different stages of females "** is the outcome of the original piece of work carried out by Sapna Rani (Registration No: 11300726) herself under my guidance and the contents of her report did not form a basis of the award of any previous degree to her and to the best of my knowledge to anybody also. The report has not been submitted by the candidate for any research degree in any other University. The training report is fit for submission to the partial fulfillment of the conditions for the award of M.Sc. in Clinical Biochemistry. Further certified that the candidate in habit and character is a fit and proper person for the award.

(Dr. Ekta Chitkara)
Internal Supervisor.

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Certificate

Certified that the data included by Sapna Rani in the training report titled "Evaluation of thyroid dysfunction in different stages of females", is genuine and relates to her work done under our supervision and guidance. To the best of our knowledge and belief, the work presented here, it has not been submitted in part or in full for the award of any degree or academic distinction to any learned body and is in accordance with the approved plan of training report.

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TO WHOM IT MAY CONCERN

This is to certify that Ms. Sapna Rani D/o Sh. Bharat Bhushan student of M.Sc (Clinical Biochemistry) from Lovely Professional University, Phagwara,Jalandhar (Punjab) has completed three months internship training in the department of Biochemistry of this college w.e.f. 23-01-2015 to 25-04-2015.

Principal & Dean
Govt. Medical College,
Jammu

Principal/Dean
Govt. Medical College
Jammu

No: JMC/UG/734
Dated: 5-05-2015

DECLARATION

This is to certify that, the work embodied in this training report was carried out by me in the Department of Biochemistry at Govt. Medical College and Hospital Jammu. Under the direct supervision of **Dr. A.S. Bhatia** HOD of Biochemistry, **Mrs. Kapila Raina** Lecturer of Biochemistry (Govt. Medical College and Hospital Jammu) and **Dr. Ekta Chitkara** Assistant professor at Lovely Professional University

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First express my gratitude to Almighty who blessed us and the most beneficent and merciful.

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Sapna Rani

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Abstract

The thyroid gland consider as largest gland in the body located below the voice box secreted two hormones thyroxine and tri-iodothyronine. These hormones are necessary for performing various functions of the body. In India 1.21 billion people believed to suffer from thyroid dysfunction and their prevalence is higher in females then male. Thyroid disorder causes various complications to the females like infertility and subfertility in reproductive females and in pregnancy causes abortion as well as intellectual impairment to the feotus. Bone mineral density decrease in menopausal females as a result of deficiency of estrogen hormone and other hormone which are responsible to affect bone metabolism this can be thyroid hormones in addition to sex steroids

In this study 144 females participated. Out of these 39(27%)females affected through subclinical hypothyroidism with raised level of TSH and normal level of T_3 and T_4 and 8(5.5%) females affected with subclinical hyperthyroidism with low TSH level and normal T_3 and T_4.These all females belonging to three different groups that were reproductive, menopausal and pregnant. Among these 39 females that were affected through hypothyroidism, 18 reproductive females affected through hypothyroidism with raised TSH level having mean values (7.50±2.4) and 14pregnant female affected through hypothyroidism with mean values (8.51±6.4) and 7 menopausal females having subclinical hypothyroidism with mean values of their TSH level (12.6±12). In 8 females that affected through hyperthyroidism 5 females belong to reproductive group 2 females belong to menopausal and only one pregnant female showing less TSH level. Thus, females mostly affected through hypothyroidism and high prevalence seen in reproductive age group. Due to thyroid dysfunction these females showed the symptoms of abnormal menstrual cycle. So, evaluation of thyroid hormones is necessary in females for diagnosis to prevent abnormalities in pregnancy and result healthy full term life birth and in reproductive females for diagnosis of infertility.

Chapter 1

INTRODUCTION

Background

Thyroid gland which is normally located in the front of the neck and vertebrate anatomy it is consider as largest gland in the body(1,2). The two hormone thyroxine(T_4) and tri-idothyronine(T_3) collectively called thyroid hormone secreted by this gland which act throughout the body influencing metabolism, growth and development and body temperature(3-5). The most prevalent endocrinopathies across the world is thyroid diseases (6). These are consider as 2nd most common glandular disorder of the endocrine system and are increasing predominately among women (7). Thyroid dysfunction is prevalent among women of reproductive age when compared with men. In India about 42 million people are believed to affectwith thyroid disorder(8). According to other estimation in India with a population of 1.21 billion, an estimated 108 million people suffer from endocrine and metabolic disorder (9). For screening and evaluating thyroid dysfunction thyroid function test pannel is commonly used. Adequate thyroid hormone (T_4 and T_3) crucially required for brain development during infancy and childhood (10-16).

Different stages in females with hormonal changes:

Reproductive stage:

On the basis of ovarian function females reproductive stage are classified into three main phases- follicular, ovulatory and luteal. These three phases are controlled by pituitary hormonal signals. A female in ovulatory phase goes through an average ovulatory menstrual cycle (MC) that last 28 days but may range from 20-45 days (17). Over the course of an ovulatory menstrual cycle hormonal fluctuations take place which is well defined predictable pattern (18, 19).Various female hormones like Lutenizing hormone (LH) and follicle stimulating hormones and the sex steroids intricate feedback mechanisms. The phases of an ovulatory differentiate by the varying concentration of estradiol and progesterone. Concentration of both hormones low during follicular phase(FP), estrogen is high and progesterone low during the ovulatory phase(OP), and both are high in the lutealphase(17).Hypothyroidism prevalence in the reproductive age(20-40 years) of females and varies from 2% and 4%(20,21). It is a broad spectrum of reproductive disorder ranging from abnormal sexual development through menstrual irregularities to infertility is associated with hypothyroidism. Since 1950s the impact of hypothyroidism on the menstrual cycle has been identified which leads to changes in cycle length and blood flow (22, 23).

Pregnant stage: In pregnancy the female hormone estrogen and progesterone helped in releasing egg from ovary and implant in the uterus lining. There is many other hormonal changes take place in pregnancy to maintain the cellular and molecular demand of maternal and physiological requirements (24-26). Like more hormone are produce when body need excess energy in certain situation which include growth, coldness or during pregnancy. In pregnancy and to sustain a healthy pregnancy normal thyroid function is necessary. There are various effect of thyroid hormone have been seen on pregnancy and reproduction (27). In normal pregnancy concentration of estrogen begins to elevate and due to its effect on liver concentration of serum TBG level also increases. As a result of increased TBG concentration the thyroid hormone (T_3 and T_4) concentration also begin to increase. As a result of over activity of thyroglobulin during pregnancy which show the hyperactivity of thyroid gland seen during normal, pregnancy with increased T_3 and T_4 concentration.(28,29)

Menopause stage:

Menopause is defined as the permanent end of menstrual cycles resulting end of female reproductive life (infertility). This occurs due to the loss of ovarian follicles (30). Females life is classified in three categories according to STRAW (staging of reproductive aging workshop) Reproductive, Menopausal transition and post Menopause. These include menstrual and qualitative hormonal criteria to define each stage. Late reproductive age being characterized by elevated early cycles and when females begin to notice change in the menstrual cycles with marks the time begin to decline. Due to changes in menstrual cyclicity critical endocrine parameter begin to change, and these hormonal changes are important in fertility assessments. This stage is subdivided into two stages. In first stage there are no changes in menstrual cycles or early follicular phase and in 2^{nd} stage shorter cycles begins (31-33). This stage is also characterized by variable but elevated early follicular phase and low AMH levels and AFC. Late menopausal transition defines by the occurrence of amenorrhea of 60 days or longer. Increase variability in cycle's length is seen in menstrual cycles in the late menopausal transition. Extreme flocculation in hormonal levels and increased prevalence of anovulations is seen in this stage. Late postmenopausal represents the periods in which further changes in reproductive endocrine functions are more limited and processes of somatic aging become of paramount concern(34-36). FSH level going to decrease after many years of menopause in old females (37, 38). Deficiency of certain hormone such as estrogen, progesterone, testosterone and dehydroeipandrosterone(DHEA)seen(39-44) in menopausal women with increase in

luteinizing hormone (LH), follicle stimulating hormone(FSH) and thyroid stimulating hormone(45-48). These hormone play individual role in the human body include cardiovascular gastrointestinal and immunological function. Emerging evidence from the ongoing research suggest that the association of high TSH levels, with two folds risk of cognitive decline as well as prevalence of abnormalities in mucoskletal seen in menopausal females(49,50).

Thyroid Hormones and its secretions

Thyroid gland is known to secrete the major catabolic hormones namely thyroxine(T_4) and tri-idotyronine (T_3). Although T_3 is produced from thyroid secretion but 80%of T_3 is produced by de-iodination of T_4 (51) most biologically active among thyroid hormones is T_3 and inter conversion of T_4 into T_3 is accomplished by the action of enzyme 5 deiodonase in the peripheral tissues of liver and kidney. T_4 consist 4 iodine atoms T_4 is transferred or changed into T_3 by the removal of one iodine atom of T_4 (52). TSH is another hormone which is formed in pituitary gland is responsible for controlling T_4 secretion by the thyroid gland. Therefore the primary responsibility or the primary role of TSH is to control the synthesis and secretion of thyroid hormones. The secretion of TSH is govern by another hormone TRH (thyrotropin releasing hormone) which is secreted by hypothalamus (master gland) (53). In conclusion synthesis and secretion of thyroid hormone is regulated by negative feedback system that involves the hypothalamus, pituitary and the thyroid gland (fig1).

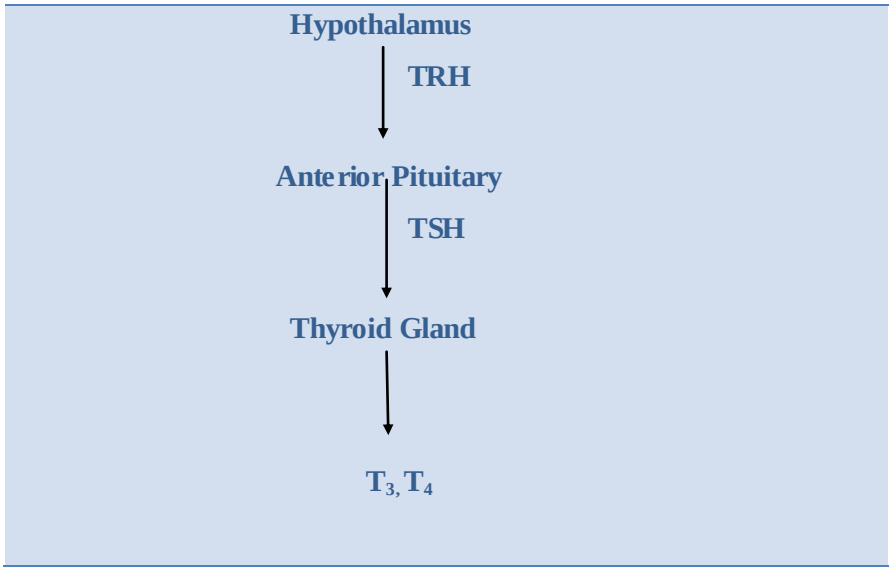


Figure 1. Showing the secretion of thyroid hormones

Structure and physical chemistry of THs (thyroid hormones) with formations

The structure of thyroid hormone is somewhat similar as examined by different studies suggesting that thyroid tissue comprises of follicular epithelium cells that covers the lumen filled with colloid. Follicles are found to be arranged together into thyroid gland in contrast to this follicles are spread in the anterior region of the body (54). The secretion of thyroglobulin protein into the central lumen of the follicles take place when thyroid epithelial cells take up iodine from the circulation. Permolecule of thyroglobulin contains more than 100 tyrosine residues out of which many tyrosine residues are being iodinated at ortho to the phenolic hydroxyl groups in order to form T_4 reflects the arrangement of thyroglobulin. In some condition of iodine deficiency more T_3 and less T_4 level are synthesized (55). Chemical structure of T_3 and T_4 hormones along with its molecular structure is depicted and the outer phenolic ring builds in a place which is perpendicular to the phenolic ring. Two iodothyronines are called as reverse T_3 (Rt_3) and 3, 5 di-iodothyronines both are being synthesized from natural tyrosine residues. Amino acid part of these molecules is L- isomer configuration. The molecular mass of T_4 is 777g/mol and T3 is 651g/mol. The only molecules in the body that are known to contain iodine are thyroid hormones and their metabolites. Iodine atom has a role in making most of the molecular mass of thyroid hormones. These iodines atoms are the heaviest elements in the body. Selenium is the next heaviest element in the body with atomic mass of 79 and is considered as important part of enzyme which role is to de-iodize the thyroid hormones. These iodine atoms have powerful electron binding energies and are electron attractive. The carboxyl groups along with amino and phenolic groups are basically ionizable parts of these molecules. About 50% of dissociation of simple phenolic groups occurs at pH of 10. The pH of phenolic group of T_3 can be reduced to 8.45 by the presence of a single iodine atom that must be electron attractant to the outer T3 ring. The presence of two iodine atom on outer T_4 ring further decrease the pH of its phenolic groups to 6.73(56). Both the T_3 and T_4 have relatively havelow solubility at acidic pH but increased solubility at acidic pH in a dramatic way. Further T_3 is more hydrophobic than T_4 because of the lack of its ionized phenolic group. Thus this becomes the base for common practice of using alkaline solution while injecting thyroid hormones. As the hormones are soluble in the injectable so, once they go inside our body-they are found back to make natural environment or even acid one and finally will be more hydrophilic in nature (57).

Importance of thyroid hormones:

The thyroid hormones have a role in maintenance BMR-basal metabolic rate and homodynamic state of most of the body cells (58). They are responsible for maintaining the normal growth development and functions of almost all the cells of the body with major effect on metabolic rate and oxygen consumption (59). BMR of hepatocytes(liver cells) are also regulated by these hormones thus maintaining the normal functioning of liver. The thyroid hormones and their metabolites regulate endocrine effect in a systemic manner (60). For both normal hepatic circulation and normal billirubin metabolism normal circulation of thyroid hormones are required.(61) These have wide variety of effects on nervous system and thyroid dysfunction correlate with pheripheral neuropathy, cognitive impairment and other disorder of the body like anxiety and depression(62). They also have an effect on the mascular system(63) Cardiovascular system bone formation (eretheropiosis) and on oxidation stress(64) implication of these thyroid disfunction can lead to high risk of morbidity and have adverse effect on health.(65,66) Thyroid hormones are closely involved in cellular metabolism and idothyrosine effect the metabolism of carbohydrates, proteins and lipids are recorded(67).

Disorder of thyroid hormones:

The most common endocrine disorder in our country is thyroid dysfunction (68). Due to primary failure of thyroid gland thyroid dysfunction occurs (69). The variety of thyroid dysfunction ranges from a condition of hypothyroidism (under active) to hyperthyroidism (over active). The clinical and biochemical classification of thyroid hormone include

- Due to thyroid gland dysfunction itself i.e. primary disorder (primary hyperthyroidism and primary hypothyroidism)
- Due to pituitary gland disorder called secondary disorder (secondary hypothyroidism and hyperthyroidism)
- As a result of hypothalamic diseases known as tertiary disorder (tertiary hyper and hypothyroidism)(70).

As a response to several thyroid disorders either excess or deficiency of thyroid hormones occurs. Both thyrotoxicosis and hypothyroidism intensely impact the cardiovascular system, digestive system, metabolism, neuro-psychological functions, muscles and cutis (71).

Hyperthyroidism:-

Hyperthyroidism or thyrotoxicosis is result due to the effect of excess thyroid hormone, there are different disorder triggered by it. In thyrotoxicosis the level of TSH low or undectable level in serum and free hormone level remain normal (72). Overt thyrotoxicosis another condition of hyperthyroidism which is defined by suppressed serum thyrotropin (TSH) and elevated free thyroid hormone concentration (73). There are multiple symptoms of hyperthyroidism present that varying according to the age of patient, magnitude of hormone excess, and presence of comorbid condition. On the basis of common sign and symptoms clinical presentation of between older and younger patients done. The diagnosis of older people becomes difficult due to presence of paucity of classic sign and symptoms (74). After a stress full illness in patients of thyroid strom (a rare presentation of hyperthyroidism) with untreated or under treatment patient of hyperthyroidism symptoms like delirium, severe tachycardia, fever, vomiting, diarrhea and dehydration seen (75).

Causes of hyperthyroidism

The most common causes include Graves disease (GD), toxic multinodular goiter (TMNG), and toxic adenoma.

Graves disease: The common cause of hyperthyroidism is Graves disease which accounts for 60 to 80 percent in all cases. This is caused by an antibody which is active against the receptor of thyroid stimulating hormone (TSH), thus stimulating the gland to synthesiz and secrete excess thyroid hormone (T3 and T4). It is a type of autoimmune disease (76).

Toxicmultinodular goiter: Approximately 5% of the cases of hyperthyroidism are caused by toxic multinodular goiter and it is commonly found in iodine deficient areas. It is mostly seen in older patients of above 4o years of age and its onset is more dangerous than Graves disease (77).

Toxic adenoma: toxic adenomas are a type of nodules that function separately. It is common in both the younger patients and in iodine deficient areas (77).

Hypothyroidism:

Hypothyroidism is categorized by low T3 and T4 and high TSH level it is common endocrine disorder due to deficiency of thyroid hormones. The most common cause of hypothyroidism is autoimmune thyroid disease (Hashimoto disease) in areas of adequate iodine intake (78).

Hypothyroidism is more common in felmales having low body mass index during childhood and small body size at the time of birth (79). Due to deficiency of thyroid hormones wide range of effect occurs. This includes either dearrangement in metabolic process or direct effect by accumulation glucosaminoglycans in the tissues. As a result of hypothyroid delayed puberty, anovulation, menstrual irregularities and infertility are common. So, TSH screening should be a routine part of any investigation into menstrual irregularities or infertility.

STATUS OF THYROID HORMONES IN DIFFERENT STAGES OF FEMALES:

In reproductive age group: Thyroid disease if remain undiagnosed and untreated it can cause infertility as well as sub fertility in females of reproductive stage. In our society these condition have important medical, economical and psychological implication. Females fertility rate are affected by abnormality of thyroid function is accomplished though various ways resulting imbalance in sex hormone, luteal phase defect , in anovulatory cycles and high prolactine (PRL) levels. Therefore in fertility, pregnancy and to sustain a healthy pregnancy normal thyroid function is necessary, even in the earliest days after conception. In any females who want to get pregnant thyroid evaluation is done with family history of thyroid dysfunction or irregular menstrual cycle or had more than two miscarriage or unable to conceive after 1 year of unprotected intercourse (80-82). In the reproductive age group prevalence of hypothyroidism is 2-4% and considers a cause of infertility and habitual abortion(83,84). By assessing TSH level in blood hypothyroidism can be easily detected. Subclinical hypothyroidism associated with a slight increase in TSH level with normal T3 and T4 level and low T3 and T4 with high TSH level indicate clinical hypothyroidism. (85)Subclinical hypothyroidism directly cause anovulation or by causing elevation in PRL. Due to increase production of thyrotropin releasing hormone (TRH) many infertile women had associated hyperprolactinemia in case of hypothyroidism (86,87). The common cause of infertility is thyroid disfunction in reproductive females which can be easily managed by correcting the appropriate level of thyroid hormones(88,89). If both hypothyroidism and hyperprolactetinemia is present firstly need to correct hypothyroidism before evaluating further cause of hyperprolactenemia. Thyroxine is the choice of hormone therapy in treatment of hypothyroidism. Menstrual cycle is normalizing by it. It also improves the fertility rate. Therefore for fertilization, the normal TSH level is the pre-requisite requirement. Autoimmune thyroid disease is most common cause of hypothyroidism in this age group(90,91). Due to numerous interaction of thyroid hormone with the female reproductive system severe hypothyroidism is commonly associated with ovulatory dysfunction. As a

result of altered GnRH pulsatile secretion delay response in LH seen and inadequate corpus luteum have been reported (92-94). By the presence of thyroid receptor on human oocyets thyroid responsitivity by the ovaries could be explained (95). To exert direct stimulating effect on granulosa cell function (progesterone production) thyroid hormone also synerigize with the FSH mediated LH/hcgreceptor(96). According to the study of Cramer et al., showed that serum TSH level become a significant predictor of failure of IVF, and it has been reported that in females who produced oocytes that failed to fertilized TSH levels significant higher (97).The impact of hypothyroidism on fertility followed by two pathways i.e. by altering the peripheral metabolism of estrogen and by decreasing SHBG production. As a result of both of this pathways abnormal feedback of the pituitary level seen. By decreasing the production of coagulation factor(factor VII, VIII, IX and XI) hypothyroidism also responsible to causing menorrhagia independently of hormonal changes(98). In hyperthyroid women SHBG production increases, as a result of hyperthyroxinemia increases the gonadotropin response to GnRH it also effect on haemostatic factors in relation to decrease in menstrual flow (99,100). Even thorough this metabolic changes, hyperthyroid women usually maintain biopsies(101) These cycles changes can frequently correct by the treatment of hyperthyroidism. The exact effect of hyperthyroidism on fertility remains ill defined (102). AITD (auto immune thyroid disease) is seen 5-10 times higher in women than in men, this is explained by genetic factor, the effect of oestrogen and perhaps choromosome X abnormalities.(103-106). For normal reproductive function synthesis of steroid hormone by oocytes is dependent on adequate level of thyroid hormones. FSH and LH action on steroid biosynthesis is modulated by T_4 and T_3 binding sites which has been identified in human and mouse oocytes(98,99). The action of estrogen is enhanced by thyroid hormones. Whereas the impact of T_3 on female reproduction or alteration in T_3 level in case of AITD have a physiological relevance in fertility further investigation is require (107,108).

IN PREGNANCY:

Thyroid dysfunction not only seen in reproductive age its prevalence is also seen in pregnancy. The prevalence of thyroid dysfunction in pregnant is 2-3% and main cause is autoimmune thyroditis. During first trimester of pregnancy both thyroid dysfunction and thyroid autoimmunity have independently been associated with adverse pregnancy outcomes (109). All circumstance should be optimal in early pregnancy, in order to achieve an optimal pregnancy outcome, namely healthy full term life birth. During the first trimester adequate functioning of maternal thyroid is required, because development of feotus brain start at this

stage and feotus not able to produce its own thyroid hormone (110). Hypothyroidism particularly caused due to the deficiency of iodine in pregnancy stage. Due to the deficiency of iodine it causes thyroid deficiency in both in mother as well as in feotus(111).It creates a situation which make difficult to determine whether the mental retardation due to maternal hypothyroidism or both maternal and feotus hypothyroidism(112). The cause of hypothyroidism is chronic autoimmune thyroditis among women in child bearing age in developed countries. Antibodies which are responsible to altering thyroid dysfunction of maternal can cross the placenta and in some instance, compromise fetal and neonatal thyroid function (113-118). Thyrotropin–receptor blocking antibody that identified by screening programs for newborn. It has been implicated in cases of transient congenital hypothyroidism (119). For the development of feotus central nervous system, the importance of maternal thyroid hormones is well established. Fetal thyroid gland cannot synthesize iodothyronine until after 10weeks of gestation at this stage therefore maternal thyroxine particularly needed. So, for normal neuro development of feotus maternal as well as fetal thyroid hormone is necessary (120). Due to severe deficiency of iodine result maternal and fetal thyroid insufficiency, thus infant has profound neurologic impairment and mental retardation(121,122). In the first trimester of pregnancy overt hypothyroidism caused by glandular failure load which is also associated with intellectuall impairment during childhood as well as pregnancy complication, it include preeclampsia, plancental abruption, preterm birth, low birth weight and fetal death(123,124). The effect of thyroid deficiency on mild maternal with a normally functioning fetal thyroid gland less clear. It is important in case of thyroid deficiency subclinical hypothyroidism caused, which is associated with elevated serum thyrotropin(thyroid–stimulating hormone) and serum free thyroxine level remain normal.(125,126) In pregnancy the prevalence of subclinical hypothyroidism seen 2 to 5% (127-129). The presence of AITD could be associated with a slight deficiency in thyroid hormones, with increase estrogen level due to inadequate response of the thyroid to adopt the changes, associated with ovarian hyper stimulation. Women had miscarriage significantly lower thyroid hormone level seen in comparison to those who delivered successfully (130). During first trimester of pregnancy thyroid binding globulin (TBG) increases in the beginning, and its value approximately reached double baseline value in the third trimester(131-133). As a result of this during third trimester a marginal fall in free T_3(FT_3) and free T_4(FT_4) seen in iodine sufficient regions, thus thyroid stimulating hormone start slighting rising. Therefore in third trimester increase level of TSH due to decrease FT_3 and FT_4 with increase in total T_3 (TT_3) and T_4 (TT_4) hormone(134-136). During pregnancy

various physiological changes take place which produce alteration in thyroid hormones but also responsible in significant alteration in metabolic process. Due to physiological demand, particularly physical mental and brain development in the first trimester of pregnancy, it should be under specific and particular medical care. To prevent any abnormalities in case of pregnancy thyroid function test is necessary (137-139).

IN MENOPAUSAL FEMALES:

In menopausal female deficiency of certain hormones seen. This include estrogen progesterone, testosterone and dehydropiandrosterone. As a result of estrogen deficiency it causes memory deficts in menopausal females (140). There are certain hormone secretion increase in this stage of female like luteinizing hormone (LH), follicle stimulating hormone and thyroid stimulating hormone.(141-144). These hormones perform various function of the human body including pulmonary, cardiovascular, gastrointestinal and immunological(145,146). Bone mineral density decrease in menopausal females as a result of deficiency of estrogen hormone and other hormone which are responsible to affect bone metabolism are thyroid hormones in addition to sex steroids(147-149). Patients having history with hyperthyroidism in past or present specially taken as a risk factor for osteoporosis, which mainly affect menopausal females. In case of hypothyroidism, the effect on bone reported that bone turnover decreased in adult. In Clinical hypothyroidism due to decrease of thyroid hormone the thickness of cortical bone increase. Whereas no difference in trabecular bone between control and hypothyroid patient(150,151). In hypothyroidism patient bone fracture risk has been reported to increase both before and after diagnosis (152). Hypothyroidism define with abnormal serum thyrotropin concentration in an asymptomatic patient with serum thyroxine(FT4) concentration is normal, it affect more than 10% females older than 60 years(153,154). TSH is consider as negative regulator of skeletal remodeling by reducing the both differentiation of oestoblasts and formation of osteaoclasts(155). Therefore mild elevation in TSH level may adversely influence bone in hypothyroid patients. Sex steroid hormones suggesting playing a role in thyroid carcinogenesis. From the support of experimental evidence estradiol help in promoting the proliferation and invasiness of thyroid cells in vitro(156,157). Reproductive factor such as age at menopause and use of oral contraceptives as exogenous sex hormone have not consistently associated with thyroid cancer.

Chapter 2

Review Literature

In year 2000 Nils Knudsen and colleagues did a comparative study of thyroid functions and types of thyroid dysfunctions on the basis of iodine status in a randomly population of 4694 from the civil registration system in between age group 18 to 65 years. Above age 40yrs patients had hyperthyroidism Ultrasound showed hyperthyroidism associated with macro nodular thyroid structure. Major differences in thyroid dysfunction were found between regions having less difference in iodine excretion. These findings suggested a higher thyroid dysfunctions among elderly persons in the regions where people were iodine deficient (158).

In 2011 Dilutpal Sharma, Akash Gupta *et al.*,2675 patients partcipated in this study and they had a history of thyroid disorder together with abnormal thyroid profile (T_3,T_4 and TSH) and they had undergone thyroid function tests b/w Dec 2012 to Dec 2013. in conclusion patients having normal T_3, T_4 and TSH were Euthyroid, those having low T_3 T_4 and high TSH hypothyroid and those having normal T_3,T_4 but low TSH were hyperthyroid particular females were found more suceptible to thyroid hormone disorder. Increased prevalence of hypothyroidism was among age gap of 26-35 years (32%) hyperthyroidism was found in age group of 36-45(7.9%). Hence this study demonstrated that hypothyroidism more prevalent in females(159).

In 2011, Ines Donangelo, *et al.*,gave a comprehensive review on subclinical hyperthyroidism is defined as less or undetectable serum TSH levels with normal FT_4 and total or FTT_3 levels. At the present time third generation assay are capable to detect TSH at levels as low as 0.01 to 0.02 mI/L Even through suppressed TSH is cost but routinely diagnosis and treatment of low but detectable TSH levels is controversial(160).

In year 2011, Isabela M. Bensenor*et al.*, did a cross section study on 1,373 partcipants(60.8% women) in order to estimate thyroid disorder among older people. A was done in order to evaluate tri idothyronine hormone and free-thyroxin hormonal levels. Prevalence of overt hypothyroidism among women was 0.8%, for men 0.4%, subclinical hyperthyroidism in men was 5.4% and in women it was found 2.8%. Further subclinical finding Hypothyroidism in men was found up to 6.1% and in women 6.7%. Since very little or no difference was found in prevalence rates according to gender but nearly 40% of women were already diagnosed and 9% of them under treatment (161).

In the year 2011 Emmy Vanden Boogaard *et al.,* gave a systemic review on significance of subclinical thyroid dysfunction and thyroid autoimmunity before conception and in early pregnancy. They observed prevalence of thyroid dysfunction were common in reproductive

age. This associated with adverse pregnancy outcomes. They conclude pregnant female mostly affected through subclinical hypothyroidism or thyroid antibodies were on a risk of complication like pre-eclampsia, perinotal mortality miscarriage (162).

In 2012, Mirella P. Hage and Sami T. Azaroberserveda relationship between thyroid dysfunction and depression. In this study, it has been explained that thyroid disturbance may significantly affect the mental health status particular depression that may be accompanied by several thyroid abnormalities. Both abnormal and insufficient thyroid hormone can lead to mood swings including depression that is associated with thyroid disorders. Patients have hypothyroidism commonly have feature of depression; while as hyperthyroidism represents a broad spectrum of depression like neuropsychiatric. Symptoms including patients having normal thyroid function do have primarly depression. Hence the underlying mechanisms behind this controversy still remain unclear but needs a further study. However recent advances in genetic, biochemical and neuro imaging fields have provided new approaches thyroid depression relationship (163).

In 2012, Alhamd Alaa Kamal &colleagees examined a clinical evaluation of T_3, T_4&TSH in first, 2nd&third trimester of pregnancy in the evaluation of hyperthyroidism & hypothyroidism must be assesses by determining serum T_3, T_4 and TSH levels. In the period of pregnancy various alterations were found in T_3, T_4 and TSH levels. Thyroid hormones levels were estimated, 35 full term pregnant women taken for study in order to analyze thyroid hormonal imbalance and another 30 groups taken as control. In first trimester of pregnancy serum TT_3 and TT_4 levels were higher than control in 2nd stage of pregnancy TT_3 and TT_4 continuously increasing then controls but TSH were significantly lower. In the third trimester TT_3 significantly increased while TSH decreased (164).

In 2013 M.A Samad M.M Haque *et.al.*, In a study of normal participants TSH,T_4, and T_3 in human serum was done in order to define their normal values were estimated. 201 assays were performed on normal individuals -comprising 26men and 44women with age group - from 2 to 84 years. The normal TSH observed were between 0.5 to 5.0mlU/L. T_4 - 55 - 169nmol/l and T_3 -1.2 -3.4nmol/L(165).

In2013, Dr. SudhaJa and Dr. Naved Ahmad conducted a study on 506 patients to find out prevalence of thyroid dysfunction in different age and sex groups of patients the study revealed that prevalence of Hypothyroidism was higher in the study population & was more prevalent in females & followed by detection & early treatment which helps to avoid

complications, as the diagnosis & management of thyroid dysfunction were still suboptimal. (166).

In 2014, Priyatarshini M *et.al* did a retrospective sudy to find out thyroid disorders among women of reproductive age group. Screening of 1192 patients was done, out of which 63.8% were euthroid, 27.5%, were had gentle or linicut TSH. Between the hyperthyroid and 20.8 were subclinical hyperthyroid. This study discovered hypothyroidism- especially subclinical one was associated with increasing the age and this prevalence of hypothyroidism was on the elevated side among the women's having infertility, PCOS and menstrual disorder(167).

CHAPTER 3

Aim & objective

OBJECTIVES

- To study the level of various thyroid hormones in females(including pregnant, reproductive and menopausal age group)
- For evaluation of the thyroid hormones, if remain undiagnosed and untreated can cause infertility and sub fertility and normal thyroid function is necessary in pregnancy, fertility and to sustain a healthy pregnancy.
- To compare and correlate the status of thyroid dysfunction including hypothyroidism and hyperthyroidism between the different stages of females.

CHAPTER 4

Material and methods

Material and method

This study was carried out in the department of biochemistry, Shri Maharaja Gulab Singh Hospital Jammu (SMGS) between 23rd Jan 2015 to 24th April 2015. A total of 144 females' participated in the study including pregnant, menopausal and reproductive age group. In the first group of 57 females of reproductive stage participated, in 2nd group 25 females who were menopausal took part and in third group 64 pregnant females enrolled for the study. Both inpatients and outpatients referred from different unit of Hospital for thyroid profile in the central clinical biochemistry enrolled in the study. The age group, clinical diagnosis and thyroid function profile were estimated which include T3, T4 and thyroid stimulating hormone for all the patients.

Evaluation of total T_3, T_4 and TSH done by using Architect system. The ARCHITECT totalT_3(TT_3), total T_4(TT_4) and TSH assay is a ChemiluminescentMicroparticle Immunoassay (CMIA) for the quantitative determination of human thyroid hormones (thyroxine and tri-iodothyronine) and thyroid stimulating hormone in human serum and plasma.

Selection of patients:

Following patients were selected for the study.

- Pregnant females for routine checkup to evaluate normal functioning of thyroid hormones.
- Females having history of menstrual irregularities, abortion, heavy bleeding and two cycles in one month
- Females having symptoms like itching on whole body, lower abdomen pain, weakness, sensation of heat in palm and sole region and swelling.
- Females having previous history of thyroid dysfunction

Requirements

Requirement for collection of sample and for performing test using ARCHITECT assay system procedure.

S.No	Materials
1	Syringes of 2ml
2	Tourniquet
3	Test tubes
4	Test tube stand
5	Centrifuge machine
6	Incubator
7	Refrigerator
8	Auto analyzer(ARCHITECHT system)
9	Cups and cup carrier
10	Reaction vials
11	Pipettes (for separating serum)

Specimen collection and preparation for analysis.

Blood serum was used (including serum collected in serum separator tubes)

Step- 1 Collection of sample:

Blood was collected through vein puncture. The three main veins were selected for collection of blood was cephalic, median cubital and median basilica veins. Firstly tourniquet was applied on to the upper arm and disinfectant was applied at the site by using spirit gauge and allows it to dry. 21 gauge needle of 2ml volume was used after collection of blood the tourniquet was released and a wad of cotton wool was placed at the puncture site. The blood was dispensed in the sample tubes.

Step- 2 Separation of serums:

Blood was placed in the incubator for 5-10 minutes for the formation of clot and then centrifuged at 3000rpm for 15 minutes at room temperature. The supernatant serum was assayed for quantitative determination of T_3, T_4 and TSH.

- While handling patient specimen to prevent cross contamination. Use of disposable pipettes or pipette tip was necessary.

Methodology

Name: ARCHITECT Total T_3, Total T_4 and TSH (Thyroid stimulating hormone)

The ARCHITECT Total T_3 (TT_3), Total T_4 (TT_3) and TSH (Thyroid stimulating hormone) assay is a Chemiluminescent Microparticle Immunoassay (CMIA) for the quantitative determination of T_3, T_4 and TSH.

For estimation of total T_3, Total T_4 and TSH.

Principles:

The ARCHITECT Total T_3, Total T_4 and TSH assay is a two step immunoassay to determine the presence of Total T_3, Total$T_4$and TSH in human serum using CMIA technology with flexible assay protocols.

In the first step, sample and anti T_3 coated paramagneticmicroparticles are combined. T_3 present in the sample binds to the anti- T_3 coated microparticles. For total T_4 estimation, sample and anti-T_4 coated paramagnetic microparicles are combined. Bound T_4 is removed from the binding sites on thyroxine binding globulin, prealbumin and albumin. T_4 present in the sample binds to the anti- T_4 coated microparticles. For TSH sample, anti-β TSH antibody coated paramagnetic microparticles and TSH Assay Diluent are combined. TSH present in the sample binds to anti- TSH antibody coated microparticles.

In the second step T_3acridiinium labeled conjugated is added after washing in case of T_3 and T_4 determination and anti-α TSH acridinium labeled conjugate is added in case of TSH determination. Pre-trigger and trigger solution are then added to the reaction mixture; the

resulting chemiluminescent reaction is measured as relative light unit (RLUS). An inverse relationship exists between the amount of total T_3, total T_4 and TSH in the sample and the RLUs detected by the ARCHITECT *i* optical system.

Figure 2: ARCHITECHT *i* optical system

REAGENTS:

1. **Microparticles(6.6ml/27.0ml):**
   For T_3 anti- T_3 (sheep) coated microparticles in MES buffer with IgG stabilizers. Minimum concentration: 0.08% solids
   For T_4 anti-T_4(sheep) coated microparticles in TRIS buffer with sheep IgG stabilizers. Minimum concentration 0.10% solids.
2. **Conjugate (5.9ml/26.3ml):** T_3acridinium–labeled conjugate in citrate buffer with NaCl and Triton X-100 stablizers. Minimum concentration for T_3 is 0.33ng/ml and for T_4 0.2ng/ml.

Fig 3. Reagent kits of TSH

Fig4. Reagent kit of T_3

Figure 5. Reagents kit of T_4

Other Reagents

- **Pre-Trigger Solution**- Pre trigger solution containing 1.32%(w/v) hydrogen peroxide.
- **Trigger Solution**- Trigger solution containing 0.35 N sodium hydroxide.
- **Wash buffer.** Wash buffer containing phosphate buffered saline solution.
- **Assay diluents(8.0/40.7ml):** TSH assay Diluent in TRIS buffer

Figure 6 showing the Trigger and Pri-trigger solution.

ASSAY PROCEDURE

- Before loading the ARCHITECT Reagent kit on the system for the first time, the microparticles bottle required mixing to resuspend microparticles that have settled during shipment.
- themicroparticles bottle were inverted 30 times.
- Visuall inspection was done until the microparticles werere suspended. If microparticles were still adhered to the bottle, bottles were continuously until microparticles had completely resuspended.
- Once the microparticles had been resuspended, remove and discard the cap. Wearing clean gloves, septum was removed from the bag. The septum was carefully snaped onto the top of the bottle.
- Load the ARCHITECT reagent kit on the ARCHITECHT *i* System. All the necessary reagent would be present. Ensured that septa should present on all reagent bottles.
- The minimum sample cup volume was calculated by the system and was printed on the Orderlist report. No more than 10 replicates may be sampled from the same sample cup.
- If using primary or aliquot tubes, sample gauge was used to ensure sufficient patient specimen.
- ARCHITECT Total T_3, T_4 and TSH calibrator were mixed by gentle inversion prior to use.
- To obtain the recommended volume requirements for the ARCHITECHT assay, the bottle were vertically holded and dispense 4 drops of each calibrator into each respective sample cup.
- Sample was loaded

Press RUN. The ARCHITECHT *i* system perform the following function:

- Moves the sample to the aspiration point
- Loads reaction vessels(RV) into the process path.
- Aspirates the transfer sample into the RV
- Advance the RV one position and transfer micropaticles into the reaction mixture.
- Adds pre-trigger and trigger solution.

- Measures chemiluminescent emission to determine the quantity of total T_3 and T_4 and TSH in the sample.

Limitations of the procedure:

- Specimen run on the ARCHITIECT assay must be processed according to the specimen test tube manufacture's instruction. Insufficient processing including deviations from recommended clotting times, centrifugation times, centrifugation speed and sample preparation techniques may cause inaccuarete results.
- For diagnostic purpose result should be used in conjunction with other data; e.g., symptoms clinical impressions, etc.
- If the T_3, T_4 and TSH are inconsistent with clinical evidence, additional testing is suggested to confirm the test.
- Some patients specimen show increased or decreased falsely result because these patients received mouse monoclonal antibodies for diagnosis or therapy may contain anti-mouse antibodies (HAMA). Additional information required for diagnosis this kind of patients (168, 169). Additional information may be required for diagnosis.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immune assay (170) Patients routinely exposed to animal or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.

Expected Values

Normal Range of TSH 0.35-4.9Uiu/ml

T_3 (total T_3) 0.58-1.59ng/ml

T_4 (total T_4) 4.87-11.72ug/dl

CHAPTER 5

Results

Results

A total no of 144 females participated in the study those females were for evaluation of thyroid profile in the laboratory of biochemistry of Shri Maharaja Gulab Singh Hospital Jammu. Evaluation of thyroid was profile done in three groups, group one of reproductive age group, group pregnant females and third group was comprised of menopausal females. A total of 57 females belong to first group of reproductive age group, group 2nd having comprised of 25 females that had gone through menopausal age and in group 3rd total 62 pregnant females participated.

Table No.1 Different groups of females

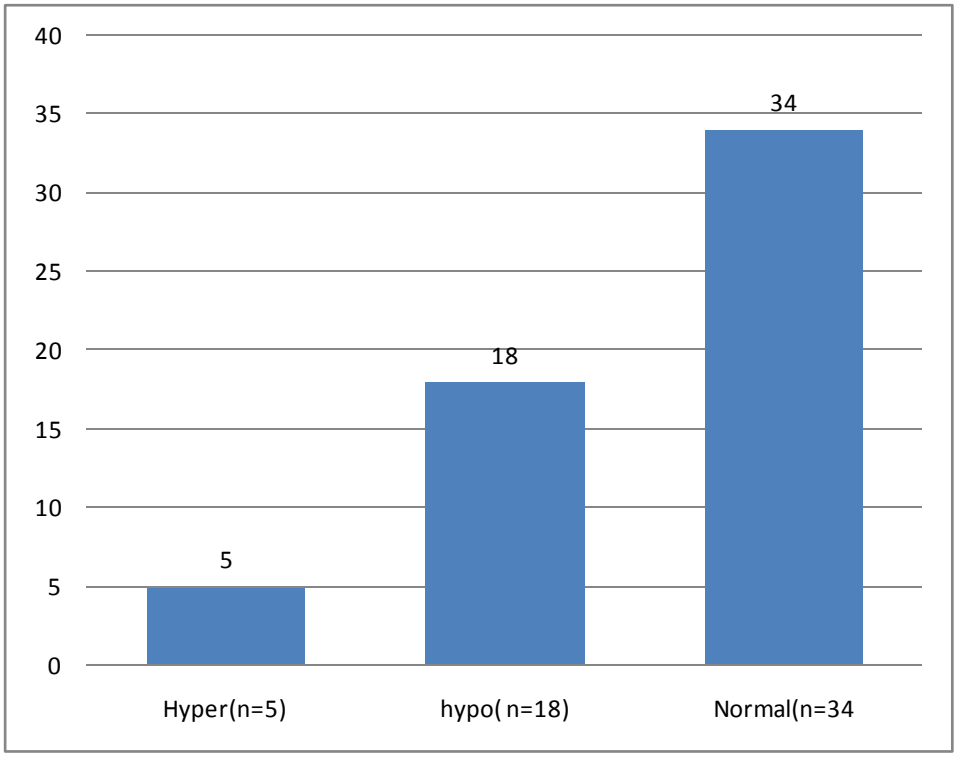
Different Stages	Total No of females (n=144)
Reproductive age group	57
Pregnant	62
Menopausal	25

In the first group 18 females showing raised level of TSH with normal level of T_3 and T_4. The mean value of TSH was 7.50±2.4, T_3 (1.235±0.31) and T4 (7.55±2.1). The female having normal T_3, T_4 and TSH level in this group were 34. The mean values of their T_3 (1.39±0.514), T_4 (6.88±2.628) and TSH (2.4±1.06). Out of these 57 only 5 females found with low TSH level with normal T_3 and T_4. The mean values of their TSH was (0.162±0.038), T_3(1.515±0.3), and T_4(12.76±1.04). Thus on the basis of TSH level distribution of subclinical hypothyroid having high TSH level were `found in 31% females, subclinical hyperthyroidism having low TSH level were in 9 % females and rest 60% were comes under euthyroid having normal T_3,T_4 and TSH level. Table 1 showing the result of T3, T4 and TSH in reproductive age group. (Refer table 1)

Table 1 Result obtained in the present study of Reproductive Age Group

Total no. of patients (n=57)	Age	TotalT_3(ng/ml)	Total T4(ug/dl)	TSH (Uiu/ml)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Hypothyroid (n=18)	28±6.29	1.23±0.30	7.55±2.1	7.5±2.4
Hyperthyroid (n=5)	24±4.9	1.515±0.30	12.7±1.04	0.16±0.038
Euthyroid (n=34)	25±4.6	1.3±0.51	6.8±2.68	2.4±1.0

Figure 7: Distribution of thyroid dysfunction in reproductive age group

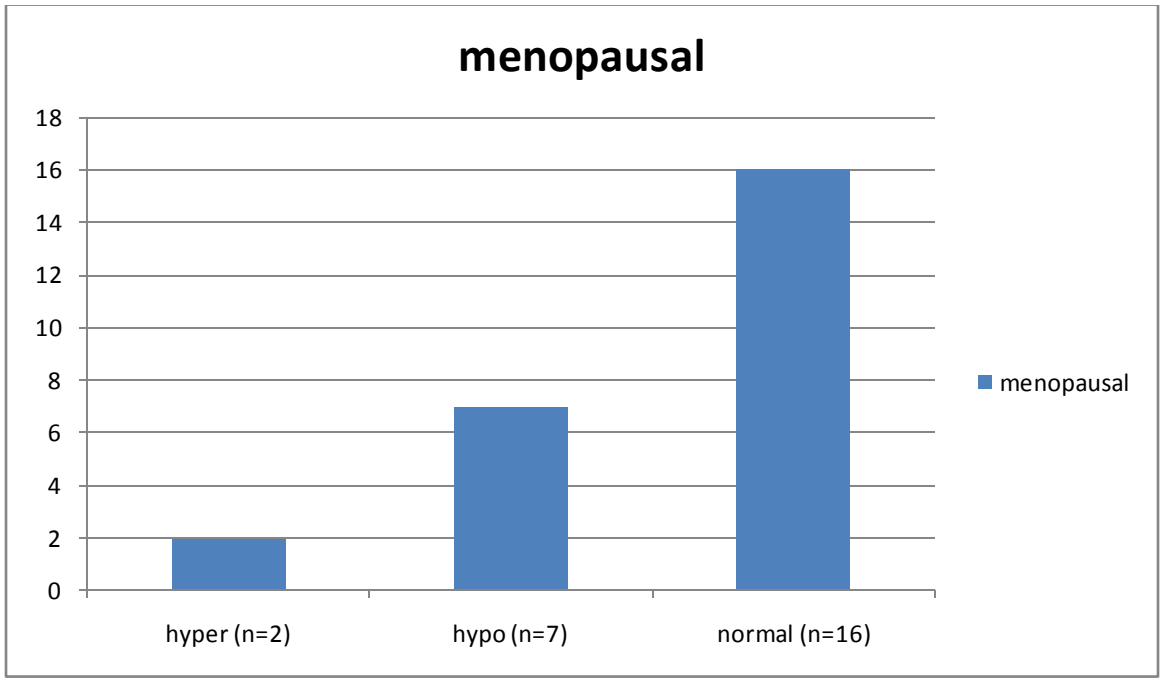


In the 2nd group of menopausal female 25 females participated. Out of this 7 females found with raised level of TSH with normal T_3 and T_4 level. The mean values of age (59.14±7.1) The mean values of total T_3 1.16±0.04, T4 was 7.97±2.16 and TSH 12.6±12. Only two females showing the low TSH level with normal T_3 and T_4 level.Restof 16 having normal level of all the parameters. Thus distribution of thyroid dysfunction in this group showing in Table 2

Table 2: showing the result of T_3, T_4 and TSH in menopausal females

Total no. of patients (n=25)	**Age**	**Total T_3(ng/ml)**	**Total T_4(ug/dl)**	**TSH (Uiu/ml)**
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Hypothyroid (n=7)	59.14±7.14	1.16±0.04	7.97±2.16	12.6±12
Hyperthyroid (n=2)	54±6.6	1.15±0.07	8.14±1.	0.18±0.02
Euthyroid (n=16)	57±9	1.2±0.53	6.9±2.67	2.155±1.0

Figure 8: Represents the menopausal females affected with thyroid dysfunction



In 3rd total number of pregnant females was 62 that took part in the study. Among all these 14 females having previous history of thyroid dysfunction affected through raised level of TSH with normal T_3 and T_4 level and rest 47 was having normal level of T_3, T_4 and TSH. Only one female belonging to this group showed the low level of TSH (Refer table 3)

Table No 3 showing the result of pregnant female

Total no. of patients (n=62)	Age	Total T_3(ng/ml)	Total T_4(ug/dl)	TSH(Uiu/dl)
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Hypothyroid (n=14)	26.1±3.4	1.4±0.34	5.9±2.1	8.5±6.40
Hyperthyroid (n=1)	27	1.6	8.0	0.07
Euthyroid (n=47	24±3.9	1.4±0.47	7.0±2.0	2.1±1.2

Figure 9: Represents the distribution of thyroid dysfunction in pregnant females

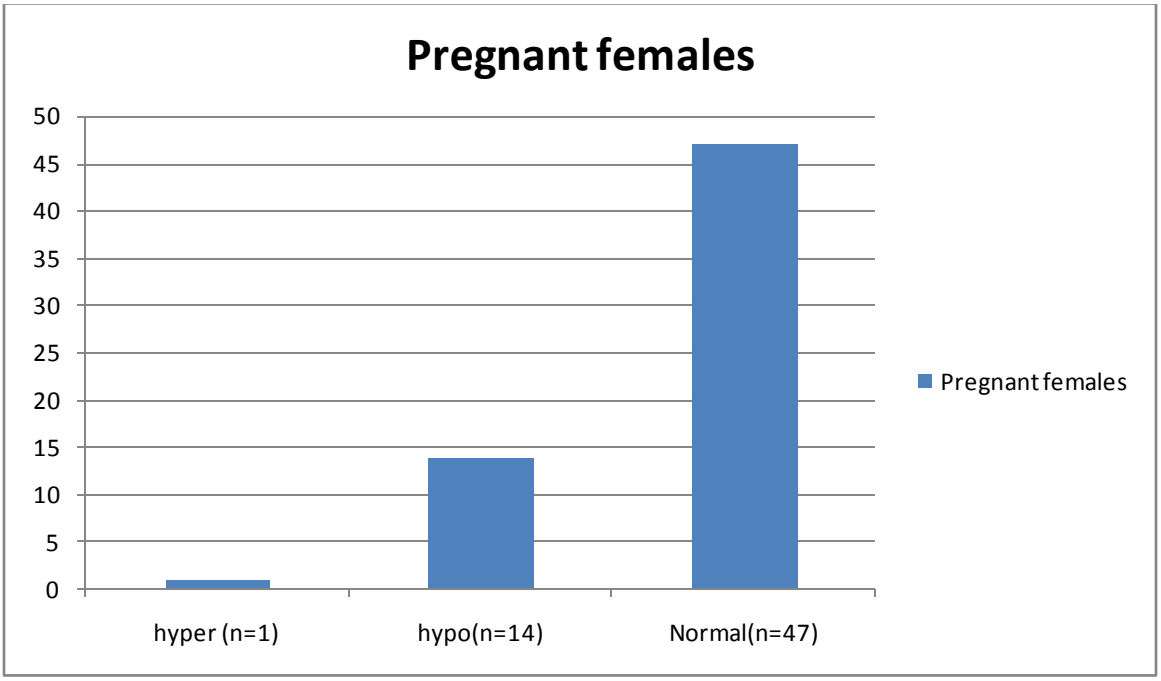
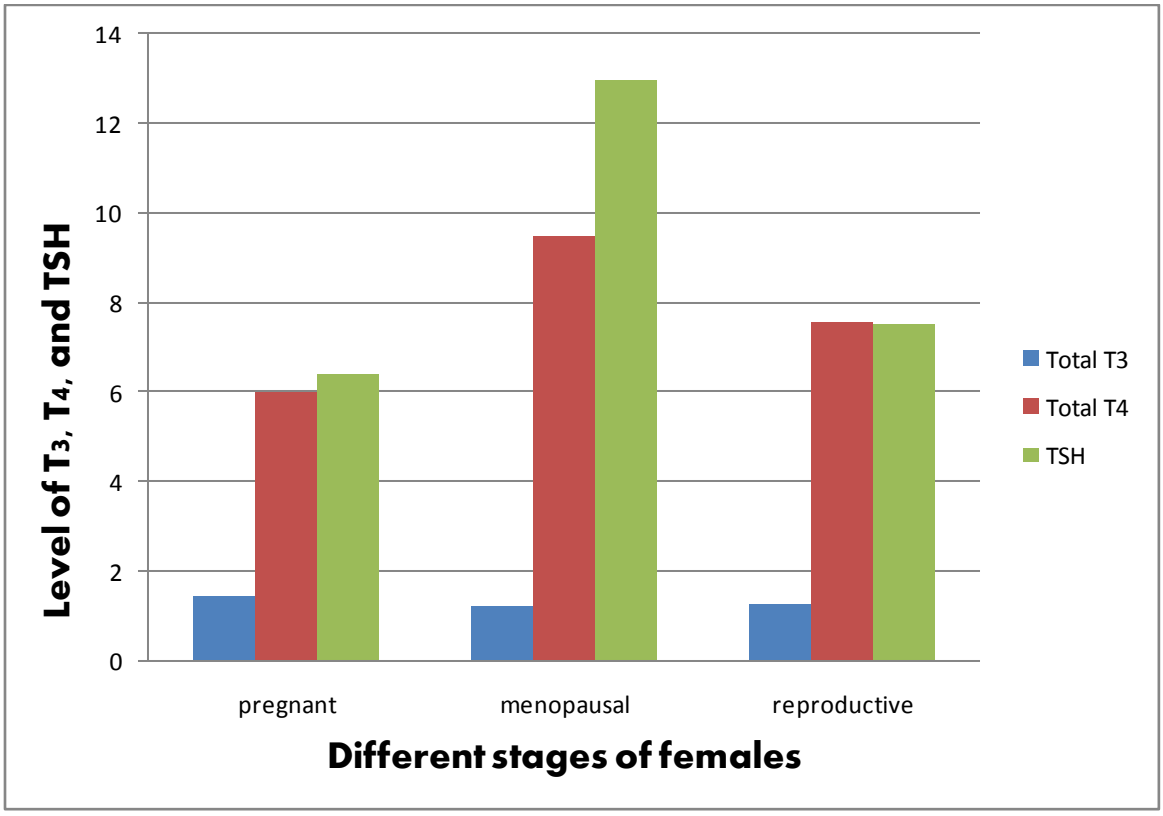


Figure10: represents the level of T_3, T_4 and TSH in hypothyroid females of all the three groups

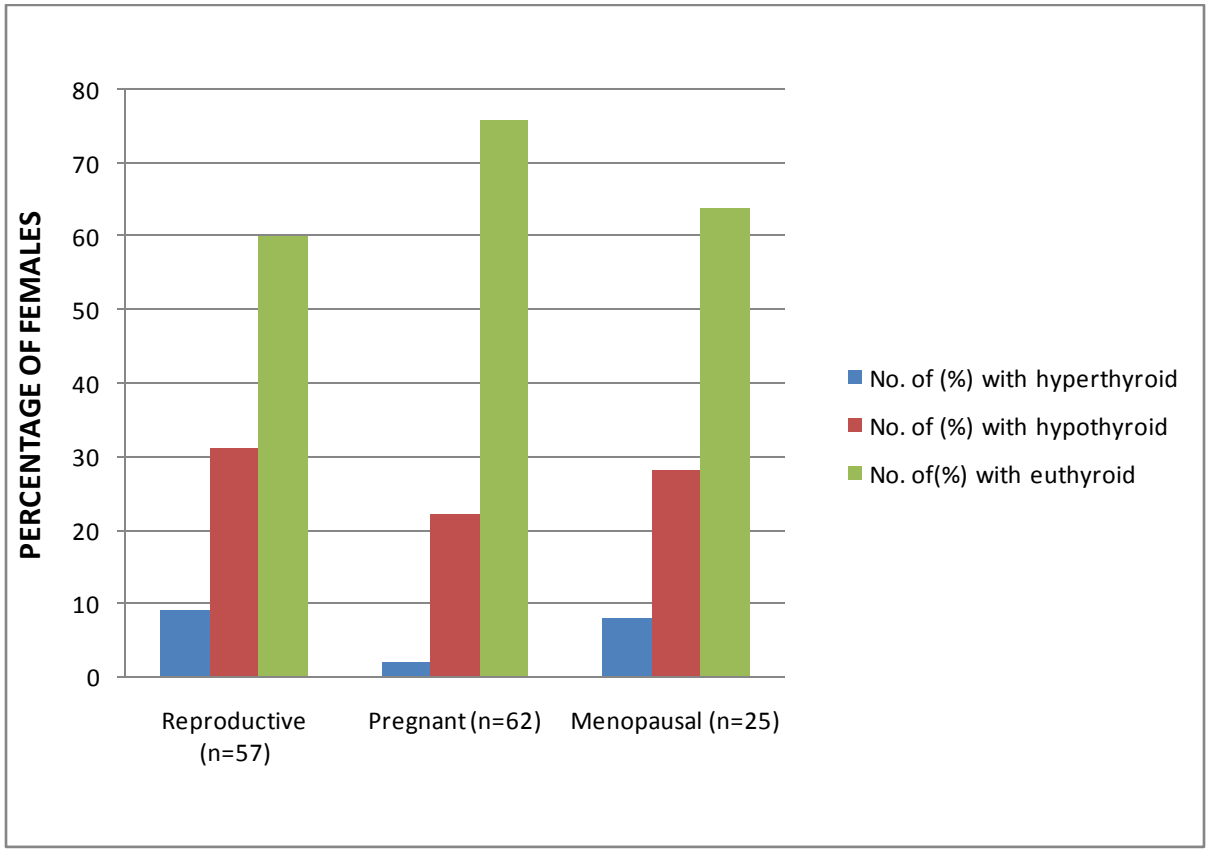


Normal Range of TSH 0.35-4.9Uiu/ml

T_3 (total T_3) 0.58-1.59ng/ml

T_4 (total T_4) 4.87-11.72ug/dl

Figure 11: Distribution of thyroid disorders in different females



CHAPTER 6

Discussion

Discussion

In the present study, we evaluate the level of T_3, $T_4$and TSH in the females of different stages of life as reproductive, pregnant and menopausal. Prevalence rate of thyroid disorder were found to increase among females in the study of Dr. SudhaJha *et al*. 2013. In our study the females of reproductive age group were mostly affected through subclinical hypothyroidism and their prevalence was higher than that of pregnant and menopausal females. Most of these females had previous history of menstrual irregularities, lower abdomen pain and some of them have undergone through abortion. The prevalence of hyperthyroidism in this group was also high in contrast to pregnancy and menopausal. In case of hypothyroidism the level of TSH raised due to increase production of TRH (thyroid stimulating hormone) and stimulation of thyroid stimulating hormone was also increased in contrast with increased production of PRL. So, by the increased production of PRL fertility rate of reproductive females altered by the impairment in the GnRH pulsatile and finally affect ovarian function in the study of Kris Poppe, *et al.*,in 2007. Our present study was also followed through this statement because many reproductive females who affected through hypothyroidism had history of menstrual irregularities and ultimately infertility.

The pregnant females who took part in our study that came for routine checkup during pregnancy. Some of them were also affected through hypothyroidism and their prevalence rate was less in comparison from other two groups. Pregnant females also seen to affect with hyperthyroidism but its prevalence was very less in comparison to hypothyroidism. Evaluation of thyroid hormones in pregnancy is necessary because hypothyroidism causes complication to the pregnant females like intellectual impairment to the feotus. In the first trimester of pregnancy feotus central nervous start developing, during this stage maternal thyroid hormones is necessary because feotus thyroid gland not able to synthesize thyroid hormones at this stage according to the study of Brain M. Casey *et al.,* So, in our study normal level of T_3, T_4 and TSH was evaluated in the pregnant females(table 2) to see any impairment in the level of thyroid hormones thus protecting the any abnormalities to the feotus life. Most of the females were found with normal level of all of these hormones in our study.

In our study very less menopausal female participated as comparison to pregnant and reproductive female. This may be due to absence of symptoms of hypothyroidism in older people or symptoms of thyroid disorder somewhat similar with ageing process according to

the study of Mustuko Nagata. *et.al,* Menopausal females were also affected through hypothyroidism in our study but its prevalence was higher than pregnant female and lesser than reproductive female. Some of these female also affected through hyperthyroidism. Prevalence of hypothyroidism in this group was also high in comparison to hyperthyroidism. According to study of Mustuko Nagata.*et.al*In case of hypothyroidism affected menopausal female level of thyroid hormones decrease thus reduction in oestoblastic and bone differentiation process. Ultimately both the level of bone formation and bone reabsorbtion start reducing but in our study there was not any kind of bone abnormalities in comparison with thyroid disorder seen. The level of thyroid stimulating hormone increase in case of clinical hypothyroidism with decrease level of T_3 and T_4 and in subclinical finding the level of TSH increase but T_3 and T_4 remain normal. In our study the level of TSH in hypothyroid menopausal female was much high in comparison to pregnancy and reproductive. The reason behind was the lack of diagnosis of thyroid disorder at initial stage in menopausal females.

Pregnant female were often screened through thyroid profile to evaluate normal function of thyroid gland to overcome the need of thyroid hormone for feotus brain development. Reproductive female generalize the symptoms of menstrual irregularities to evaluate the cause of menstrual irregularities these females were also screened through thyroid profile. Thus in the initial stage these two groups screened but menopausal female not screened at initial stage. So level of TSH in subclinical hypothyroid menopausal female was higher than other two group in our finding (figure10). Prevalence of hypothyroidism found higher in reproductive females as comparison to other two groups (figure 11) This is due to numerous interaction of thyroid hormone with normal reproductive function both by directly affecting the function of ovaries or indirectly by interacting with sex hormone binding proteins. Further this work need more sample size and statistical analysis to give a scientific judgement and stating the exact evaluation of thyroid dysfunction in different stages of females.

Conclusion

In our present study, the prevalence of thyroid dysfunction was high in Reproductive stage of females as compared to other two groups viz. Pregnant and Menopausal Females. These females presented the symptoms of menstrual irregularities. Most of the pregnant females who participated in the study were having normal level Thyroid Profile. In case of menopausal females there were very few patients screened for thyroid profile as the symptoms of thyroid dysfunction were similar to ageing, like fatigue, tiredness, lack of concentration and dry skin. Although very less menopausal female were screened than other two groups but prevalence of thyroid dysfunction was higher than pregnant females but lesser than reproductive females. In case of menopausal females who were affected with subclinical hypothyroidism the level of TSH was much higher than other two groups. This is due to screening process delay due to paucity of symptoms. Further studies work required more sample size and long term follows up to confirm the variation in TSH level in different females.

CHAPTER 7

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