

**EFFECTS OF THYROID PROFILE CHANGES ON THE
BIOCHEMICAL MARKERS OF BONE, LIVER AND
RENAL FUNCTION IN NON-PREGNANT WOMEN OF
KHYBER PAKHTUNKHAWA, PAKISTAN**



Ph.D Thesis

By

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INSTITUTE OF CHEMICAL SCIENCES

UNIVERSITY OF PESHAWAR

(Session: 2011-12)

**INSTITUTE OF CHEMICAL SCIENCES
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ABSTRACT

This hospital based cross sectional study was conducted from 01 March 2014 to 31 March 2015 in Khyber Teaching Hospital (KTH), a tertiary care hospital in Khyber Pakhtunkhawa (KP) province of Northern Pakistan. The study group comprises of 259 non pregnant women in the age group of 18-75 years. Informed consent was sought from each patient and all human dignity was respected throughout the study period in accordance with the international norms involving human as an experimental subjects. 5 mL of fresh venous blood (fasting sample) was taken from each patient. It was divided into two portions- one portion was used for the determination of thyroid profile markers by Elisa methods and the other portion was used for the determination of serum alkaline phosphatase, calcium, Zinc, urea, creatinine, SGPT and SGOT level on autoanalyser (Erbamannhein chemistry autoanalyser, Germany) by using Erba kits and Standard protocols.

After the determination of thyroid profile status, patients having normal thyroid profile (n=54, TSH $\leq$ 6.0, normal T3 and T4 levels) were taken as controls. While the hypothyroid patients (n=96) and hyperthyroid group (n=109) constituted the study group. The patients in the hypothyroid groups were further sub divided into Sub clinically hypothyroid (Sh) (n=48) and Overtly hypothyroid (Oh) (n=48). Similarly the patients in hyperthyroid groups were also subdivided into Overtly Hyperthyroid (OH) (n=58) and Sub clinically Hyperthyroid (SH) (n=51).

Purposive sampling method was employed for the collection of the relevant data. The total no of patients who met the purpose of the study were 259.

The data about age, BMI and all the required biochemical parameters for each patient was collected on a well-designed data entry form.

The data so obtained was statistically analyzed using SPSS for windows 21.0 software and Microsoft Excel. Values were reported as mean $\pm$ standard error of mean. Pearson's correlation of the data was also carried out to look for association between variables. A, p value of < 0.05 was considered to be significant.

The mean age of patients in the control group (N) was 42.15 ± 1.86 years, 46 ± 1.38 years for Overtly hypothyroid (Oh), 45.97 ± 1.93 years for Sub clinically hypothyroid (Sh), 49.74 ± 1.62 years for Overtly Hyperthyroid (OH) and 48.94 ± 1.87 years for the Sub clinically Hyperthyroid (SH). The control group were 54, of which 61.11% (33) were Menopausal (M, age below 45 years), 18.52% (10) were Early Post-Menopausal (EPM, age 45-50 years) and 20.37% (11) were Late Post-Menopausal (LPM, age above 50 years). The %age of M, EPM and LPM in the Oh (48) and Sh (48) group were, 54.16%(26), 12.5%(06), 33.83%(16) and 45.83%(22), 22.92% (11), 31.25%(15) respectively. Similarly the %age of M, EPM and LPM in the OH (58) and SH(51) were 34.48% (20), 27.58%(16), 37.93%(22) and 43.14%(22), 07.84%(04), 49.07%(25) respectively.

The mean serum level of TSH was found to be lowest in OH group ($0.17 \pm 0.01 \mu\text{IU}/\text{mL}$) and highest in the Oh group ($25.89 \pm 2.86 \mu\text{IU}/\text{mL}$). Serum T3 level was highest in OH group ($2.37 \pm 0.01 \text{ng}/\text{mL}$) and lowest in Oh group ($0.94 \pm 0.09 \text{ng}/\text{mL}$). Highest serum T4 level was found for OH group ($12.07 \pm 0.46 \mu\text{g}/\text{dL}$) and lowest in Oh group ($4.30 \pm 0.28 \mu\text{g}/\text{dL}$).

Mean serum creatinine was highest in N group ($1.47 \pm 1.25 \text{mg}/\text{dL}$) as compared to OH ($1.29 \pm 0.01 \text{mg}/\text{dL}$) and SH group ($1.34 \pm 0.15 \text{mg}/\text{dL}$). Serum Urea level was found elevated in OH ($35.35 \pm 1.75 \text{mg}/\text{dL}$) and SH ($32.98 \pm 1.63 \text{mg}/\text{dL}$) as compared to N group ($32.60 \pm 1.22 \text{mg}/\text{dL}$). Serum creatinine showed positive relation with TSH, in

all the study groups, negative relation with T3 in the N group and OH and positive in SH group. Serum creatinine showed a significant positive correlation with TSH in the OH group ($p= 0.05$). Serum creatinine showed a significant negative correlation with T3 in the N group ($p= 0.05$). No significant correlation with T4 was found either for creatinine or Urea in any of the study groups.

A minor decrease in the serum creatinin level in the Oh (1.10mg/dL) and Sh group (1.18 mg/dL) was found as compared to N group (1.47mg/ dL). Serum urea was found to be slightly decreased in the Sh group (23.22 mg/dL) as compared to N group (32.60mg/dl) and Oh group (30.11mg/dL). A very significant positive correlation was found between Serum creatinine and T3 in the Sh group ($p= 0.005$) and significant negative correlation with T3 in the N group ($p= 0.05$). Urea was significantly negatively correlated with TSH in Sh group ($p= 0.05$).

Mean Serum Calcium was highest in OH group (9.80 ± 0.90 mg/dL) and lowest in Oh group (8.95 ± 0.10 mg/ dL). Serum total alkaline phosphatase was found to be maximum in N group (159 ± 7.61 U/L) and minimum in Sh group (128 ± 6.42 U/L). There were no significant differences in the Serum Zinc level of all the diseased groups.

Calcium showed significant positive correlation with TSH in Oh ($p=0.01$) and OH ($p=0.04$). Serum Calcium was significantly negatively correlated with T3 in OH ($p=0.01$). Serum alkaline phosphatase was significantly negatively related with T4 in OH ($p=0.01$). Serum Zn showed a very significant positive correlation with T3 in SH ($p=0.02$).

Significance differences were found in the SGPT and SGOT level in all the five groups. SGPT level was found to be 33.14 ± 2.98 in OH, 29.50 ± 1.69 in SH group as

compared to 29.69 ± 3.28 IU/L in N group. SGOT level was 33.70 ± 5.12 IU/L in N group, 30.68 ± 2.7 in OH group and 30.38 ± 2.6 IU/L in SH group. A very significant positive correlation was found between SGOT and TSH in the N group ($p = 0.002$). A very significant positive correlation was also found between BMI and T3 in N group and OH group ($p = 0.01$). SGPT level was found to be 24.35 ± 1.11 IU/L in the Oh, 16.85 ± 0.55 IU/L in Sh as compared to 29.69 ± 3.28 IU/L in N group. SGOT level was 33.70 ± 5.12 IU/L in N group 23.65 ± 1.22 IU/L in Oh group and 17.86 ± 0.98 IU/L in Sh group respectively. SGOT was not significantly negatively correlated with TSH in the Oh and Sh groups. No significant correlation was found between SGPT and thyroid profile markers (TSH, T3 and T4) in any of the study groups.

In conclusion, thyroidal dysfunction is a common problem in northern Pakistan affecting women more as compared to other gender due to poverty, lack of education, social taboos and lack of health facilities in rural areas. Moreover there is a lack of adequate amount of iodine in drinking water and iodized salt due to lack of strict quality control. There is a complex interaction between thyroid kidney, liver and bone turnover markers. It was concluded from this study that bone resorption occurs at a higher rate in hyperthyroid patients and bone turnover is increased in favor of resorption. Opposite changes are observed in hypothyroid women. Also hyperthyroidism may affect renal function in the long run if left untreated, while hypothyroidism may not affect renal functions in non-pregnant women.

It was also concluded from this study that SGOT is directly affected by the change in the serum level of TSH in the normal individual (N). Serum T3 directly affected BMI in N and OH groups. It was also found that hypothyroidism may decrease while Hyperthyroidism may increase the rate of synthesis of liver enzymes. Due to this

complex inter play between these vital organs of the body and thyroid hormone, multi system approach should be adopted in the diagnosis of clinical conditions affecting either organs.

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LIST OF ABBREVIATIONS

T4	Tertraiodothyronine, (Thyroxine,)
T3	Triiodothyronine
BMR	Basal Metabolic Rate
TSH	Thyroid Stimulating Hormone
TRH	Thyrotrophic Releasing Hormone
ft3	free thyroxin
TBG	Thyroxin Binding Globulin
ft4	free tetraiodothyronin
OH	Overt hyperthyroidism
Oh	Overt hypothyroidism
AF	Atrial Fibrillation
TRT	Thyroxin Replacement Therapy
Sh	Subclinical hypothyroidism
SH	Subclinical Hyperthyroidism
AST	Aspartate Aminotransferase
SGOT	Serum Glutamic Oxolo transaminase
ALT	Alanine Aminotransferase
SGPT	Serum Glutamic Pyruvic Transaminase
TRT	Thyroid Replacement Therapy
NIH	National Institute of Health
BUN	Blood Urea Nitrogen
BTP	β -Trace Protein
TH	Thyroid Hormones
GFR	Glomerulus Filtration Rate
NS	Nephrotic syndrome
KTH	Khyber Teaching Hospital
NPW	Non- pregnant Women
TMB	Tetra Methyle Benzidine

HRPO	Horse Radish Peroxidase
OD	Optical Density
GLDH	Glutamate Dehydrogenase)
NADH	Nicotinamide Adenine Dinucleotide
α-KG	α -ketoglutarate
ALP	Alkaline Phosphatase
N	Normal
Oh	Overtly hypothyroid,
Sh	Sub clinically Hypothyroid
SH	Sub clinically hyperthyroid,
SE	Standard Error
SD	Standard Deviation
M	Menopausal
EPM	Early Postmenopausal
LPM	Late Postmenopausal
UW	Underweight
OW	Overweight
O	Obese
BMI	Body Mass Index

CHAPTER-1

INTRODUCTION

1.1 THYROID GLANDS

1.1.1 Anatomy and Physiology of Thyroid gland

The thyroid is a shield shape gland, located at the proximal part of the wind pipe or trachea. The main functions of the Thyroid Glands (TG) is to synthesis, store and secretes Thyroid Hormones (TH), which regulate Basal Metabolic Rate (BMR) and play a key role in the metabolism of biofuel i.e carbohydrates, proteins and fats. They are also essential for the maintenance and development of both mental and physical functions. Thyroid Hormone (TH) has its effects on every organ and system of the human body and hence its imbalance may cause various physiological disorders [1].

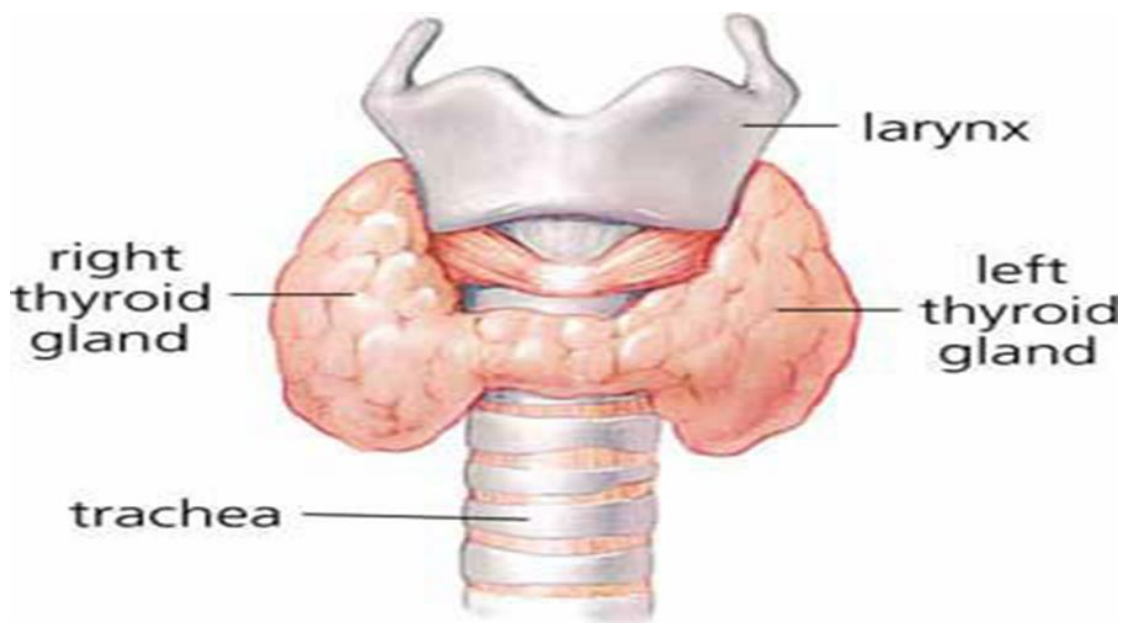


Fig-1 Showing Thyroid glands

1.1.2 Regulation of Thyroid Hormones

Thyroid hormones are synthesized by the TG under the direct influence of hypothalamus. This coordination is brought about by Thyroid Stimulating Hormone (TSH) or thyrotrophin through a negative feed-back regulatory mechanism. Anterior pituitary gland secretes TSH in response to a decrease in the concentration of circulating Thyroid Hormones level, which stimulate the release of Thyrotrophin Releasing Hormone (TRH) by the hypothalamus. TSH stimulates the activity of TG, till its level become normal. This inverse relationship between serum TSH and free thyroxin (fT3) will result in an exponential increase in serum TSH concentrations, which encourages the TG to increase the absorption of iodide ions and hence encourage the synthesis of the hormones and its storage [2].

Thyroid hormones are released in to the general blood circulation of the body, where they are bonded to their transport proteins mainly Thyroxin Binding Globulin (TBG), albumin and pre-albumin. A very small portion of the hormone however remains unbound and is termed as free tetra and tri-iodothyronin (fT₄ and fT₃) which are biologically more active and act on the cells and body tissue for production of energy, growth and development of body cells and tissues. Both fT₄ and fT₃ have the same metabolic and physiological effects [3].

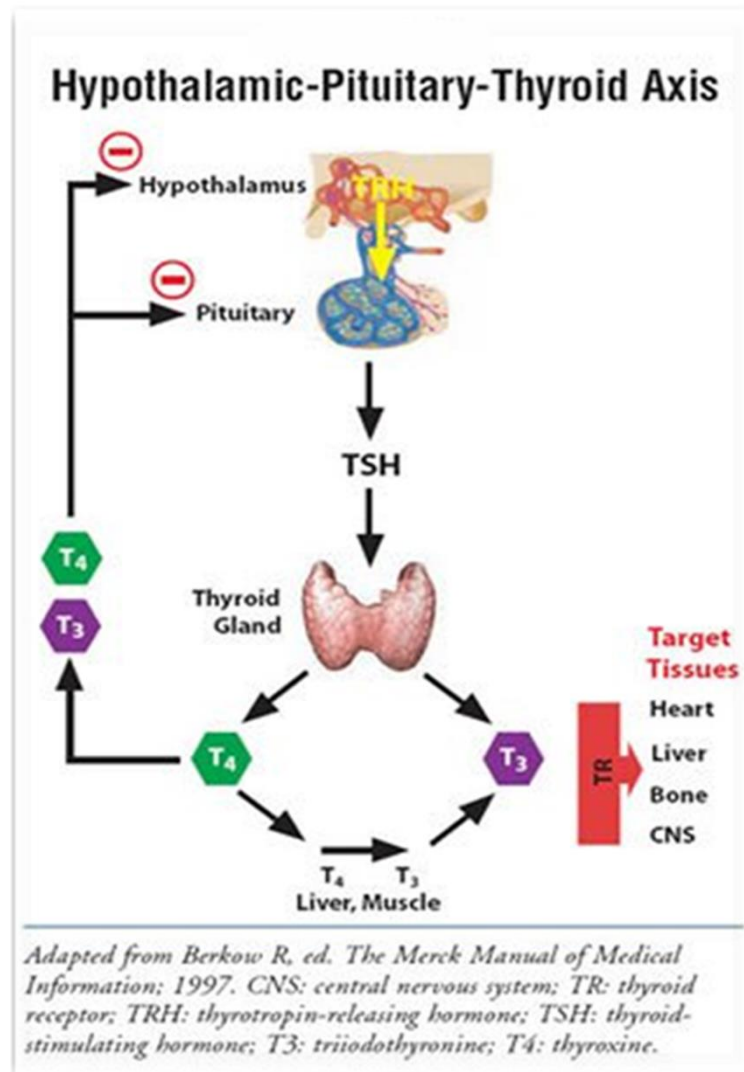


Fig-2 Showing the Hypothalamic- Pituitary- Thyroid axis

1.1.3 Thyroid gland dysfunctions and symptoms

Dysfunctions of the thyroid glands are considered either under- or over-activity of the glands, depending upon the concentration of thyroid hormones released. In Overt Hyperthyroidism (OH) there is higher serum concentration of thyroid hormone than the normal range values while TSH is below the normal range of the population under study. In Overt Hypothyroidism (OH) there is insufficient thyroid hormone while the serum concentration of TSH is above the normal range.

The symptoms of OH include intolerance to heat, palpitation, breathlessness, excessive perspiration, weight loss, tremor, muscular weakness, fatigue, and irritability [4, 5]. Physical signs are fine tremor, Atrial Fibrillation (AF) and rapid pulse [6]. In the long run, OH is associated with osteoporosis, cardiac arrest and AF [7]. OH is also linked with high risk of morbidity especially from cerebrovascular and cardiovascular causes [8].

The symptoms of Oh include weight gain, intolerance to cold, constipation, dry skin, puffy eyes, hoarse voice, poor memory and muscle cramps [9, 10].

1.1.4 Pathophysiological effects of Overt hyperthyroidism

Hyperthyroidism increases the rate of Basal Metabolic Rate (BMR) which results in more heat production in the body [6]. This will increase the rate of metabolism of carbohydrate and fat. Hyperthyroidism leads to heat intolerance, decrease in overall muscle mass, muscle weakness and also weight loss. Bone metabolism is also impaired in hyperthyroidism with bone desorption being affected more than its synthesis. This will lead to higher risk for the onset of osteoporosis and fracture [11]. OH is found to be associated with anxiety, depression, and changes in mood [12, 13].

1.1.5 The pathophysiological effects of overt hypothyroidism

The pathophysiology of Oh is totally opposite to those of OH. Lower rate of metabolism is associated with lower heat production; metabolism of carbohydrates and fats is reduced, resulting in glycogen storage and ultimate weight gain. Concentration of plasma lipids is elevated, which increases the risk for the onset of atherosclerosis. This may also enhance the chances of hypertension associated with overt hypothyroidism. A large no of studies have also reported an association between Oh depression and dementia with poor quality of life [14, 15]. Oh has also been linked with increased risk for osteoporosis and other bone diseases [16]. In the long

run, Oh has adverse effects on hearts like atherosclerosis, angina, myocardial infarction, coronary heart disease, and high cardiovascular mortality risk [17].

1.1.6 Prevalence and aetiology of Overt thyroid dysfunctions

Overt thyroid dysfunctions are considered as the common disorders of endocrine glands throughout the world. About 3% of the population in UK was prescribed TRT for the treatment of Oh in 2006 [18]. A UK population based cross-sectional study show that Oh is more prevalent in female than in male (1.4% versus 0.1%) [19] while prevalence of OH was found to be 2.0% in women and 0.2% in men respectively. The prevalence rates in different areas of the world differ with age, ethnicity and other factors [20].

Iodine deficiency and sufficiency between different geographical areas is also one of the main causes of the difference in the prevalence data from around the world.

The principal causes of Oh are Hashimoto's thyroiditis or a destructive treatment for Overt OH which can lead to decrease in the activity of the TG and lower production of TH.

Similarly, causes of OH, includes Graves' disease, toxic multinodular goiter, or excessive use of TRT.

1.1.7 Subclinical thyroid dysfunctions

In subclinical Thyroid dysfunction, the serum level of TSH is abnormal and concentrations of TH (T_3 & T_4) are normal [21]. In subclinical hyperthyroidism (SH) there is a decrease in the concentration of serum TSH while the concentrations of the Thyroid Hormones (T_4 or T_3) are within the normal range. While in case of subclinical hypothyroidism (Sh), there is high serum TSH concentration while serum T_4 and T_3 concentration is within the normal reference range [22].

Subclinical thyroidal dysfunction is considered by some experts to be a moderate type of overt thyroid dysfunctions and have shown the symptoms which are characteristics of overt thyroid dysfunctions [23].

1.1.8 Prevalence and Aetiology of subclinical thyroid dysfunction

The prevalence rate for both Sh and SH varies due to factors like dietary intake of iodine ethnicity, and the frequency distribution of Anti-thyroid Antibodies of the target populations under study [24]. Generally, the prevalence of Sh is higher in iodine deficient areas and SH is more frequent in areas that are iodine sufficient [25]. The prevalence of subclinical thyroid dysfunctions in females in iodine sufficient and iodine deficient areas are found to be higher than the male populations, especially in older individuals. The etiology of SH can be classified into two types; that is exogenous and endogenous. The main cause of exogenous SH is the use of high doses of TH. The endogenous type is mostly due to Autonomous thyroid dysfunction. It is still unclear whether endogenous and exogenous subclinical hyperthyroidism exerts equivalent effects or not.

1.2 THYROID AND LIVER AXIS

1.2.1 Physiological Role of Liver

Liver weigh about 1500 g, and is considered the largest organ of the human body. It performs many important metabolic functions [26] like glycogenesis and glycol-genolysis, synthesis of urea, plasma proteins and clotting factors which are then released into the blood circulation. It also regulates amino acids levels in blood. Liver parenchyma cells serves as a store house for fat, fat soluble vitamins and glycogen. It also secretes bile which removes and excretes toxic substances from the body [27].

1.2.2 Role of liver in metabolism and transport of Thyroid Hormone

In healthy individual, the TG usually secretes 110 and 10 nmol/day of T₄ and T₃ respectively [28]. The affinity of T₃ for nuclear receptors is ten times higher than T₄.

Thus T_4 is considered as a pro-hormone and should be deiodinated to biologically more active T_3 [29]. This inter conversions is regulated by a group of three enzymes, constituting the seleno-deiodinase enzyme system (D1, D2 and D3). These enzymes are present in the skin, liver and Central Nervous System (CNS), where they catalyze the interconversion of T_4 to rT_3 and T_3 to T_2 ; it also converts rT_3 to rT_2 . They also activate of T_4 to T_3 and inactivate of T_4 to rT_3 and also convert rT_3 and T_3 to T_2 as shown in Figure 3.

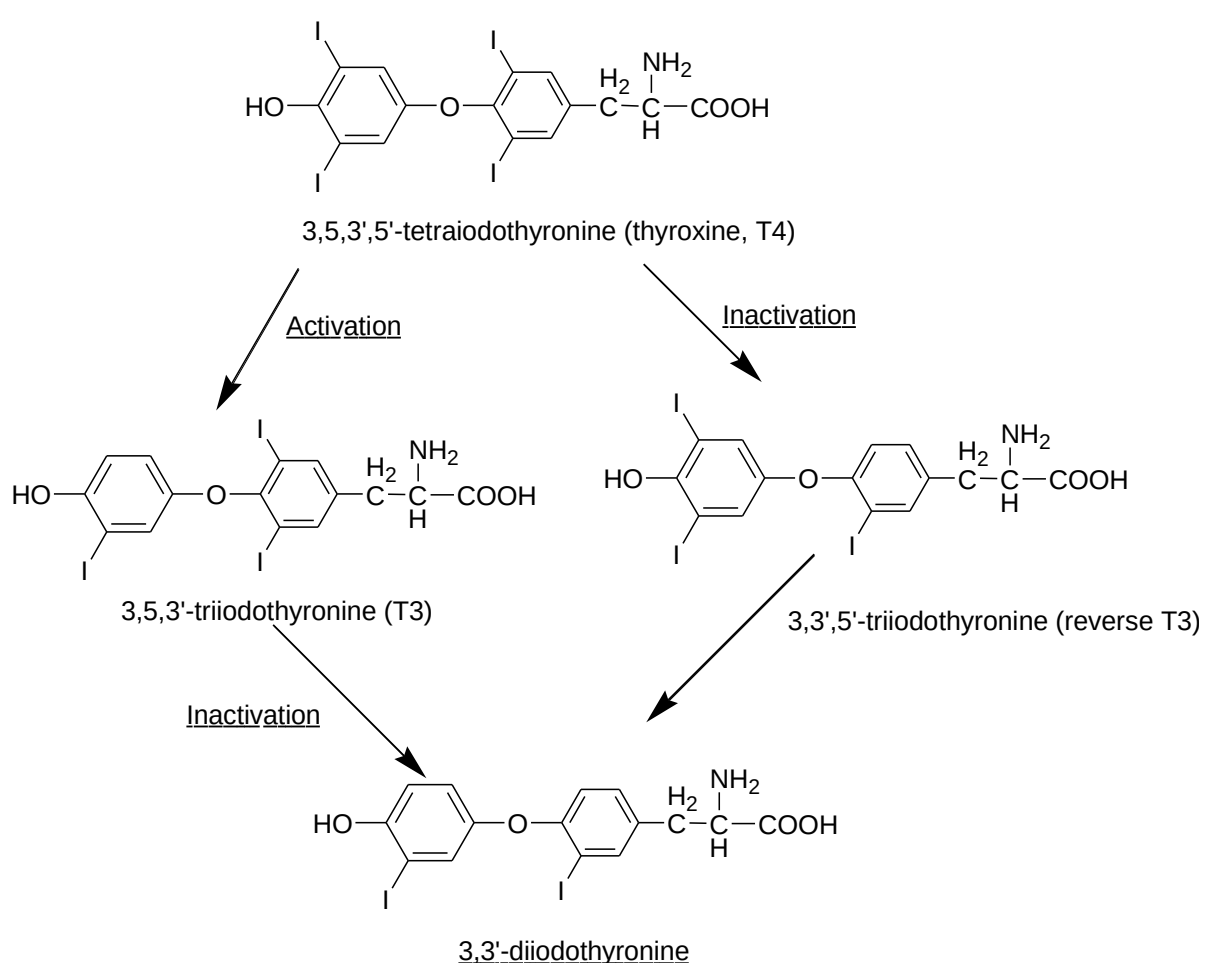


Figure 3. Structures and interconversion of principal thyroid hormones

The important inactivator is thought to be the D3 deiodinase system, but the other two enzyme system can also cause inactivation of T_4 and T_3 by inner-ring deiodination [30].

Liver is also involved in the transport of thyroid hormones. Different studies show that liver extracts 5–10% of plasma T_4 during a single passage, which is much greater than the amount of ft_4 transported to the liver, showing that a considerable amount of protein-bound T_4 is available for uptake [31]. The transport of TH (T_4 and T_3) across the liver cells membrane is believed to occur through a stereospecific mechanism and this process require the input of energy [32].

A number of plasma proteins are synthesized by liver which bind the lipophilic thyroid hormones.

The TH are 99% bound to Thyroxin-Binding Globulin (TGB), Thyroxin-Binding Pre-albumin and Albumin in plasma. The free and bound form of the hormone is in equilibrium within the plasma, and it is the free form which is responsible for the physiological activities of the hormone. The level of ft_4 and ft_3 are at steady state, and varies according to the transport and the activity of deiodinase within a particular tissue [33].

1.2.3 Biochemical markers of liver functions

Liver enzymes are the biochemical markers used in assessing the liver health. The most widely used liver enzymes are the Amino-Transferases, which catalyze trans-amination reactions in the cells. These are enzymes, Aspartate Aminotransferase (AST/ (SGOT) and Alanine Aminotransferase (ALT/ (SGPT) [34]. Normal serum levels of AST and ALT are 5 to 40 U/L and 7 to 65 U/L respectively. These enzymes are released into blood whenever the liver is damaged; the rise in the level of these enzymes in the blood is considered as a sign of liver damage.

AST and ALT are sensitive biochemical markers of hepatic diseases, but abnormal levels of these liver enzymes, does not necessarily means liver damage. ALT and AST levels depend on the clinical interpretation of all the signs and symptoms.

Analysis of ALT and AST level should be carried over certain period of time, till it can be established that the elevation of the serum levels are stagnant or have dropped [35].

As AST and ALT enzymes are very important biochemical markers in various clinical conditions affecting liver, hence they are the most widely studied markers in liver diseases.

1.2.4 Liver abnormalities in thyroid disease

Normal activity of TH is necessary for the normal development, growth, and the regulation of energy metabolism within cells, and it is dependent on a normally working TG and liver axis. Various thyroidal dysfunctions may cause liver abnormalities.

1.2.4.1 Effects of Hypothyroidism on liver functions

The symptoms associated with hypothyroidism may mimic liver disease. These symptoms include myalgia's, fatigue and muscle cramps and elevated level of AST [36] coma accompanied by hyper- ammoniaemia in myxedema coma, [37, 38] Myxedema ascites, has been widely considered as an intrinsic liver defect . However, in some other studies normal right heart pressure was reported and it was proposed that Oh actually caused increased permeability of vascular endothelium which resulted in the onset of ascites [39] it was observed that myxoedema ascites resolves over a short period following initiation of TRT.

Liver anatomy and physiology are also affected by hypothyroidism. Hypothyroidism has been associated with cholestatic jaundice. In one experimental induced hypothyroidism, the activity of bilirubin UDP-Glucuronyl transferase was found reduced, which results in the decrease excretion of bilirubin from the body [40].The decrease in the flow of bile is the result of an increase in the ratio of membrane cholesterol and phospholipid and the decrease in fluidity of membrane which can

affect a large number of membrane transporters and also a number of enzymes. Gallstones formation is encouraged in hypothyroid patients due to lowered bilirubin excretion, hypercholesterolemia and hypotonia of the gall bladder [41]. Recent reports have however indicated that the liver abnormalities caused by hypothyroidism can be reversed in a short time following TRT, with no residual hepatic damage [42, 43].

1.2.4.2 Effect of hyperthyroidism on liver functions

Hyperthyroidism causes liver injury in human and can be classified into two types i.e. hepatitis and cholestasis. In hepatic injury, it was found that 27% of the patients have elevated AST while 37% has elevated ALT respectively, despite the fact that these patients showed no other abnormal biochemical tests of hepatic diseases [44]. In few patients suffering from thyrotoxicosis, liver failure is also reported [45].

In cholestatic injury, level of serum ALP was found to be elevated in 64% of patients suffering from Oh [46] but as this enzyme can also originate from bone and liver, the result may be misleading. Thus it is also important to look at elevation in the level of bilirubin (5%) and enzyme c-glutamyl Trans peptidase (17%) as a biomarker of cholestasis. There also reports that untreated hyperthyroidism in some patients may cause focal necrosis with fatty change to cirrhosis [47]. The developments in modern medicines have made hepatic abnormalities a very rare complication of OH. In most of the cases, the liver abnormalities linked with hyperthyroidism are thought to be reversible; if it is early diagnosed and timely treatment is followed [48].

1.3 BIOCHEMICAL MARKERS OF RENAL FUNCTION TESTS

Biochemical markers play a very important role in correct diagnosis of pathological condition. A biochemical marker as “a characteristic which is measured objectively and tested as a marker of normal, pathologic, biological processes, or pharmacologic responses to a therapeutic intervention” (National Institute of Health (NIH)). [49] Bio

markers of kidney Functions are Uric acid, Creatinine, Urea, and electrolytes which are used in routine clinical analysis, markers like, β -Trace Protein and Cystatin C are also considered to be useful.

1.3.1 Creatinine

Creatinine is the waste product of creatine phosphate metabolism in muscles, which is produced in the body at a constant rate [50]. Creatinine is most commonly used in routine clinical laboratory as a marker of renal function. The normal Creatinine Clearance Test (CCT) value is 110-150 mL/min and 100-130 mL/min in male and female respectively [51]. Glomerular Filtration Rate (GFR) is calculated from Serum Creatinine concentration (National Kidney Disease Education Program) [52]. The CCT is usually used to monitor the progress in kidney diseases. When the Serum Creatinine concentration is above the upper limit of the “normal” level, it is considered as a sign of kidney failure. In chronic kidney failure and uremia, a gradual decrease in the excretion of creatinine occurs by both the glomerulus and renal tubule [53]. The concentration of creatinine is not constant and may change as its production is influenced by activity, diet muscle function, its chemical composition and the health condition of the individual [54]. A false negative value of serum creatinine is found in some patients with kidney dysfunction due to higher tubular secretion of creatinine [55]. The higher serum values of creatinine are also observed in paralysis, hyperthyroidism, leukemia, anemia and muscular dystrophy. The lowered values are observed in glomerulonephritis, acute tubular necrosis, polycystic kidney disease, congestive heart failure, shock and dehydration [53].

1.3.2 Urea

Urea is considered the principal breakdown product of amino acid and protein catabolism, produced by liver. Urea is filtered out of blood by kidney glomeruli and is partially reabsorbed with water [51]. Serum urea concentration is the most often used clinical markers for testing kidney function. It is useful in differentiating acute renal

failure and pre renal condition where Blood Urea Nitrogen–creatinine (BUN) ratio is elevated [56]. Elevation in BUN is found to be associated with renal disease or kidney failure. High serum level of BUN is observed in severe renal damage (above 100 mg/dl) while lower BUN is seen in fluid excess. Lower values are also observed in malnutrition, opioids, trauma, steroid use and surgery [57].

1.3.3 Cystatin C

The cystatin C is a low molecular weight, non-glycosylated protease inhibitor. Cystatin is considered as a biomarker, because it is synthesized by all the nucleated cells at a constant rate. It is filtrated by the glomerulus and is completely broken down in proximal renal tubules. Its level is largely determined by GFR, and is thus considered an endogenous biomarker of GFR [58]. Dharnidharka et al [59] in a Meta-analysis that found cystatin C was a superior marker than serum creatinine for GFR. In some patients with cirrhosis following liver transplantation, Cystatin C is found to be an important biomarker for GFR [60]. In diabetic patients of both type 1 and type 2 cystatin C is considered as an effective marker for the early diagnosis of early renal impairment [61]. Cystatin C is also associated with moderate renal dysfunction, peripheral arterial disease, cardiovascular events, and cardiac failure [62].

1.3.4 β -Trace Protein (BTP)

This important protein is fairly filtered out at glomeruli and then may be reabsorbed in the proximal tubule or may be excreted in urine and hence fulfill the criteria for its use as a biomarker of GFR [51]. It has been reported to be a superior indicator than serum creatinine in decreased GFR [63].

Serum BTP was found higher in patients with kidney diseases [64]. Cystatin C is still considered a superior marker than Serum BTP [65].

1.3.5 Proteinuria

Proteinuria is the appearance of protein in urine in a significant quantity and is considered an early sign of all type of renal diseases. Normal excretion of protein in healthy adults individuals is between 20-150 mg in 24 hrs urine. Urinary excretion higher than 3.5 g/day of protein is considered an early sign of Nephrotic Syndrome (NS). Quantitative Proteinuria can be predicted from Protein/Creatinine ratio in 24 hour urine collection [66].

1.3.6 Electrolyte

Electrolytes are also important bio markers of renal function and involve the measurement of potassium, sodium, bicarbonate and chloride, both for management and diagnosis of renal, acid-base, endocrine and water balance. Potassium is considered as a better electrolyte biomarker of renal failure. The level of plasma potassium is elevated in low filtration and its lower secretion in distal tubule during renal failure. Hyperkalemia is the life-threatening complication of renal failure [67].

1.3.5 Effect of Thyroid Dysfunctions on Renal Functions:

The interactions between renal and thyroid functions are well known for many years [68]. TH plays a very significant role in development, growth, and functions of the kidney [69]. Thyroidal dysfunction disturbs the kidney to body weight ratio (hypothyroidism decreases while hyperthyroidism increases the mass of kidney) as compare to its body weight [70]. The kidney is also involved in the regulation of metabolism and elimination of Thyroid Hormone (TH).

Thyroid dysfunctions produce significant changes in renal functions. Both hyperthyroidism and hypothyroidism affect renal blood flow, tubular function Glomerulus Filtration Rate (GFR), electrolyte pump functions, electrolytes homeostasis, and kidney structure [71].

1.3.5.1 Hypothyroidism

The common kidney complications linked with hypothyroidism includes elevation of serum creatinine levels, renal plasma flow (RPF), decrease in GFR, excretion of free water and hyponatremia [72].

Overt hypothyroidism (Oh) causes a reversible increase of serum creatinine in both children and adults [73, 74]. Some researchers have also reported an increase of Serum Creatinine in Sh [75]. Oh is also linked with a decrease in GFR and RPF which become normal after TRT [74].

1.3.5.2 Hyperthyroidism

Hyperthyroidism or thyrotoxicosis results in an increase of GFR and RPF and decrease of serum creatinine levels [76]. These changes are reversed after appropriate treatment [71]. The decrease in serum creatinine has been also reported in SH [75].

The various types of renal diseases can be associated with both hypothyroidism [77] and hyperthyroidism [78]. These include membranous glomerulopathy associated with NS and IgA glomerulonephritis [79, 80].

1.4 THYROID AND BONE

1.4.1 Chemical Composition of Bone

Bone is a connective tissue which consists of minerals and organic matrix. Dry weight of bone is mainly composed of minerals, mainly hydroxyapatite. Other minerals, such as sodium, magnesium and carbonate are absorbed principally on the hydroxyapatite crystals. The major portion of calcium (99 %) and phosphate (80 %) in the body are stored in the skeleton, and are then released from there when required. The type I collagen is the major component (90%) of bone organic matrix. Bone organic matrix also contains other non- collagenous proteins, like bone sialoprotein and osteocalcin. In addition, Osteoblasts produce proteins which play a role in cell adhesion. Bone matrix also contains cytokines and many growth factors required for differentiation of

bone cells, which are synthesized by both the cells in the bone marrow and osteoblasts. Cells usually absorb about 2% of the bone organic matrix [81].

1.4.2 Markers Reflecting Bone Formation

Bone formation markers are generally proteins which are synthesized by the osteoblast, and released into the blood circulation. The concentration of these markers can be determined in the blood and hence may be used as bone turnover markers. Until recently the total activity of ALP, the concentration of osteocalcin, type I procollagen, amino- and carboxy-terminal propeptides have been widely used as markers of bone formation rate. Serum Ca and Zn also play an important role in bone formation.

1.4.2.1 Serum Calcium

Calcium is one of the major components of mineralized tissues and is necessary for normal growth and maintenance of bone. Abnormal Serum Calcium level has serious effects on neurological, gastrointestinal and renal functions. The normal serum calcium level is maintained by the kidney, bone and intestinal tract under the influence of parathyroid hormone and 1, 25-dihydroxyvitamin D₃. Serum calcium abnormalities are due to a number of disorders. Treatment of both hypocalcaemia and hypercalcaemia depends upon the type of disorder, level of calcium, and the associated symptoms. Deficiency of calcium causes osteoporosis which is one of the leading causes of morbidity and mortality in older people. Calcium supplements play an important part in the prevention or treatment of osteoporosis [82]. The Recommended Dietary Allowances (RDAs) for calcium range from 700 to 1300 mg/d for various age groups [83].

In older people the deficiency of vitamin D causes muscle weakness, impairs mobility and is the major risk factor for bone fracture due to falling. Vitamin D and

calcium supplements improve mobility and muscle mass strength in female geriatric patients [84].

1.4.2.2 Serum Zinc

Zinc is an important cofactor needed for many important enzymes like carbonic anhydrase, liver alcohol dehydrogenase and ALP [85] which metabolize a number of hormones.

The role of zinc in TH secretion, regulation and metabolism in human and other animal's model is not still very. Studies in human and animals have shown conflicting results. In some patients low T_3 is found to be associated with zinc deficiency. Thyroid function influences zinc metabolism. Bellisola et al, found a very significant negative relationship between zinc, iodine, selenium, TH and TSH blood levels [86]. In one German population based study, a significant negative relationship was found between zinc levels and thyroid volume in adult patients [87]. Graves' disease is also believed to change erythrocyte zinc level in some patients and thus erythrocyte zinc levels could be used as an index of thyroid status and for the differentiation between other forms of thyrotoxicosis and Graves' disease [88].

In some studies it has been found that Zinc has little effects on serum TSH and TH levels. Moreover, zinc supplements improve thymic and immune system functions in children with Down's syndrome (DS) [89] it has been reported that children with DS and low zinc levels are at higher risk for of Sh and higher TSH levels and can be improved by zinc supplementation [89]. In a study Maxwell and Volpe observed that zinc supplementation increases fT3, fT4 and total hormone levels in some subjects [90]. In another such study, it was found that zinc lower TSH levels in healthy individuals [91].

1.4.2.3 Serum Alkaline Phosphatase

Alkaline phosphatases are hydrolase enzymes which participate in bone metabolism like bone reabsorption and osteogenesis. They catalyze the hydrolysis of aliphatic and aromatic phosphate esters to remove phosphates group. These enzymes are active at alkaline and acidic pH respectively. These are widely distributed in nature and also found most of the animals and plant world. ALP is mainly concentrated in liver, kidney, bile duct, placenta and bone. ALP is involved in the bone development and growth. It promotes the bone mineralization process by providing an alkaline environment necessary for calcium crystallization, growing the bones and achieves their strength. Alkaline phosphatase is usually used as a diagnostic tool for bone diseases. The normal concentration of alkaline phosphatase in human is 60-170 IU/L standardized in laboratory. An abnormally low level is observed in thyroid patients [92].

1.4.2.4 Effects of thyroidal dysfunctions on bone metabolism

Thyroid hormones are believed to affect bone formation and desorption equilibrium which are necessary for the normal maintenance of bone [93]. Thyroid diseases are considered to be one of the major causes of Secondary Osteoporosis. This effect is mediated through different biochemical mechanisms. Many studies have revealed that thyrotoxicosis causes bone diseases [94]. Data show that hypothyroidism delay the bone remodeling process and also reduces bone turnover. Large population based studies have shown that hypothyroidism increases the fracture risk two- to three-fold [95].

Thyroid hormones are also necessary for the development of bone mass to maintain the optimal bone strength. Population based studies have shown that thyroidal dysfunction are associated with a high risk of bone fracture [96].

Biochemical bone biomarkers shows significant changes in the metabolism of bone minerals associated with thyroidal dysfunctions. Hypothyroidism decreases rate of bone formation and also destroy bone architecture. Hypothyroidism for more than one year will decrease bone age [97]. Bone density is higher than normal in adult hypothyroid patients. In hypothyroid patients serum ALP is either normal or low while serum calcium and phosphorus concentrations are normal [98]. Hypothyroid patients may develop hypercalcaemia due to intolerance to oral calcium. This defect is due to the decrease in the rate of calcium disposition, rather than its excess absorption [99].

In hyperthyroid patients there are elevated serum levels of calcium and ionized calcium (ICa) with low bone mineral density [100-102].

In thyroidal dysfunction the serum ALP level is found to be abnormal. Bone specific isoenzyme of ALP is a superior biomarker than Osteocalcein [103]. Its level is usually elevated in hyperthyroidism [104]. It has been reported that bone loss due to thyrotoxicosis does not depends upon the concentration of circulating TSH levels. Thyroid hormones are believed to influence calcium metabolism hyperthyroidisms elevate while hypothyroidism lower serum calcium level [105].

1.5 Objectives of the Present Study

Thyroidal dysfunctions are more prevalent in females and its adverse effects in pregnant females are well documented. The data about the prevalence of thyroidal dysfunctions and its ill effects in Non-Pregnant Women (NPW) is limited especially in south Asia, in general, and Pakistan, in particular. The main objective of the present work is to generate a data about the prevalence of thyroidal dysfunction in Non-Pregnant Women (NPW) in Khyber Pakhtunkhawa Pakistan and also study the effects of these clinical conditions on their bone, renal and liver health in the selective group of general population.

CHAPTER-2

EXPERIMENTAL

2.1 ETHICAL APPROVAL OF THE STUDY

The present cross sectional control study was approved by the concerned ethical committee of the Khyber Teaching Hospital (KTH) Peshawar Pakistan through its letter no 1876/KTH/ P.S. (annexure1) .

2.2 MATERIALS AND METHODS

2.2.1 Study Period and Study Population

This cross sectional and analytical study was carried out between 01 March 2014 to 31 March 2015 in KTH. The total number of patients visiting various outpatient departments during this period was 539968. Out of these 281,311 were female and 258,657 male. The total referral cases from these departments for thyroid screenings were 15667. Out of these 3010 were found to be newly diagnosed cases of thyroidal dysfunctions, 2303 were female and 707 were male. The ratio of female and male was 3.26:1. Out of total females patients 607 were premenopausal, 540 were pregnant, 952 females were sufferings from various other diseases like kidney, liver, bone, heart ailment, diabetes and hence were excluded from this study.

2.2.2 Study Groups

The study group comprises of 205 non pregnant women in the age group of 18-75 years. The control group consisting of 54 non pregnant women was selected from the patient having normal thyroid profile.

2.2.3 Data Collection

Data was collected from the patients using a well-designed data entry form. Informed consent was sought from each patient directly or through her attendant. All ethical

principles were followed according to Helinisky resolution no 196/96 on research involving human subjects.

Data Entry Form

A) Personal Information

1. Name _____ 2.Age (Years) _____

3. Marital Status

Married	Unmarried	Single	Widowed
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4. No of children _____

5. Menopausal status

Pre	Menopause	Post
-----	-----------	------

6.

Lactating	Non lactating
-----------	---------------

7. Weight (kg) _____ 8.Height (m) _____

9. BMI _____

B. Medical history:

1. Thyroid disease

Euthyroid(N)	Hyperthyroid(H)	Hypothyroid(h)
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2. Other diseases

S no	Disease	Response	Response
1	Diabetic	Yes	No
2	Renal	Yes	No
3	Liver	Yes	No
4	Hypertensive	Yes	No
5	Other	Yes	No

2.2.3 Blood Collection

Five mL of fresh fasting venous blood sample was taken from each individual. After coagulation of blood, it was centrifuged for 10 minutes at 3000 rpm to get a clear cell free serum. Serum was divided into two portion- one portion was analyzed for the determination of thyroid profile markers by Elisa techniques on Dia 710 micro plate reader (Made in Australia) and the other was used for the determination of biochemical markers of renal functions, liver functions and bone formation markers using Standard protocols and standard Erba kits on autoanalyser (Erbamannhein Chemistry Autoanalyser, Germany).

2.3 BIOCHEMICAL TESTS FOR THYROID FUNCTIONS

2.3.1 Determination of TSH in human serum by Sandwich Enzyme immunoassay ^{106, 107}.

Kits: Biocheck

Catalog number: BC-1001

Reagents in the kits

- Microtiter wells coated with murine monoclonal anti-TSH.
- Lyophilized Standards set of 0, 0.5, 2.5, 10 and 25µIU/ml.
- Enzyme conjugate solution.
- TMB solution, 11mL
- Stop solution (1.0 N HCl), 11mL.

Test Principle

The working principle of this test is a solid phase enzyme- linked immunosorbent assay ^{108, 109} in which, mouse monoclonal anti-TSH antibody is immobilized on the solid phase (microtiter wells).The conjugate solution contain the goat anti-TSH antibody. The TSH molecule in the sample is sandwiched between enzyme –linked antibodies and the solid phase. The wells are incubated for about one hour at 25° C and then washed to remove the residual labeled antibodies. The TMB reagent is added for colour development (blue) and is incubated for 20 minutes. The stop solution is

added to prevent further colour development and the colour is changed to yellow. The absorbance of the solution is measured Spectrophotometrically at 450 nm.

Procedure

All the reagents were set at 25°C. The standard (lyophilized) was diluted with 1.0 mL of double distilled water and allowed to stand for 20 minutes. 100µL of standard; control and specimen were taken in appropriate well. 100µL of the enzyme conjugate solution was taken in all wells and was thoroughly mixed for thirty seconds. The contents were incubated at 25°C for about one hour. The mixture was removed and the microtitre wells were washed five times with double deionized water. The wells were dried by mean of absorbent papers. 100µL of TMB solution was added to each well and was mixed for ten seconds gently. The mixture was again incubated for twenty minutes. 100 µL of stop solution was added into each well to terminate the reaction. The contents were again gently mixed till the blue color was completely changed to yellow. The optical density (OD) was noted spectrophotometric ally at 450 nm.

Results Calculation

The mean absorbance value (A_{450}) was determined for each set of patient samples, standards and controls. A standard graph was drawn by plotting the average absorbance vs. concentration (µIU/mL). The concentration of TSH in µIU/mL in the patient sample was calculated from the standard curve.

Normal range: 0.4- 6.0 µ IU/mL.

2.3.2 Determination of T₃ and T₄ in human serum using competitive Enzyme immunoassay ¹¹⁰.

2.3.2.1 Principle of competitive ELISA

A known volume of test serum, anti-T₃/ anti-T₄ antibody and T₃/T₄ linked with HRPO (Horseradish Peroxidase) are added to the microtitre wells coated with a

secondary antibody (anti-mouse IgG). The mouse anti-T3/ T4 antibody is attached to the secondary antibody present on the wells during incubation and T3/T4 and conjugated T3/T4 also compete for the very limited attachment sites on the respective anti-T3/T4 antibody. The wells are incubated for 60 minutes and are then washed with deionized water five times to remove the unbound T3/T4 conjugate. The TMB reagent is added and then incubated for twenty minutes to develop blue colour. The stop solution is added to stop colour development and the OD of the colour is determined spectrophotometric ally at 450 nm.

2.3.2.2: Determination of T₃

Kits: Biocheck USA

Catalog number: BC-1005

Reagents in the kits:

- Microtiter wells coated with goat anti mouse IgG.
- T₃ Standards set of 0, 0.75, 1.5, 3.0, 6.0 and 10 ng/mL, lyophilized.
- Enzyme conjugate concentrate (11×1.3 mL).
- TMB solution, 11mL
- Stop reagent (1.0 N HCl), 11mL.
- Enzyme conjugate solution, 13 mL
- Antibody reagent, 7 mL

Assay procedure

All the reagents were brought to 25°C. 0.1 mL solution of T₃ linked with HRPO was added to 1.0 mL of T₃ conjugate solution and was mixed well to make working conjugate. 50µL of standard; specimen and control were taken in appropriate well. 50µL of antibody reagent was taken in all wells and was thoroughly mixed for 30 seconds. 100 µL of working conjugate reagent was taken in all wells and mixed thoroughly for thirty seconds. The contents were incubated at 25°C for 60 minutes. The mixture was removed and the microtitre wells were washed five times with

double deionized water. The wells were dried by mean of absorbent papers. 100µl of the TMB solution was added to each well and was then mixed vigorously for ten seconds. The mixture was again incubated at 25°C for twenty minutes. 100 µl of stop solution was then added into each well to terminate the reaction. The contents were again gently mixed till the blue colour was completely changed to yellow. The OD was noted at 450 nm spectrophotometrically.

Results calculation

The average absorbance value (A_{450}) was determined for the standards, patient samples and controls. A standard graph was constructed by plotting the average absorbance vs. concentration (µIU/mL). The concentration of T_3 in ng/mL in the patient sample was determined from the standard curve.

Normal range: 0.6-1.85 ng /mL.

2.3.2.3 Determination of Tetraiodothyronine (T_4)¹¹¹.

Kits: Bio check USA

Catalog number: BC-1007

Reagents in the kits:

- Microtiter wells coated with sheep anti- T_4 mouse.
- Lyophilized T_4 Standards set of 0, 2, 5, 10, 15 and 25 µg/dL.
- Enzyme conjugate concentrate (11×1.3 mL).
- TMB solution, 11mL.
- Stop solution (1.0 N HCl), 11mL.
- Enzyme conjugate diluent, 13mL.

Assay procedure

All the solutions were set at 25°C. 0.1 mL of T_4 -HRPO linked concentrate was added to 1.0 mL of T_4 conjugate diluent and was mixed well to prepare working conjugate reagent. 25µL of standard; specimen and control were taken in appropriate well.

100µL of working conjugate solution was taken in all wells and was thoroughly mixed for thirty seconds. The contents were incubated at 25°C for about one hour. The mixture was removed and the microtiter wells were washed 5 times with double deionized water. The wells were dried by mean of absorbent papers. 100µL of TMB solution was added into all wells and was mixed for ten seconds gently. The mixture was again incubated at 25°C for twenty minutes in the dark. 100 µL of stop solution was added into each well to terminate the reaction. The contents were again gently mixed till the blue color was completely changed to yellow. The OD was noted at 450 nm spectrophotometric ally (model and specification).

Results calculation

The mean absorbance value (A_{450}) was determined for the patient samples, standards and controls. A standard graph was plotted between mean absorbance and concentration ($\mu\text{g/dL}$). The concentration of T3 in $\mu\text{g/L}$ in the patient sample was determined from the standard curve.

Normal range: 4.8-12.0 $\mu\text{g/dL}$.

2.4 THYROID PROFILE STATUS OF THE STUDY GROUPS

Based on the thyroid function tests, the patients were categorized into two main groups.

Hyperthyroid patients (H) (n=109) & Hypothyroid patients (h) (n=96). Hypothyroid patients(n=96) were further sub divided into Sub clinically hypothyroid(Sh) (n=48; TSH above 6.1 $\mu\text{IU/mL}$ with normal T_4 and T_3 levels) and Overtly hypothyroid(Oh) (n=48) with TSH above 6.0 $\mu\text{IU/mL}$ and/ or abnormally low T_4 (below 4.8 $\mu\text{g/dL}$) and T_3 (below 0.6 ng/mL) levels. Hyperthyroid patients (n=109) were also further sub divided into Sub clinically hyperthyroid(SH) (n=51); TSH $\leq$ 6.1 $\mu\text{IU/mL}$ with normal T_4 and T_3 levels) and Overtly hyperthyroid(OH) (n=58) with TSH $\leq$ 6.0 $\mu\text{IU/mL}$ and/ or abnormally high T_4 (above 4.8 $\mu\text{g/dL}$) and T_3 (above

0.6ng/mL) levels. Subjects having normal thyroid profile were taken as control (n=54, TSH $\leq$ 6.0, normal T₃ and T₄ levels).

2.5 BIOCHEMICAL TESTS FOR RENAL FUNCTIONS

2.5.1 Serum Creatinin determination in human serum ¹¹².

Kits: ERBA

Cat. No. BLT00019

Kit composition

Pack	Contents	Concentration	Quantity
R1	Sodium Hydroxide	394 mmol/L	2x250 mL
R2	Picric Acid	11 mmol/L	2x250 mL
R3	Standard		2x5 Ml

Principle

Alkaline picrate forms an orange-yellow colour complex on reaction with Creatinine (the Jaffe's reaction). The absorbance of this complex is directly proportional to the concentration of creatinine in the test sample and is determined spectrophotometrically at 490-510 nm.

Assay procedure

The reagents were mixed in a Cuvette of 1 cm length according to the following table.

The absorbance was noted at 510 nm after 10 seconds (A₁) and 60 seconds (A₂).

Reagent	Blank Reagent	Standard	Sample
Reagent 1	1.0 mL	1.0 mL	1.0 mL
Sample	-	-	0.1 mL
Standard	-	0.1 mL	-
Distilled water	0.10 mL	-	-

Results calculations

$$\text{Creatinine (mg/dl)} = \frac{\text{Absorbance of the sample} \times \text{C.S}}{\text{Absorbance of the standard}}$$

Where C.S= Concentration of the standard

Absorbance of the sample = $A_1 - A_2$

Normal range: 0.6-1.3 mg/ dL

2.5.2 Serum Urea determination [113].

Kit specification: ERBA

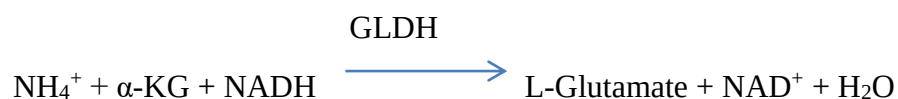
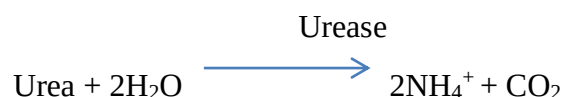
Cat. No.	Pack Name	Packaging
BLT00060	UREA 1000	R1: 4 x 200 ml, R2: 1 x 200 mL
		R3 standard 1 x 5 Ml

Kit composition

R1	Contents	Concentration
	Tris Buffer	100 mmol/L
	α -Ketoglutarate	5.49 mmol/L
	Jack Bean Urease	≥ 10 U/L
	GLDH (Glutamate Dehydrogenase)	≥ 3.8 U/L
R2	NADH (Nicotinamide Adenine Dinucleotide)	1.66 mmol/L
R3	Standard	-

Principle

Urea undergoes hydrolysis in the presence of Urease and water to liberate ammonia. Ammonia reacts with α -ketoglutarate (α -KG) in the presence of GLDH and reduced NADH to form L-Glutamate. The concentration of urea in the sample is directly proportional to change in absorbance over a fixed period of time.



The progress in the rate of reaction is followed by determining the change in absorbance at 340 nm, as NADH is oxidized to NAD^+ .

Assay procedure

The reagents were mixed according to the following table and after 60 seconds incubation (at 37 °C) Reagent 2 (R_2) was added.

Reagent	Blank	Standard	Sample
Reagent 1(R_1)	1.0 mL	1.0 mL	1.0mL
Sample	-	-	0.01 mL
Standard	-	0.01 mL	-
Distilled water	0.01 mL	-	-
Reagent 2(R_2)	0.25 mL	0.25 mL	0.25 mL

The reagents were mixed and the initial absorbance was measured after 30 sec (A_1). Timer was started simultaneously and second absorbance was measured exactly after 1 min (A_2). Absorbance was also measured for reagent blank. Absorbance change was calculated $\Delta A = (A_2 - A_1)/\text{min}$.

Results calculations

Urea (mg/dl) = (Absorbance of the sample $\times$ C.S) / (Absorbance of the standard)

Where C.S= Concentration of the standard

Absorbance of the sample = $A_1 - A_2$

Reference range: 5-45 mg/ dL

2.6 BIO CHEMICAL TESTS FOR BONE FORMATION MARKERS

2.6.1 Serum Alkaline Phosphatase ¹¹⁴.

Cat. No.	Pack Name	Packaging
BLT00004	ALP AMP 500	R1: 4 x 100 mL, R2: 1 x 100 ML

Reagent composition

	Content	Concentration
R1	AMP buffer, pH 10.4	434 mmol/L
	Magnesium acetate	2.48 mmol/L
	Zinc sulfate	1.24 mmol/L
	HEDTA	2.48 mmol/L
R2	p-Nitro phenyl phosphate	19.5 mmol/L

Principle

The method is based on recommendations of IFCC, utilizing 4-Nitrophenyl phosphate as the substrate. ALP present in the test sample catalyzes the hydrolysis of the substrate to form a yellow coloured compound, 4-Nitrophenol. The rate of reaction is monitored by observing the change in absorbance at 405 nm or 415 nm which is directly proportional to the concentration of ALP in the test sample.



Assay procedure

Content	Blank Reagent	Standard	Sample
Working reagent	1.0 mL	1.0mL	1.0 mL
Sample	-	-	0.02 mL
Standard	-	0.02 mL	-
Distilled water	0.02mL	-	-

The reagents were mixed according to the above table and were then incubated for 1 minute at 37°C and then the initial absorbance of standards and samples was measured at 420 nm in 1 cm Cuvette against blank reagent. Change in absorbance was measured exactly after 1, 2 and 3 min. change in absorbance after one minutes was Calculated ($\Delta A/\text{min}$).

Results calculations

Alkaline phosphatase activity in the sample in U/l= $\Delta A/\text{min} \times 3433$

Reference value: 50-190 U/l

2.6.2 Serum Calcium Determination ¹¹⁵

Cat. No.	Pack Name	Packaging
BLT00016	CA 250	R1: 1 x 250 mL, R2 standard: 1 x 5 mL

Reagent composition

	Content	Concentration
R1	Arsenazo III	0.10 mmol/L
	Phosphate buffer (pH 7.8 ± 0.1)	50 mmol/L
R2	Standard	-

Principle

Calcium ions in the test sample react with Arsenazo III at pH 6.5 to form a coloured complex. The absorbance of the complex is determined at 650 nm (650-660 nm) which is directly proportional to the concentration of calcium in the test sample.

Procedure

The reagents were mixed well according to the following table and incubated for 10 minutes at 37°C and the absorbance was noted at 650 nm in 1 cm Cuvette.

Content	Blank reagent	Standard (Cal.)	Sample
Reagent 1	1.0 mL	1.0 mL	1.0 mL
Sample	-	-	0.01 mL
Standard	-	0.01 mL	-
Distilled water	0.01 mL	-	-

Results calculation

$(\text{Absorbance of the Sample} \times \text{C.S}) / (\text{Absorbance of the standard})$

Where C.S= Concentration of the standard

Reference value: 8.6-10.3 mg/ dL

2.7 BIOCHEMICAL TESTS FOR LIVER MARKERS

2.7.1 ALT Determination in Human Serum¹¹⁶

Kit: Erba Mannheim System Packs

Composition of the kit

Reagent	Active ingredients	Concentration
R1	Tris Buffer(pH=7.5)	137.5 mmol/L
	L-Alanine	709mmol/L
	LDH(microbial)	$\geq 2000\text{U/L}$
R2	CAPSO	20 mmol/L
	2- Oxologlutarate	85 mmol/L
	NADH	1.05 mmol/L

Principle of the assay

This assay procedure is based on the recommendations of the IFCC without pyridoxal phosphate. The series of reactions are as follow

L-Alanine + 2-oxologlutarate $\xrightarrow{\text{SGPT}}$ Pyruvate+ L-glutamate

Pyruvate + NADH $\xrightarrow{\text{LDH}}$ L-Lactate + NAD⁺

Sample pyruvate+ NADH $\xrightarrow{\text{LDH}}$ L-Lactate+ NAD

Assay procedure

The Enzyme Reagent (R₁) and Starter Reagent (R₂) were taken into a clean dry test tube and mixed according to the following table. The initial absorbance A₀ was noted and the absorbance reading was noted after every minute. The change in average absorbance per minute was calculated (D A/ min).

Reagent	Amount
Enzyme solution(R ₁)	0.8 mL
Sample	0.2 mL
Starter solution (R ₂)	0.2 mL

Now the following reagents were taken in a clean dry test tube and mixed well.

Reagents	Amount	
Working Reagent (L1)	1.0 mL	1.0 mL
Sample	0.2 mL	0.1 mL

The initial absorbance A₀ was noted after 1 minute and, the absorbance reading was noted after every 1, 2, & 3 minutes. The change in average absorbance per minute was calculated (D A/ min).

Results calculations

$$\text{ALT activity in U/L at } 37^{\circ}\text{C} = \text{D A/ min.} \times 1746$$

Reference range: 5-45 IU/L

2.7.2 AST determination [117]

Composition of the kits

Content	Reagent	Quantity	
R1	Enzyme solution	20 mL	60 mL
R2	Starter solution	5mL	15mL

Principle of assay

AST catalyzes transamination reaction between Ketoglutarate and L-Aspartate to form Glutamate and Oxaloacetate. The Oxaloacetate so formed reacts with NADH to form NAD in the presence of enzyme Malate Dehydrogenase. The rate of reaction is measured as a change in the absorbance which is directly proportional to the activity of AST in the test sample.



Assay procedure

The Enzyme Reagent (R₁) and Starter Reagent (R₂) were taken into a clean dry test tube and mixed according to the table. The initial absorbance A₀ was noted at 340 nm and the absorbance reading was noted after every 1, 2, & 3 minutes. The change in average absorbance per minute was calculated (D A/ min).

Reagent	Amount	
Enzyme Reagent (R ₁)	0.8 mL	0.8 mL
Test sample	0.2 mL	0.1 mL
Starter Reagent (R ₂)	0.2 mL	0.2 mL

Now the following reagents were taken in a clean dry test tube and mixed well.

Reagents	Amount	
Working Reagent (R1)	1.0 mL	1.0 mL
Sample	0.2 mL	0.1 mL

The initial absorbance A₀ was noted after 1 minute and the absorbance reading was noted after every 1, 2, & 3 minutes. The mean absorbance change per minute (D A/min) was calculated.

Results calculations

$$\text{SGOT (AST) activity in U/L at } 37^{\circ}\text{C} = \text{D A/min.} \times 1746$$

Reference range: 5-45 IU/

2.8 STATISTICAL ANALYSIS OF THE DATA

The data obtained for all the groups was statistically analyzed on SPSS 21.0 software (SPSS Inc. Chicago, IL, USA). Values were reported as mean $\pm$ standard error of mean. Pearson's correlation analysis was also applied to look for the possible association between the required variables. A two-tailed p value < 0.05 was considered statistically significant.

CHAPTER-3

RESULTS AND DISCUSSION

3.1 BASE LINE CHARACTERISTIC OF THE STUDY GROUP

3.1.1 Age and Menopausal Status of the Study Group

The study group and the control group were composed of age matched 259 Non-Pregnant Women (NPW). The mean age of patients in the control group (N) was 42.15 ± 1.86 years, 46 ± 1.38 years for Overtly hypothyroid (Oh), 45.97 ± 1.93 years for Sub clinically hypothyroid (Sh) 49.74 ± 1.62 years for Overtly Hyperthyroid (OH) and 48.94 ± 1.87 years for the Sub clinically Hyperthyroid (SH). The control group was composed 54, of which 61.11% (33) were Menopausal (M, age below 45 years), 18.52% (10) were Early Post-Menopausal (EPM, age 45-50 years) and 20.37% (11) were Late Post-Menopausal (LPM, age above 50 years). The %age of M, EPM and LPM in the Oh (48) and Sh (48) group were, 54.16% (26), 12.5% (06), 33.83% (16) and 45.83% (22), 22.92% (11), 31.25% (15) respectively. Similarly the %age of M, EPM and LPM in the OH (58) and SH (51) were 34.48% (20), 27.58% (16), 37.93% (22) and 43.14% (22) 07.84% (04), 49.07% (25) respectively. The frequency distribution of the study population as group ID, age wise and menopausal status is given in table 3.1 - 2 and shown in figures 3.1-2 and 3.3 a-e respectively.

Table 3.1: Frequency distribution of thyroid function status of the study groups

S no	Group ID	Frequency(n)	%age	Years range	Mean age (year)	SE	SD
1	N	54	20.85	20-73	42.15	1.86	13.67
2	Oh	48	18.53	20-72	46.18	1.83	12.67
3	Sh	48	18.53	25-74	45.97	1.93	13.36
4	OH	58	22.39	20-72	49.74	1.62	12.33
5	SH	51	19.70	21-75	48.94	1.87	13.40

N: Normal, Oh: Overtly hypothyroid, Sh: Sub clinically hypothyroid OH: Overtly Hyperthyroid, SH: Sub clinically hyperthyroid, SE: Standard Error, SD: Standard Deviation

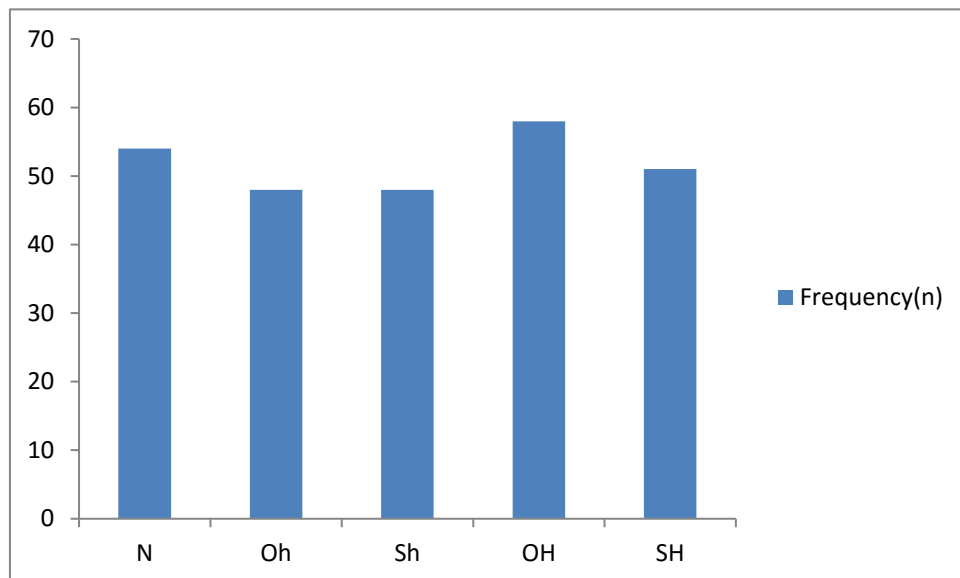


Figure 3.1: Frequency distribution of thyroid function status of the study group.

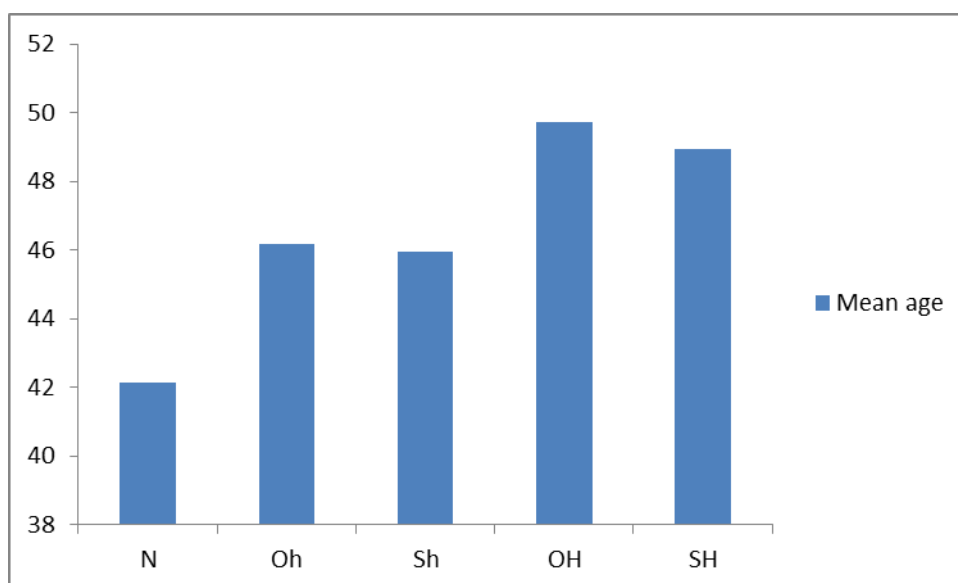


Figure 3.2: Mean age of the study group.

Table 3.2: Frequency distribution of menopausal status of the study groups.

S no	Group ID	% M (n)	% EPM (n)	% LPM (n)	TOTAL(N)
1	N	61.11(33)	18.52(10)	20.37(11)	54
2	Oh	54.16(26)	12.50(06)	33.83(16)	48
3	Sh	45.83(22)	22.92(11)	31.25(15)	48
4	OH	34.48(20)	27.58(16)	37.93(22)	58
5	SH	43.14(22)	07.84(04)	49.07(25)	51

M: Menopausal, EPM: Early Postmenopausal, LPM: Late Postmenopausal.

Figure 3.3a-e: Frequency distribution of menopausal status of the study group:

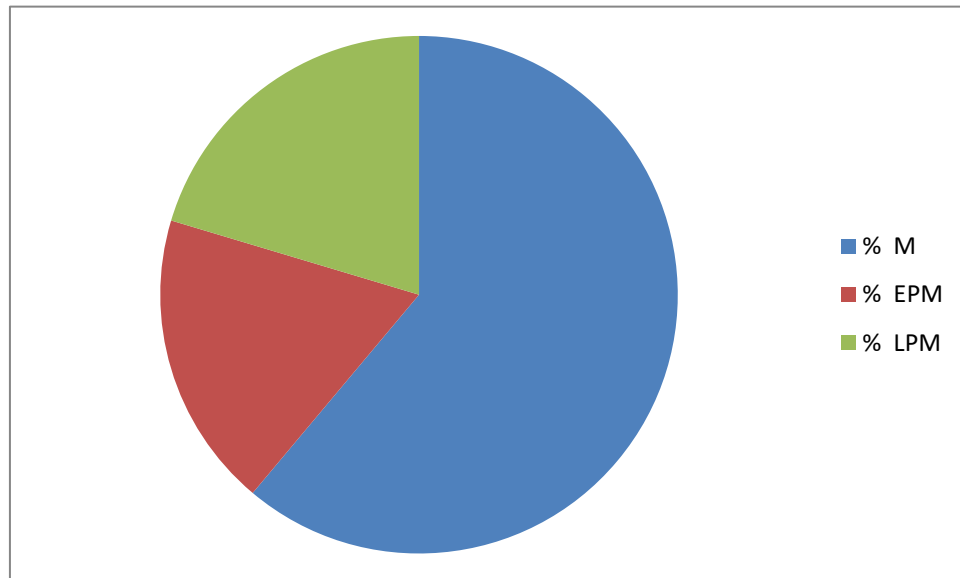


Fig 3.3 a Frequency distribution of menopausal status of the N group

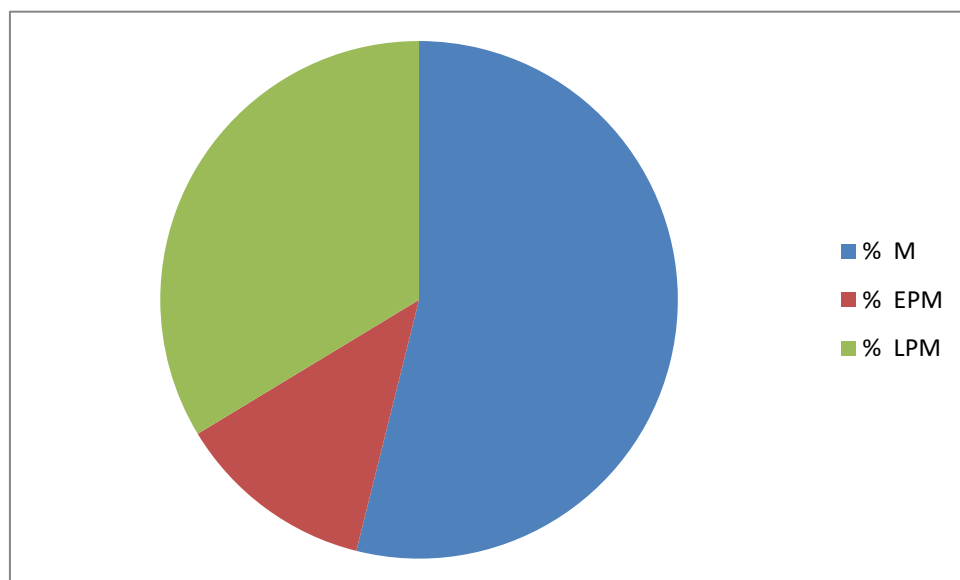


Fig 3.3 b Frequency distribution of menopausal status of the Oh group

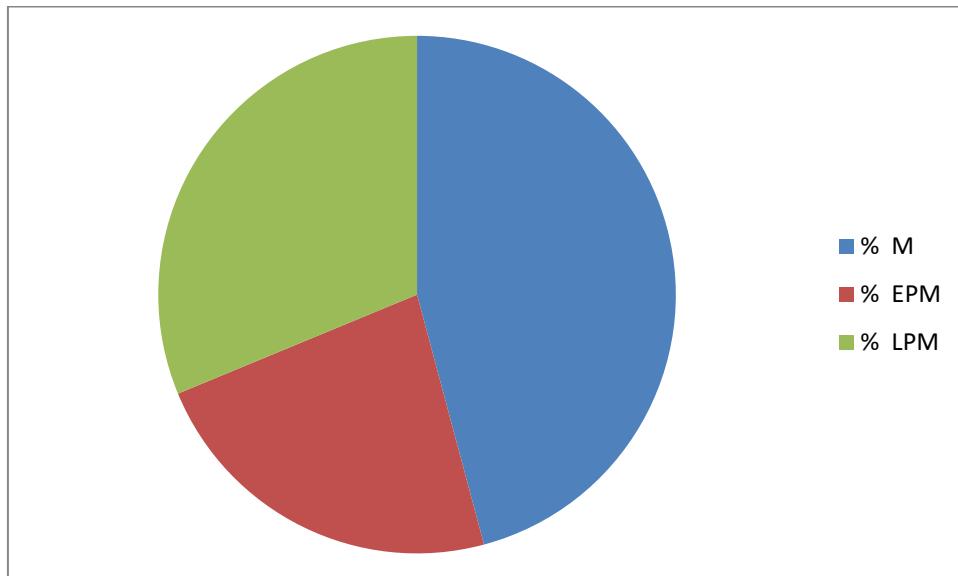


Fig 3. 3c Frequency distribution of menopausal status of the Sh group

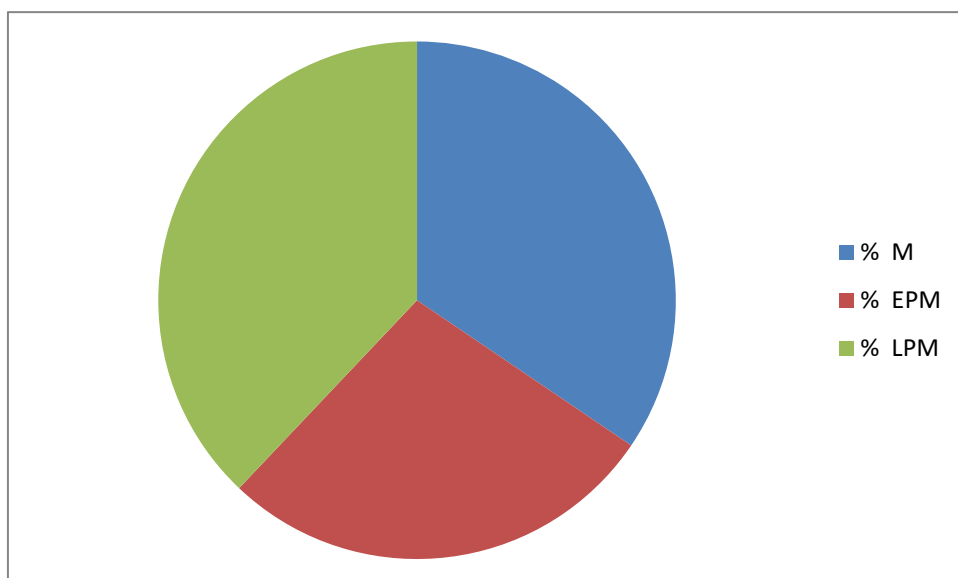


Fig 3.3 d Frequency distribution of menopausal status of the OH group

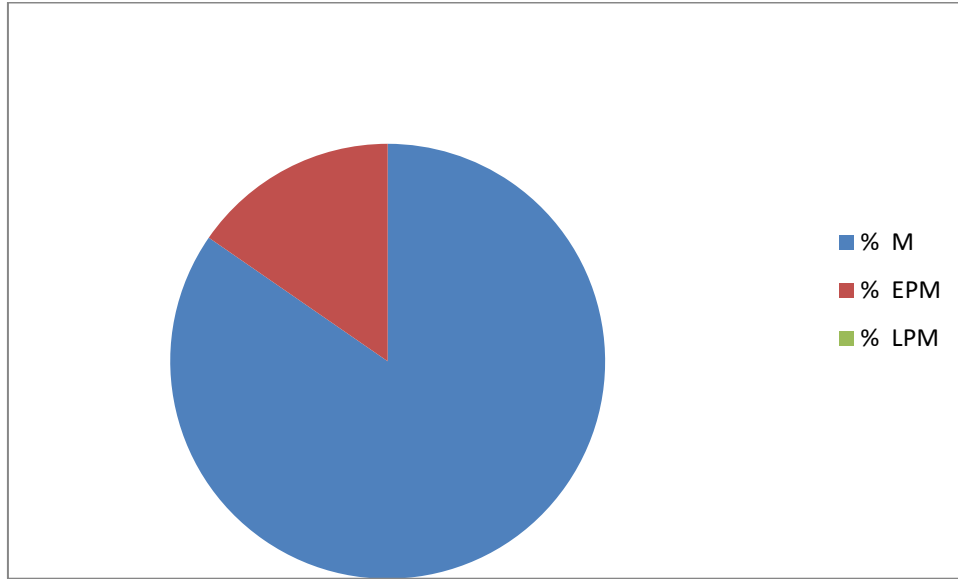


Fig 3.3 e Frequency distribution of menopausal status of the SH group

3.1.2 BMI of the Study Groups

The obesity of an individual is expressed in term of BMI (Kg/ m^2). The values of standard BMI are given in table 3.3a [118]. According to the standard table, the %age of obese individual was highest in N (42.60%) and lowest in OH (06.70%). The %age of Overweight (OW), Normal(N) and Underweight (UW) were highest in Sh (37.50%), (39.58%) and OH (13.80%) and lowest in N (14.81%), Oh (35.42%) and Oh (0.00%) respectively. The results of BMI, frequency distribution of the study population are given in tabl3.3 b and shown in figures 3.4 a-e.

Table 3.3 a: Standard BMI values.

Nomenclature	BMI(Kg/m^2)
Underweight(UW)	<18.5
Normal(N)	18.5-22.9
Overweight (OW)	23-25
Obese(O)	>25

Table 3.3 b: Frequency distribution of BMI status of the study group.

S no	Group ID	% O (n)	% OW(n)	% N(n)	% UW(n)	TOTAL(N)
1	N	42.60(23)	14.81(08)	37.03(20)	05.55(03)	54
2	Oh	29.17(14)	35.42(17)	35.42(17)	0.00(0)	48
3	Sh	22.92(11)	37.50(18)	39.58(19)	0.00(0)	48
4	OH	06.70(04)	27.60(16)	51.70(30)	13.80(08)	58
5	SH	13.72(07)	29.41(15)	47.06(24)	09.80(05)	51

O: Obese, OW: Over Weight, N: Normal, UW: Under Weight.

Fig 3.4 a-e: Frequency distribution of BMI status of the study group

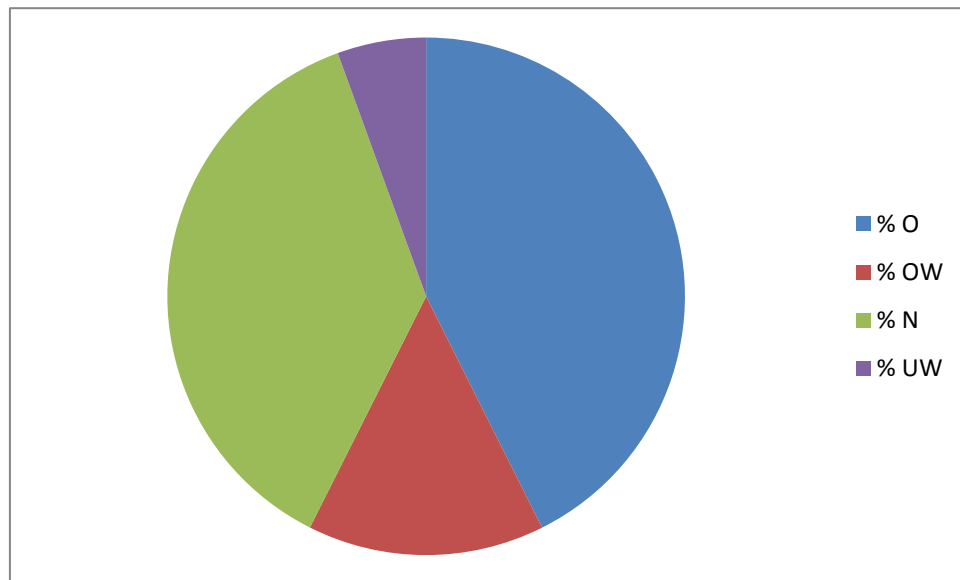


Fig 3.4 a: Frequency distribution of BMI status of the N group

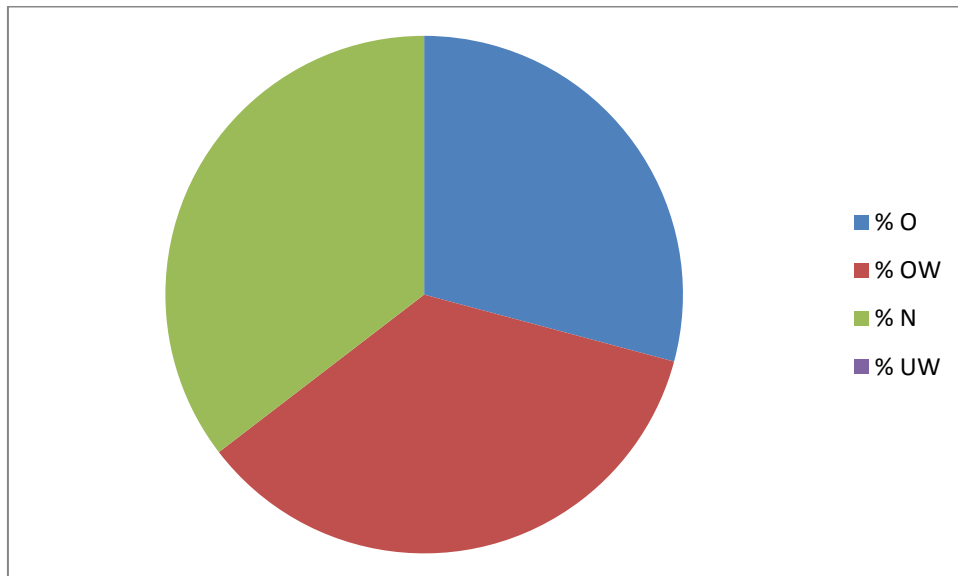


Fig 3. 4 b: Frequency distribution of BMI status of the Oh group

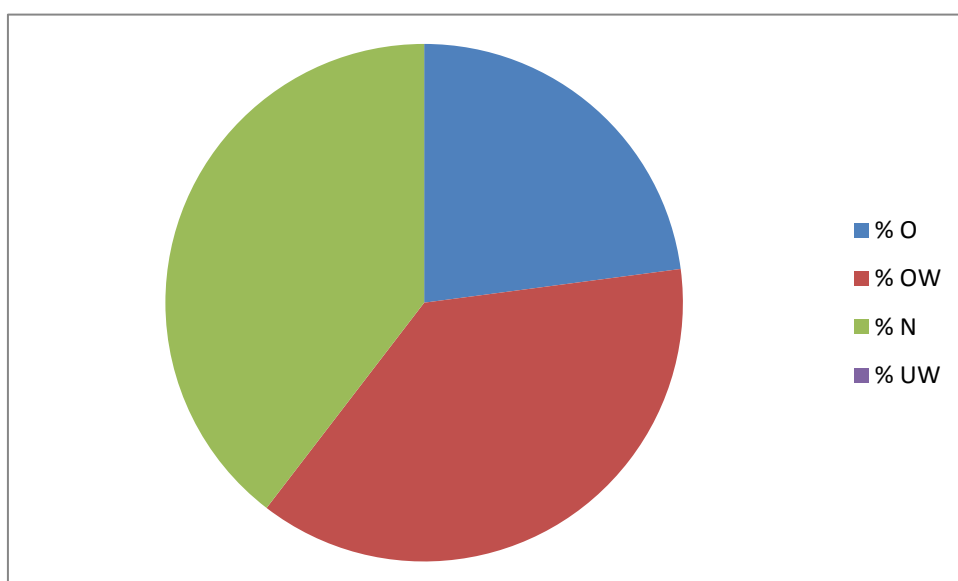


Fig 3. 4 c: Frequency distribution of BMI status of the Sh group.

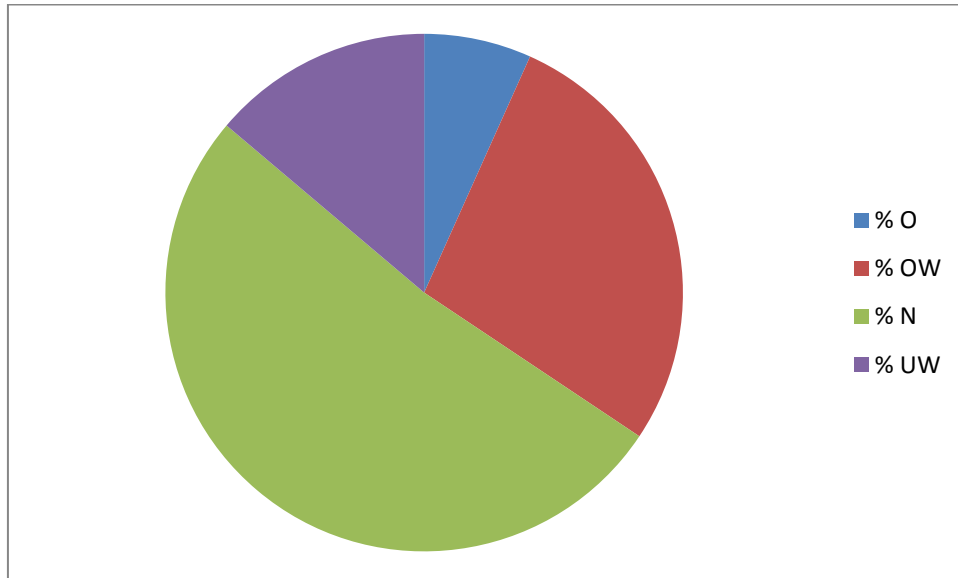


Fig 3. 4 d: Frequency distribution of BMI status of the OH group

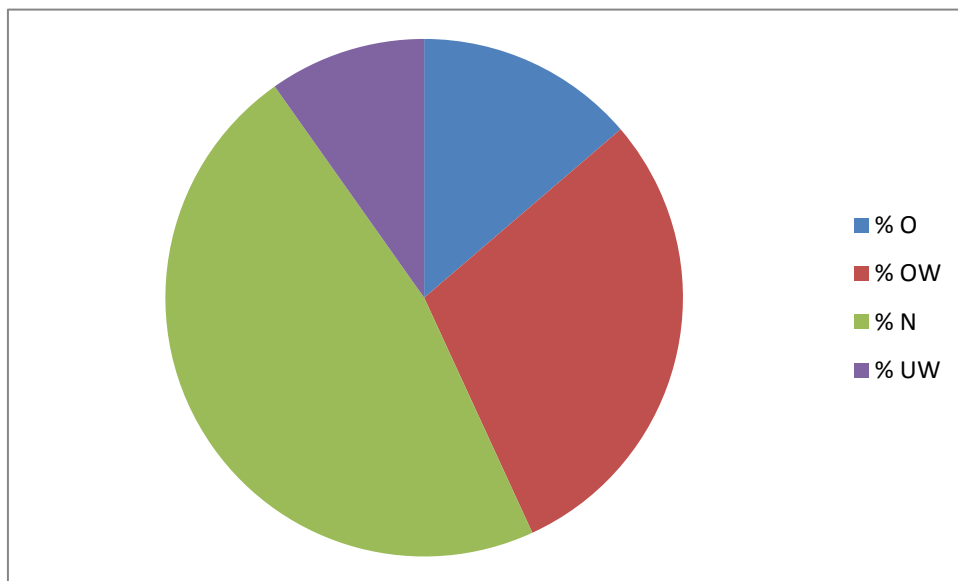


Fig 3. 4 e: Frequency distribution of BMI status of the SH group

3.2 COMPARISON OF THYROID PROFILE MARKERS, RENAL, LIVER AND BONE MARKERS OF THE STUDY GROUP

The values of thyroid hormones, biochemical markers of renal, liver function and bone formation markers are presented in table 3.4, 3.5, 3.6 and 3.7 and shown in figures 3.5 a- c, 3.6- 3.9.

The mean serum level of TSH was found to be lowest in OH group ($0.17 \pm 0.01 \mu\text{IU/mL}$) and highest in the Oh group ($25.89 \pm 2.86 \mu\text{IU/mL}$). Serum T_3 level was highest in OH group ($2.37 \pm 0.01 \text{ ng/mL}$) and lowest in Oh group ($0.94 \pm 0.09 \text{ ng/mL}$). Highest serum T_4 level was found for OH group ($12.07 \pm 0.46 \mu\text{g/dL}$) and lowest in Oh group ($4.30 \pm 0.28 \mu\text{g/dL}$). The mean serum creatinine was found highest in N group ($1.47 \pm 1.25 \text{ mg/dL}$) and lowest in Oh group ($1.10 \pm 0.01 \text{ mg/dL}$).

Serum Urea level was found elevated in OH ($35.35 \pm 1.75 \text{ mg/dL}$) and lowest in Sh group ($23.22 \pm 0.88 \text{ mg/dL}$).

The mean SGPT level was highest in OH group ($33.14 \pm 2.98 \text{ IU/L}$) and lowest in the Sh group (16.85 ± 0.55). The mean SGOT was found highest in N group ($33.70 \pm 5.12 \text{ IU/L}$) and lowest in Sh group ($17.86 \pm 0.98 \text{ IU/L}$).

For Serum Calcium the highest values were found in OH group ($9.80 \pm 0.90 \text{ mg/dL}$) and lowest in Oh group ($8.95 \pm 0.10 \text{ mg/dL}$). Serum total alkaline phosphatase was found to be maximum in N group ($159 \pm 7.61 \text{ U/L}$) and minimum in Sh group ($128 \pm 6.42 \text{ U/L}$). No significant difference was found in the Serum Zinc level of all the diseased groups.

Table 3.4: Thyroid profile of the study groups.

S no	Group ID	TSH	T_3	T_4
1	N	2.38 ± 0.49	1.68 ± 0.05	8.50 ± 1.59
2	Oh	25.89 ± 2.86	0.94 ± 0.09	4.30 ± 0.28
3	Sh	19.04 ± 2.22	1.47 ± 0.08	7.07 ± 0.38
4	OH	0.17 ± 0.01	2.37 ± 0.01	12.07 ± 0.46
5	SH	0.18 ± 0.01	1.64 ± 0.04	8.16 ± 0.24

Fig 3.5 a-c: Comparison of thyroid profile markers of the study groups

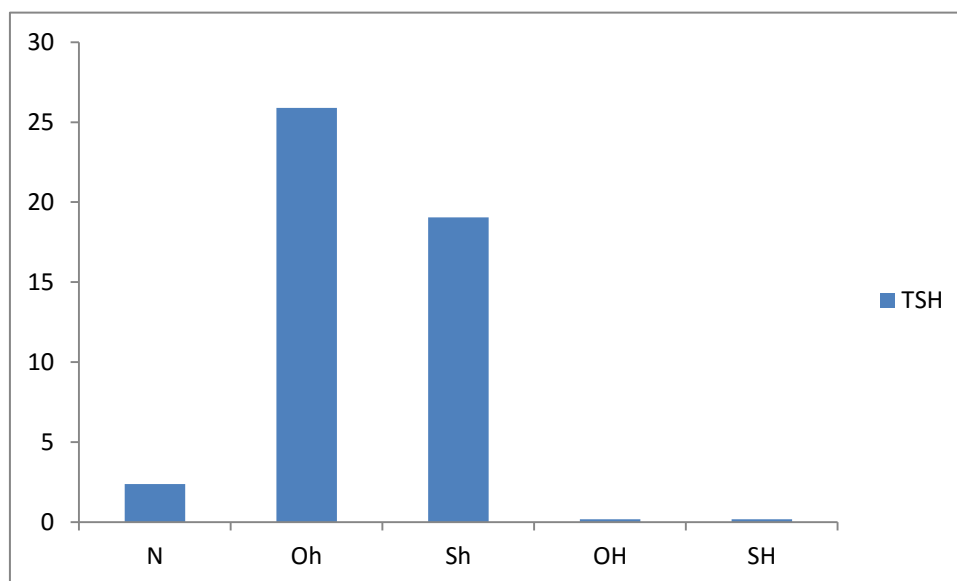


Fig 3.5 a: Comparison of TSH of the study groups

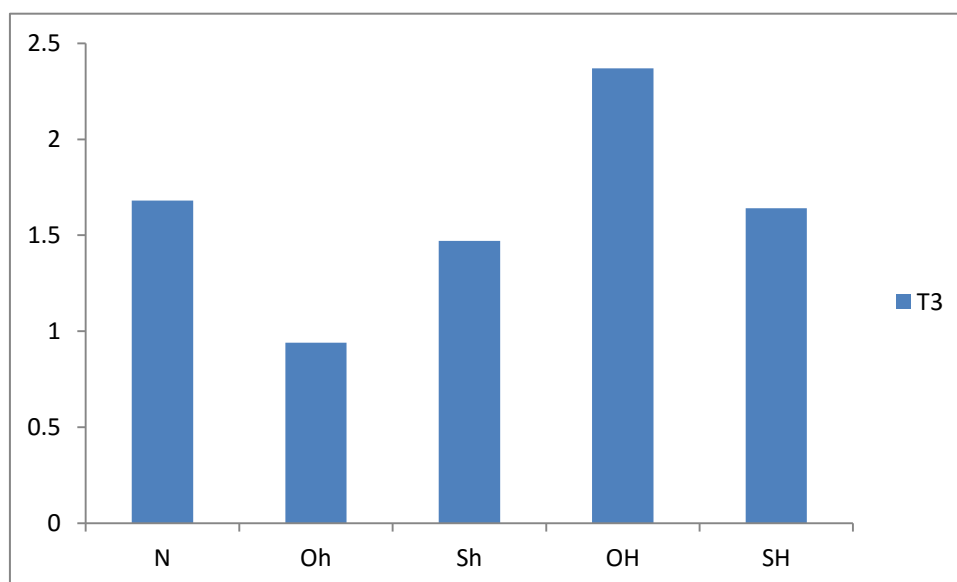


Fig 3. 5 b: Comparison of T₃ study groups.

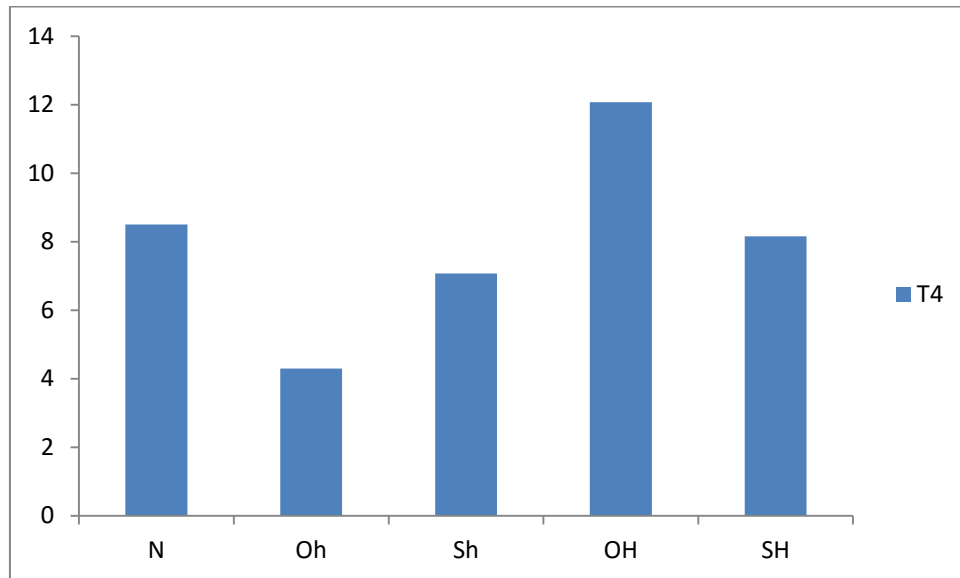


Fig 3. 5 c Comparison of T₄ study groups

Table 3.5: Comparisons of the renal markers of the study groups.

S no	Group ID	S. Creatinine	S. Urea
1	N	1.47±1.25	32.60±1.22
2	Oh	1.10±0.01	30.11±1.25
3	Sh	1.18±0.04	23.22±0.88
4	OH	1.29±0.01	35.35±1.75
5	SH	1.34±0.15	32.98±1.63

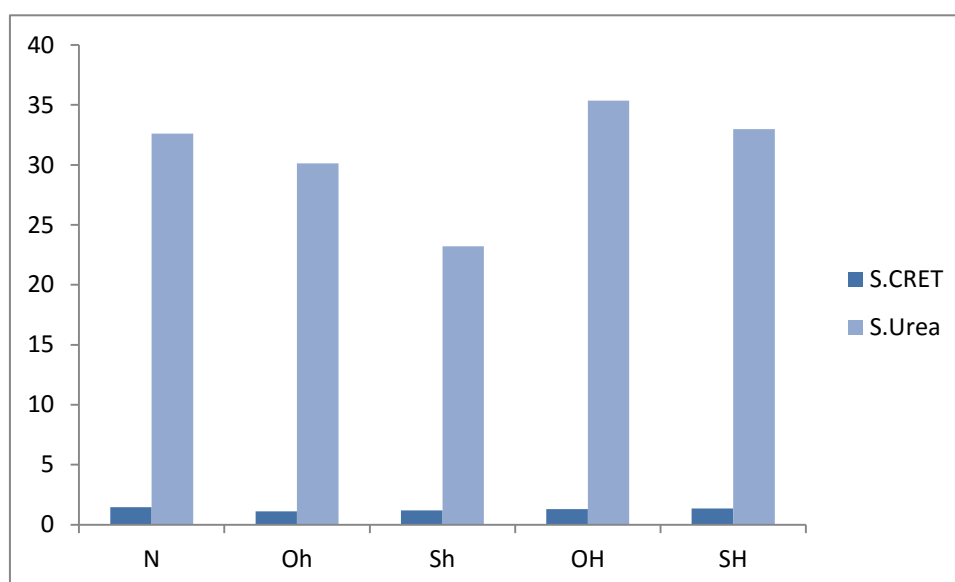


Fig 3. 6: Comparisons of the renal markers of the study group

Table 3. 6: Comparison of the liver markers of the study groups.

S no	Group ID	SGPT	SGOT
1	N	29.69±3.28	33.70±5.12
2	Oh	24.35±1.11	23.65±1.22
3	Sh	16.85±0.55	17.86±0.98
4	OH	33.14±2.98	30.68±2.7
5	SH	29.50±1.69	30.38±2.60

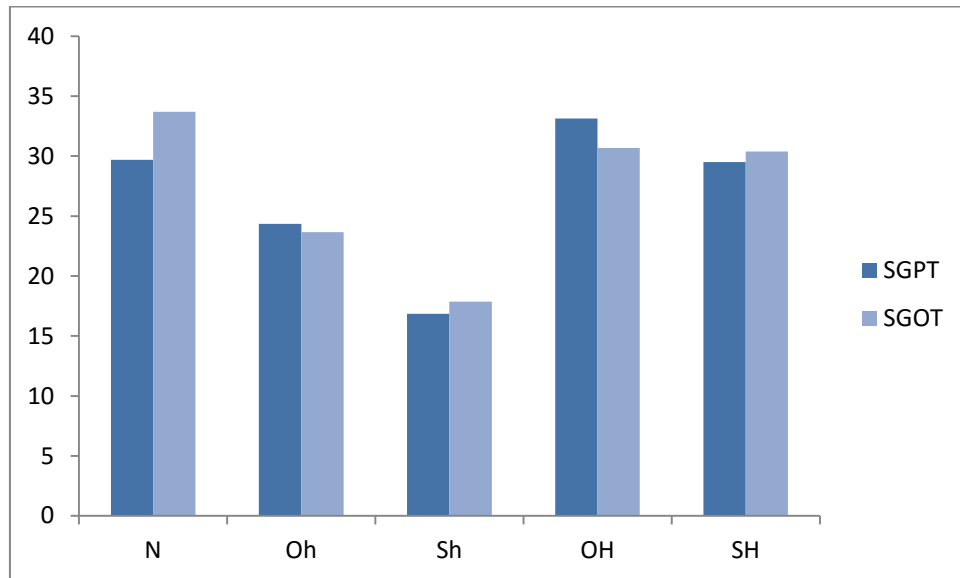


Fig 3.7: Comparison of the liver markers of the study group.

Table 3.7: Comparison of the bone formation markers of the study groups.

S no	Group ID	Bone turn over Markers		
		S.Ca	S. AIP	S.Zn
1	N	9.20±0.75	159±7.61	0.45±0.11
2	Oh	8.95±0.10	138±5.30	0.56±0.08
3	Sh	8.96±0.08	128±6.42	0.60±0.10
4	OH	9.80±0.90	144±7.12	0.61±0.11
5	SH	9.58±0.10	142±4.97	0.61±0.11

S: Serum, ALP: Alkaline Phosphatase.

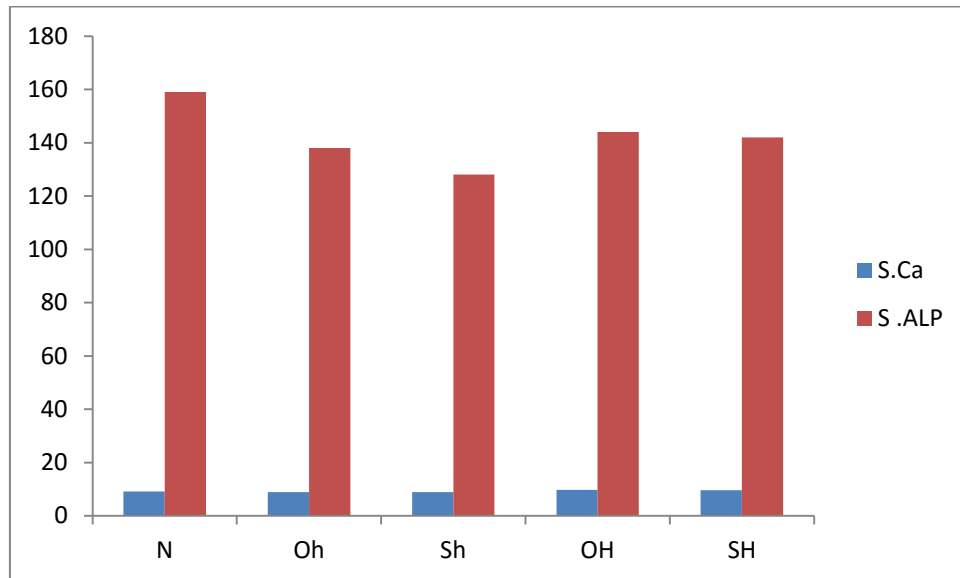


Fig 3. 8: Comparison of serum Calcium and ALP of the study groups.

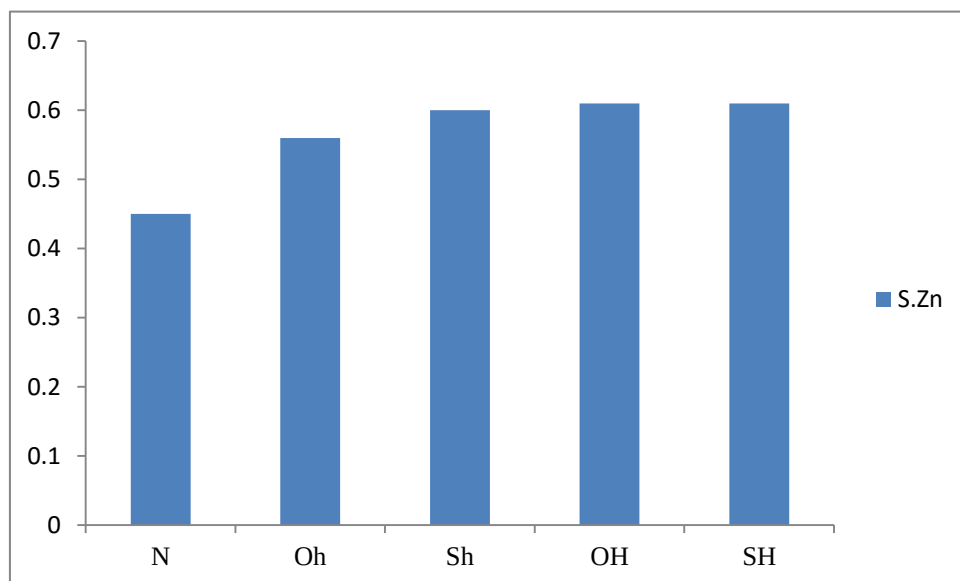


Fig 3.9: Comparison of serum Zn of the study groups.

3.3 STATISTICAL ANALYSIS OF THE DATA

3.3.1 Regression Analysis

Regression analysis is a statistical procedure for determining the kind of relationship between variables. It defines the difference of the dependent variable around the regression function ¹¹⁹. Multiple linear regression analysis of the thyroid profile markers (TSH, T₃ and T₄) as independent variables with BMI, biochemical markers of renal functions (Creatinine and Urea), liver (AST & ALT) and bone formation markers (ALP, Ca & Zn) as dependent variables was carried out. The results are discussed below.

3.3.1.1 Regression Analysis of BMI with Thyroid Profile Markers

The results of regression analysis of BMI with thyroid profile markers of the study groups is shown in table 3.8.

The results show that BMI was positively related with TSH in all the study groups except in SH where it was negatively related. With T₃ it was negatively related in N and Oh and positively in Sh, OH and SH group respectively. Negative relation was found for T₄ in all the study groups. The cumulative effect of thyroid profile change on BMI was found to be highest in Oh (19.7%) and lowest in SH group (8.3%).

Table 3. 8: Regression analysis of BMI with thyroid profile markers

Parameter	Group ID	Constant		Thyroid profile markers(IV)						%B
				TSH		T ₃		T ₄		
BMI (DV)		Unstandardized coefficients								
		B	SE	B	SE	B	SE	B	SE	
		N	30.18	3.22	0.35	0.36	- 0.12	1.29	-0.8	
	Oh	23.28	1.10	0.05	0.02	-0.4	0.52	- 0.08	0.10	19.7
Sh	23.44	1.51	0.01	0..02	0.73	0.63	- 0.18	0.13	5.8	
OH	23.21	1.72	5.15	3.78	0.6	0.53	- 0.30	0.11	16.3	
SH	23.82	1.54	- 7.03	3.45	0.64	0.69	- 0.11	0.15	8.3	

IV: Independent Variable, DV: Dependent Variable, SE: Standard Error

B: Unstandardized coefficients

3.3.1.2 Regression Analysis of the Renal Markers with Thyroid Profile in the Study Group

The results of regression analysis of thyroid profile with renal markers of all the 5 study groups are presented in table 3.9 to 3.13.

Serum creatinine showed positive relation with TSH, in all the study groups, negative relation with T₃ in the N group and OH and positive in SH group. Positive relation was observed for creatinine with T₄ in N and SH and negative in OH group. Serum urea showed positive relation with TSH in all groups, with T₃ in N and negative in SH and OH groups. Serum urea showed negative relation with T₄ in all groups.

Table 3.9: Regression analysis of thyroid profile with renal markers in the Normal group (N).

Model		Unstandardized coefficients			
DV		S.Creatinine		S.Urea	
Constant		B	SE	B	SE
		2.60	0.92	38.92	8.91
I V	TSH	0.12	0.33	0.03	0.98
	T ₃	-0.75	0.47	0.86	3.56
	T ₄	-0.01	0.02	-0.92	0.80

Dependent variable: DV, Independent Variable: IV, Standard Error: SE

Table3.10: Regression analysis of thyroid profile with renal markers in the Overtly hypothyroid group (Oh).

Model		Unstandardized coefficients			
DV		S. Creatinine		S. Urea	
Constant		B	SE	B	SE
		0.58	0.35	30.00	4.35
IV	TSH	0.01	0.01	0.02	0.07
	T ₃	0.03	0.16	-3.27	2.04
	T ₄	0.09	0.06	0.66	0.72

Table3.11: Regression analysis of thyroid profile with renal markers in the sub clinically hypothyroid group (Sh).

Model		Unstandardized coefficients			
DV		S. Creatinine		S. Urea	
Constant		B	SE	B	SE
		083	0.17	24.70	4.39
IV	TSH	0.002	0.00	-0.04	0.07
	T ₃	0.19	0.07	2.50	1.84
	T ₄	0.006	0.02	-0.64	0.39

Table 3.12: Regression analysis of thyroid profile with renal markers in the Overtly hyperthyroid group (OH).

Model		Unstandardized coefficients			
DV		S.Creatinne		S.Urea	
CONSTANT		B	SE	B	SE
		1.13	0.43	4.34	9.00
IV	TSH	1.67	0.95	8.83	19.78
	T ₃	-0.004	0.13	-3.91	2.77
	T ₄	-0.01	0.03	-0.02	0.56

Table 3.13: Regression analysis of thyroid profile with renal markers in the sub clinically hyperthyroid group (SH).

Model		Unstandardized coefficients			
DV		S.Creatinne		S.Urea	
Constant		B	SE	B	SE
		0.63	0.66	44.97	7.47
IV	TSH	2.58	1.48	0.75	16.70
	T₃	0.13	0.30	-4.66	3.33
	T₄	0.04	0.06	-0.41	0.73

3.3.1.3 Regression Analysis of Liver Markers

Result of regression analysis between the thyroid profile markers (independent variable) and liver markers (dependent variable) for the 5 study groups are shown in table 3.14-3.18. It was observed that both SGPT and SGOT are positively related with TSH, T₃ and negatively related with T₄ in N group. SGPT show positive relation with TSH and T₃ in Oh group and negative with T₄, while it was negatively related with all thyroid markers. Similarly in Sh group SGPT showed positive relation with TSH and T₄ and negative with T₃. In case of SGOT, it was found to be negatively related with all thyroid profile markers in N Oh and Sh group. Both SGPT and SGOT are positively related with TSH in OH and SH groups. SGPT show positive relation with T₃ in OH group and positive in SH group. SGOT was a negatively related in OH and SH group. It show positive relation with T₄ in OH and negatively related in SH group.

Table 3.14: Regression analysis of thyroid profile with liver markers in the Normal group (N)

Model		Unstandardized coefficients			
DV		SGPT		SGOT	
Constant		B	SE	B	SE
		30.16	23.11	28.56	13.33
IV	TSH	4.01	2.55	4.80	1.47
	T ₃	10.43	9.24	3.97	5.33
	T ₄	-3.04	2.08	-1.82	1.20

Table 3.15: Regression analysis of thyroid profile with liver markers in the Overtly hypothyroid group (Oh)

Model		Unstandardized coefficients			
DV		SGPT		SGOT	
Constant		B	SE	B	SE
		25.28	4.00	27.00	4.38
IV	TSH	0.01	0.06	-0.05	0.07
	T ₃	0.62	1.87	-1.16	2.06
	T ₄	-0.38	0.66	-0.23	0.72

Table 3.16: Regression analysis of thyroid profile with liver markers in the Sub clinically hypothyroid group (Sh)

Model		Unstandardized coefficients			
DV		SGPT		SGOT	
Constant		B	SE	B	SE
		17.03	2.83	22.30	5.01
IV	TSH	0.01	0.05	-0.08	0.08
	T ₃	-1.11	1.18	-1.77	2.10
	T ₄	0.18	0.25	-0.13	0.45

Table 3.17. Regression analysis of thyroid profile with liver markers in the Overtly hyperthyroid group (OH).

Model		Unstandardized coefficients			
DV		SGPT		SGOT	
Constant		B	SE	B	SE
		10.18	15.41	23.10	13.97
IV	TSH	34.5	33.86	30.53	30.69
	T ₃	3.88	4.74	-3.62	4.3
	T ₄	0.66	0.95	0.92	0.86

Table 3.18: Regression analysis of thyroid profile with liver markers in the Sub clinically hyperthyroid group (SH).

Model		Unstandardized coefficients			
DV		SGPT		SGOT	
Constant		B	SE	B	SE
		16.00	10.10	51.36	15.3
IV	TSH	28.4	18.60	38.81	28.17
	T ₃	3.55	6.11	-16.16	9.25
	T ₄	0.33	1.12	-0.18	1.69

3.3.1.4 Regression Analysis of Bone Formation Markers

Regression analysis of thyroid profile with bone markers in the study groups are carried out and the results are shown in table 3.18 to 3.22. Serum calcium was observed to be negatively related with TSH in N, OH and SH while positively in Oh and Sh groups. Serum calcium was positively related with T₃ in all groups except OH where it was negatively related. With T₄ it was negatively related in Sh and SH groups and positively related in the rest of the groups.

Serum alkaline phosphatase had negative relationship with TSH in Sh and SH and positive in the other groups. It was negatively related with T₃ except in Sh group. With T₄ it was positively related in Oh and SH and negative in the rest of the groups.

Serum Zinc was positively related with TSH in all the groups. With T₃ it was negatively related in N and Sh and positive in the rest of the groups. It was negatively related with T₄ in SH, Sh and positively related in the other groups.

Table 3.19: Regression analysis of thyroid profile with bone markers in the Normal group (N).

Model		Unstandardized coefficients					
DV		S.Ca		Alk.p		S.Zn	
Constant		B	SE	B	SE	B	SE
		8.64	0.74	236.04	53.91	0.32	0.11
IV	TSH	-0.06	0.08	3.53	5.94	0.01	0.01
	T ₃	0.02	0.30	-29.91	21.56	-0.02	0.04
	T ₄	0.08	0.07	-3.86	4.86	0.02	0.01

Table 3.20: Regression analysis of thyroid profile with bone markers in the Overtly hypothyroid group (Oh).

Model		Unstandardized coefficients					
DV		S.Ca		Alk.p		S.Zn	
Constant		B	SE	B	SE	B	SE
		8.16	0.34	132.91	18.55	0.38	0.06
IV	TSH	0.02	0.01	0.01	0.29	0.001	0.001
	T ₃	0.25	0.16	-10.68	8.71	0.04	0.03
	T ₄	0.04	0.06	3.01	3.05	0.00	0.001

Table 3.21: Regression analysis of thyroid profile with bone markers in the sub clinically hypothyroid group (Sh).

Model		Unstandardized coefficients					
DV		S.Ca		Alk.p		S.Zn	
Constants		B	SE	B	SE	B	SE
		8.45	0.37	133.32	33.14	0.51	0.08
IV	TSH	0.01	0.01	-0.29	0.54	0.00	0.001
	T ₃	0.39	0.16	2.42	13.92	-0.004	0.03
	T ₄	-0.04	0.03	-0.46	2.95	-0.01	0.01

Table3.22: Regression analysis of thyroid profile with bone markers in the Overtly hyper thyroid group (OH).

Model		Unstandardized coefficients					
DV		S.Ca		Alk.p		S.Zn	
Constant		B	SE	B	SE	B	SE
		10.16	0.58	198.33	34.86	0.49	0.08
IV	TSH	2.08	1.26	81.48	76.60	0.19	0.17
	T ₃	-0.38	0.18	-7.39	10.73	0.03	0.02
	T ₄	0.02	0.04	-4.16	2.15	0.001	0.01

Table 3.23: Regression analysis of thyroid profile with bone markers in the sub clinically hyperthyroid group (SH).

Model		Unstandardized coefficients					
DV		S.Ca		Alk.p		S.Zn	
Constant		B	SE	B	SE	B	SE
		10.23	0.60	134.12	29.71	0.45	0.09
IV	TSH	-1.26	1.10	-14.68	54.74	0.11	0.17
	T ₃	0.39	0.36	-19.60	17.93	0.12	0.06
	T ₄	-0.13	0.07	5.29	3.29	-0.01	0.01

3.3.2 Correlation Analysis

Correlation analysis is a statistical method for the verification of a relationship among two variables and is expressed in term of coefficient of correlation(r). Its values vary from -1 to 0 to +1. A positive relation means a direct dependence and negative mean an inverse relation [120].

3.3.2.1 Correlation Analysis of BMI and Thyroid Markers

The Pearson's correlations of biochemical markers of thyroid function with BMI of the study groups are given in table 3.24.

BMI was strongly positively correlated with TSH in Oh group (p=0.003) and has significant negative correlation with T₄ in N (p=0.01) and OH (p=0.01). No significant correlation was observed between T₃ and BMI in any group.

3.3.2.2 Correlation Analysis of Renal and Thyroid Markers

The Pearson's correlations of biochemical markers of renal function with the different thyroid profile markers were evaluated. The results are shown in table 3.25-3.27. A very significant positive correlation was found between Serum creatinine and T₃ in the Sh group ($p= 0.005$), while significant negative correlation of serum creatinine with T₃ in the N group was also found ($p= 0.05$). Urea was found to be significantly negatively correlated with TSH in Sh group ($p=0.05$) while Serum creatinine showed a significant positive correlation with TSH in the OH group ($p= 0.05$). No significant correlation with T₄ was found either for creatinine or Urea in any of the hyperthyroid groups (OH & SH group).

Table 3.24: Correlation analysis of thyroid profile markers with BMI in the study groups

Parameter	Group ID	Thyroid profile markers					
		TSH		T ₃		T ₄	
		r	p	r	p	r	p
BMI	N	0.16	0.25	-0.12	0.40	0.37**	0.01
	Oh	0.42**	0.003	-0.18	0.22	-0.22	0.13
	Sh	0.07	0.62	0.07	0.63	-0.17	0.24
	OH	0.22	0.10	-0.04	0.78	0.35**	0.01
	SH	-0.25	0.05	0.08	0.56	-0.04	0.74

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table3. 25: Correlation analysis of renal markers with TSH in the study groups.

Parameter	Group ID	Biochemical markers of renal function			
		S.Creatinne		S.Urea	
		r	p	r	p
TSH	N	0.19	0.17	0.004	0.98
	Oh	0.06	0.67	0.02	0.90
	Sh	-0.13	0.4	-0.01**	0.05
	OH	0.25*	0.05	0.11	0.41
	SH	0.23	0.08	0.001	0.10

***. Correlation is significant at the 0.05 level (2-tailed).**

****. Correlation is significant at the 0.01 level (2-tailed).**

Table 3.26: Correlation analysis of renal markers with T₃ in the study groups.

Parameter	Group ID	Biochemical markers of renal function			
		S.Creatinne		S.Urea	
		r	p	r	p
T₃	N	-0.27 [*]	0.05	0.004	0.98
	Oh	0.07	0.65	-0.21	0.15
	Sh	0.400 ^{**}	0.005	0.16	0.22
	OH	-0.08	0.55	-0.22	0.91
	SH	0.10	0.45	-0.23	0.09

***. Correlation is significant at the 0.05 level (2-tailed)**

****.** Correlation is significant at the 0.01 level (2-tailed).

Table 3.27: Correlation analysis of renal markers with T₄ in the study groups.

Parameter	Group ID	Biochemical markers of renal function			
T₄		S.Creatinne		S.Urea	
		r	p	r	p
	N	0.13	0.33	-0.16	0.26
	Oh	0.19	0.19	0.07	0.63
	Sh	0.18	0.21	-0.12	0.40S
	OH	-0.11	0.44	-0.10	0.44
	SH	0.09	0.51	-0.15	0.25

***. Correlation is significant at the 0.05 level (2-tailed).**

****. Correlation is significant at the 0.01 level (2-tailed).**

3.3.2.3 Correlation Analysis of Liver Markers with Thyroid Profile Markers

The results of correlation analysis of liver markers with thyroid profile markers are presented in table 3.28- 3.30. SGPT was found to be positively correlated with TSH in the entire study groups, but the correlation was insignificant. A very significant positive correlation was found between SGOT and TSH in the N group ($p = 0.002$), while SGOT was insignificantly negatively correlated with TSH in the Oh and Sh groups.

SGPT was positively correlated T₃ in N, Oh and negatively in Sh groups to the insignificant extent. Similarly SGOT was positively correlated with T₃ in N and

negatively correlated with Oh and Sh group. SGPT showed insignificant correlation with T₄ in N and Oh group and

Positive in Sh. SGOT showed a non-significant negative correlation with T₄ in all the hypothyroid groups.

SGPT showed negative correlation with T₃ and T₄ and positive correlation in OH and SH group. All these correlations were found to be non-significant. SGOT was insignificantly negatively correlated with TSH in the OH and positive within SH group.

SGOT was negatively correlated in non-significant way with T₃ in OH and SH group. Similarly SGOT was negatively correlated with T₄ in SH group and positively correlated with OH group. BMI was found to be very significantly correlated with T₄ in N and OH group ($p = 0.01$).

Table 3.28: Correlation analysis of Liver markers with TSH in the study groups.

Parameter	Group ID	Biochemical markers of liver function			
		SGPT		SGOT	
		r	p	r	p
TSH	N	0.20	0.16	0.41**	0.002
	Oh	0.04	0.80	-0.09	0.57
	Sh	0.05	0.71	-0.64	0.67
	OH	0.09	0.49	-0.64	0.67
	SH	0.06	0.63	0.13	0.32

***. Correlation is significant at the 0.05 level (2-tailed).**

****. Correlation is significant at the 0.01 level (2-tailed).**

Table 3.29: Correlation analysis of liver markers with T₃ in the study groups.

Parameter	Group ID	Biochemical markers of liver function			
		SGPT		SGOT	
		r	p	r	p
T ₃	N	0.27	0.05	0.06	0.68
	Oh	0.03	0.87	-0.08	0.57
	Sh	-0.13	0.40	-0.08	0.60
	OH	0.13	0.33	-0.09	0.50
	SH	0.65	0.63	-0.6	0.66

*

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed)

Table 3.30. Correlation analysis of liver markers with T₄ in the study groups.

Parameter	Group ID	Biochemical markers of live function			
T₄		SGPT		SGOT	
		r	p	r	p
	N	-0.18	0.18	-0.20	0.15
	Oh	-0.07	0.56	-0.04	0.81
	Sh	0.04	0.77	-0.03	0.85
	OH	0.12	0.36	0.08	0.56
	SH	0.01	0.94	-0.01	0.92

***. Correlation is significant at the 0.05 level (2-tailed).**

****. Correlation is significant at the 0.01 level (2-tailed).**

3.3.2.4 : Correlation Analysis of Bone Formation Markers with Thyroid Markers

Correlation analysis of thyroid profile markers and bone formation markers are presented in table 3.31 to 3.33. Serum Calcium showed significant positive correlation with TSH in Oh (p=0.01) and OH groups (p=0.04). Serum Calcium was significantly negatively correlated with T₃ in OH (p=0.01). No significant correlation was found between serum Calcium and T₄ in any group.

Serum alkaline phosphatase was significantly negatively related with T₄ in OH (p=0.01). No significant correlation was found for it in the other study groups. Serum Zn showed a very significant positive correlation with T₃ in SH (p=0.02), while no significant correlation was found for it with other thyroid profile markers in the other study groups.

Table 3. 31: Correlation analysis of bone markers with TSH in the study groups.

Parameter	Group ID	Bone markers					
		S.Ca		ALP		S.Zn	
TSH		r	p	r	p	r	p
	N	-0.13	0.36	0.15	0.28	0.09	0.51
	Oh	0.34*	0.01	0.02	0.88	-0.17	0.24
	Sh	0.133	0.39	-0.01	0.50	-0.02	0.92
	OH	0.269*	0.04	0.22	0.11	0.10	0.46
	SH	-0.21	0.11	-0.11	0.40	0.14	0.33

ALP: Alkaline Phosphatase, S.Ca: Serum Calcium, S. Zn: Serum Zinc

***. Correlation is significant at the 0.05 level (2-tailed).**

****. Correlation is significant at the 0.01 level (2-tailed)**

Table 3. 32: Correlation analysis of bone markers with T₃ in the study groups.

Parameter	Group ID	Bone markers					
T₃		S.Ca		ALP		S.Zn	
		r	p	r	p	r	p
	N	0.07	0.60	-0.24	0.07	-0.40	0.80
	Oh	0.19	0.21	-0.15	0.30	0.08	0.58
	Sh	0.21	0.15	0.07	0.64	0.21	0.15
	OH	-0.33*	0.01	-0.24	0.07	0.18	0.18
	SH	-0.01	0.96	0.03	0.80	0.32*	0.02

***. Correlation is significant at the 0.05 level (2-tailed).**

****. Correlation is significant at the 0.01 level (2-tailed).**

Table 3.33: Correlation analysis of bone markers with T₄ in the study groups.

Parameter	Group ID	Bone markers					
		S.Ca		ALP		S.Zn	
		r	p	r	p	r	p
T₄	N	0.17	0.22	-0.15	0.27	0.21	0.13
	Oh	0.01	0.97	0.01	0.53	0.11	0.47
	Sh	-0.12	0.41	0.03	0.84	0.14	0.34
	OH	-0.11	0.43	-0.34**	0.01	0.08	0.53
	SH	-0.15	0.25	0.19	0.16	0.08	0.56

***. Correlation is significant at the 0.05 level (2-tailed).**

****.** Correlation is significant at the 0.01 level (2-tailed).

3.4: Correlation analysis of thyroid hormones

Table 3.34 show the correlation analysis of T₃ & T₄ with TSH in the study groups. Very significant negative correlation was observed between TSH & T₄ in Oh (p=0.02) & Sh group (p=0.00). Similarly TSH showed significant negative correlation with T₃ in N (p=0.03) & Sh groups (p=0.00).

3.5 THE EFFECT OF THYROID PROFILE CHANGE ON BMI, RENAL AND LIVER HEALTH IN THE STUDY GROUP

Table 3.35 and 3.38 show the relative effect of thyroid profile change on BMI, renal and liver and bone formation markers. Maximum effect (19.7%) was observed on BMI of Oh group and minimum (5.7%) in Sh group. The highest effect on serum creatinine and urea was observed in Sh group (17.2%, 8.6%) and lowest in Oh (5.2%) and in N (2.6%). The effect on SGPT (9%) and SGOT (21%) was significant in N group only. Serum Calcium was more affected in Sh group (20%) and least affected in N group (3%). S.ALP was found more affected in N group (9%) and least in Sh (01%). Highest contribution was found for S. Zn in Oh (16%) and lowest in N group (8%).

Table 3. 34: Contribution of thyroid profile on BMI and Renal markers.

SNO	GP ID	Markers					
		BMI		Creatinine		Urea	
		B	% B	B	% B	B	% B
1	N	0.157	15.7	0.12	12%	0.026	2.6
2	Oh	0.197	19.7	0.056	5.6	0.062	6.2
3	Sh	0.058	5.7	0.172	17.2	0.086	8.6
4	OH	0.163	16.3	0.07	07	0.054	5.4
5	SH	0.083	8.3	0.068	6.8	0.054	5.4

B: Contribution

Table 3. 35. Contribution of thyroid profile on liver markers.

SNO	GP ID	SGPT		SGOT	
		B	% B	B	% B
1	N	0.09	9	0.21	21
2	Oh	0.01	01	0.02	02
3	Sh	0.03	03	0.02	02
4	OH	0.04	04	0.04	04
5	SH	0.05	05	0.05	05

Table 3.36: Contribution of thyroid profile on bone formation markers.

S no	GP ID	Markers					
		S.Ca		S.ALKP		S.Zn	
		B	% B	B	% B	B	% B
1	N	0.03	3	0.09	9	0.08	8
2	Oh	0.05	5	0.06	6	0.16	16
3	Sh	0.2	20	0.01	01	0.11	11
4	OH	0.05	5	0.07	7	0.14	14
5	SH	0.05	5	0.06	6	0.11	11

DISCUSSION

Diseases of the thyroid gland are one of the most common disorders of the endocrine glands in the world, especially in Asia [121, 122]. Thyroidal dysfunctions are more prevalent in females and its adverse effects in pregnant females are well documented. Hypothyroidism is 10 times more common in women than in men [123]. Hypothyroidism has been found to occur in around 2.5% of otherwise normal pregnancies [124]. In pregnant woman hypothyroidism is associated with anemia, postpartum hemorrhage, preeclampsia, spontaneous abortion and fetal mortality. The prevalence rate of hyperthyroidism in women is found to be between 0.5 and 2%, and its frequency is 10 times higher in female than in male [125]. It is found in 0.2% of all pregnancies.

The purpose of this cross sectional study was to generate a data about the prevalence of thyroidal dysfunction in Non-Pregnant Women (NPW) in Khyber Pakhtunkhawa Pakistan and also to study the effects of these clinical conditions on their bone, renal and liver health in the selective group of general population. The study groups comprise 259 NPW in the age group of 18-75 years. These subjects were subdivided into five sub groups based on the thyroid profile tests as N, Oh, Sh, OH and SH respectively.

Thyroidal dysfunctions have adverse effects on renal physiology. It has been reported that hypothyroidism decreases and hyperthyroidism increases the kidney-to-body weight ratio [126]. Also children with Congenital Hypothyroidism (CH) have a high prevalence of congenital kidney abnormalities [127]. Kidneys also play an important role in the metabolism and removal of thyroid hormone from the body [128]. Elevated values for creatinine are also seen in hyperthyroidism [129].

Kidney and thyroid function and dysfunction are interrelated through complex mechanisms. Thyroid dysfunction causes remarkable changes in glomerular and

tubular functions and electrolyte and water homeostasis. Excessive levels of thyroid hormone (TH) level result in an increase in Glomerular Filtration Rate (GFR) and Renal Plasma Flow (RPF). The association of different types of glomerulopathies with both hyper- and hypo functions of the thyroid has been reported. Nephrotic Syndrome (NS) is accompanied by changes in the concentrations of TH due to loss of protein in the urine. Some authors have reported associations between thyroid cancer and kidney tumors and each of these organs can develop metastases into the other. Data from recent research show that TH, especially T_3 , can be considered as a marker for survival in patients with kidney disease.

In the present cross sectional hospital based study we evaluated the biochemical markers of renal function in Normal(N), Non-Pregnant Women (NPW) and compared it with hypothyroid (Oh & Sh group) and hyperthyroid (OH & SH group) Non-Pregnant Women. In our study we found a minor decrease in the serum creatinin level in the Oh (1.10mg/dl) and Sh group (1.18 mg/dl) as compared to N group(1.47mg/ dl).This is in agreement with the notion that serum creatinine virtually remain unchanged due a balance created between the decrease in renal clearance and decrease generation [130]. Some studies have reported the elevation of serum creatinine in the hypothyroid subjects but it includes all the sexes as against our study groups which includes only NPW ¹³¹. Serum urea was found slightly decreased in the Sh group (23.22 mg/dl) as compared to N group (32.60 mg/dl) and Oh group (30.11mg/dl). This minor decrease was insignificant and was in line with the other studies in which no significant effects were observed on serum urea levels in hypothyroid subject [132]. Hyperuricemia in hypothyroid subjects has been reported in a few studies done around the world [133, 134]. A few surveys report the effect of hypothyroidism on Serum creatinine and Serum Urea levels, literature search did not show any formal studies reporting such correlations between Serum creatinine, urea and hypothyroidism. Some studies have reported negative correlation

of creatinine with T_3 and T_4 and positive correlation with TSH [135]. In our study Serum creatinine showed a very significant positive correlation with T_3 in the Sh group ($p= 0.005$). Serum creatinine showed a significant negative correlation with T_3 in the N group ($p= 0.05$). Urea was significantly negatively correlated with TSH in Sh group ($p= 0.05$).

Mean serum creatinine was found to be highest in N group as compared to OH and SH group. Serum Urea level was found higher in OH and SH as compared to N group. Other similar studies have reported that hyperthyroidism or thyrotoxicosis results in an increase of GFR and RPF and decrease of serum creatinine levels [136]. These changes are supposed to be normalized after appropriate treatment [137]. The decrease in serum creatinine has also been reported in subclinical hyperthyroidism¹³⁸. The different types of kidney diseases can be associated with various disorders of thyroid function. We found in our study that BMI was positively related with TSH and negatively related with T_3 and T_4 in N group. BMI showed negative relation with T_4 in OH and SH groups. Serum creatinine showed positive relation with TSH, in all the study groups, negative relation with T_3 in the N group and OH and positive in SH group. Serum creatinine showed a significant positive correlation with TSH in the OH group ($p= 0.05$). Serum creatinine showed a significant negative correlation with T_3 in the N group ($p= 0.05$). No significant correlation with T_4 was found either for creatinine or Urea in any of the study groups [139].

Thyroid hormones were also observed to have significant effects on the physiology of the liver. There are reports that hypothyroidism may affect the liver anatomy and physiology. Hypothyroidism is linked in a few cases with cholestatic jaundice [140]. In one study in hyperthyroidism, the level of Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) was found to be 27% and 37% higher than normal level, despite the fact that these patients were showing no sign of liver disease [141]. The data presented here show the effect of biochemical markers of thyroid functions

on liver functions. It shows how the change in the serum level of one marker will affect the level of other markers in the study groups.

In our study significance differences were observed in the SGPT and SGOT level in all the study groups but the level of these enzymes were found lower in the Oh and Sh as compared to N group. These findings are in contradiction of earlier studies in which elevation of these markers were reported [142]. Earlier studies also report that hypothyroidism may directly affect liver.

A few studies have also reported cholestatic jaundice in the hypothyroid group due to the decrease in the activity of UDP-glucuronyl transferase resulting in the decrease of bilirubin excretion. Some studies have shown that the liver abnormalities associated with hypothyroidism are mostly reversible and can be corrected easily in a few weeks with Thyroxin Replacement Therapy (TRT), with no apparent hepatic damage [143]. In this study a f very significant positive correlation was observed between SGOT and TSH in the N group ($p = 0.002$). SGOT was non-significantly negatively correlated with TSH in the Oh and Sh groups. SGOT showed a non-significant negative correlation with T_4 in all the study groups. No significant correlation was found between SGPT and thyroid profile markers (TSH, T_3 and T_4) in any of the study groups. Similar results have been observed in a retrospective study of 10,292 outpatients where similar correlations were seen with Gamma Glutamyl Transferase and ALT [144]. SGPT level was found higher while SGOT level was depressed in OH group as compared to N group. These findings are in good agreement with earlier studies in which elevation of these markers was reported [145].

A very significant positive correlation between BMI and T_3 in N group and OH group was observed ($p = 0.01$). No significant correlation was found between liver enzyme and thyroid profile markers in any of the study groups.

The findings of our study are important in understanding the intricate interactions between the thyroid gland and liver. Further it provides a multisystem approach to treat patients with diseases affecting either thyroid or liver.

Thyroid function disorders affect bone metabolism [146, 147] and are the main causes of secondary osteoporosis [148]. Biochemical bone markers show important changes in bone mineral metabolism in various thyroid function disorders. Hypothyroidism decreases rate of bone formation [149]. Adult patients tend to show higher bone density. The serum level of phosphorus, calcium and alkaline phosphatase (ALP) is either normal or low [150].

In most population based studies, it has been found that both over activity (hyperthyroidism) and under activity (hypothyroidism) of thyroid gland are associated with high risk of bone fractures.

In the present study an attempt has been made to assess the changes in bone formation markers in various thyroid functional disorders. The markers included in our study were simple and can be easily analyzed in routine clinical laboratory except Zn, which is measured by atomic absorption. The study group comprises of a total of 259 Non pregnant women (NPW). These were subdivided into five groups based on their thyroid profile status as N, Oh, Sh, OH, and SH respectively.

Mean Serum Calcium was highest in OH group (9.80 ± 0.90 mg/dl) and lowest in Oh group (8.95 ± 0.10 mg/ dl as compared to N group (9.20 ± 0.75 mg/dl). Serum Calcium was positively related with T_3 in all groups except OH group where it was negatively related. Serum Calcium showed significant positive correlation with TSH in Oh ($p=0.01$) and OH ($p=0.04$). Serum Calcium was significantly negatively correlated with T_3 in OH ($p=0.01$). No significant correlation was observed between serum Calcium and T_4 in any group. These results are similar to the earlier reports [151-153]

in which a decrease in serum level of Calcium has been reported in hyperthyroid subjects.

Elevated level of thyroid hormones, in hyperthyroid subjects, accelerate the rate of bone desorption and consequently bone loss occurs which is observed as high serum calcium values in hyperthyroid patients.

In hypothyroidism, serum level of Calcium is low due to slow mineral metabolism, high urinary excretion of Calcium and decreased absorption of Calcium from the gut [154]. This explains the low calcium level in hypothyroidism.

Thyroid hormones are believed to stimulate both osteoclast and osteoblast; stimulation of the first one appears to be indirect. The exact mechanism by which TH increases the activity of osteoclast is not yet unknown.

In hypothyroidism a very low level of TH (especially T_3) in serum suppress the activation of osteoclasts. This results in the decrease of bone turnover process. Subsequently the rate of bone loss is decreased thereby serum level of Total Ca as well as Ionized Ca is reduced. In hyperthyroidism, all the processes which occurs in hypothyroidism, take place in opposite or reverse fashion.

Total ALP level in serum is not a good biomarker of deranged bone metabolism but give a gross idea because of its low specificity. The HL-ALP has high specificity as it originates from osteoblasts in bone. Therefore, serum levels of this isoenzyme fraction reflect osteoblastic activity. Bone specific ALP is a superior marker than osteocalcein. It has long half-life, relatively unaffected by diurnal changes, has least biological, individual &/or analytical variations, in addition to being simple and economical investigation compared to other markers [155]. Serum total alkaline phosphatase was found to be higher in N group (159 ± 7.61 U/l) and OH group (144 ± 7.12 U/l) and lower in Sh group (128 ± 6.42 U/l).

The changes in ALP levels in thyroid disorders are not due to altered liver function. Since LFTs such as, ALT, AST were found in our study not much different from the controls (N) [156]. This confirms that the changes in ALP are not due to abnormality in liver function. It is more likely due to altered bone metabolism. Serum levels of the HL-ALP reflect osteoblastic activity. Elevated levels in hyperthyroidism imply increased osteoblastic activity and thus increased bone desorption and formation. This indicates high degree of bone turnover or remodeling activity. These observations are consistent with other similar studies [157]. No significant difference was found in the Serum Zinc level of all the diseased groups. Serum Zinc was positive with TSH in all the groups. With T₃ it was negative in N and Sh and positive in the rest of the groups, it was negatively related with T₄ in SH and positive in the other groups.

Serum Zn showed a very significant positive correlation with T₃ in SH ($p=0.02$). No significant correlation was found for it with other thyroid profile markers in the other study groups. It is reported that most markers of bone turnover are decreased in patients with hypothyroidism [158, 159]. In the present study it was observed that serum total Ca levels were lower in hypothyroid than hyperthyroid patients, which indicate that bone desorption was accelerated much more in hyperthyroid patients and had direct relationship with thyroid hormone excess state.

CONCLUSIONS

Thyroidal dysfunction is a common problem in northern Pakistan affecting women folks more as compared to other gender due to poverty, lack of education, social taboos and lack of health facilities in rural areas. More over there is a lack of adequate amount of iodine in drinking water and iodized salt due to lack of strict quality control. There is a complex interaction between thyroid kidney, liver and bone turnover markers. It was concluded from this study that that bones desorption occurs at higher rate in hyperthyroid patients and Bone turnover is increased in favor of desorption. Opposite changes are observed in hypothyroid women. Also Hyperthyroidism may affect renal function in the long run if left untreated, while Hypothyroidism may not affect renal functions in non-pregnant women.

It was also concluded from this study that SGOT is directly affected by the change in the serum level of TSH in the normal individual (N). Serum T_3 directly affected BMI in N and OH groups. It was also found that hypothyroidism may decrease while Hyperthyroidism may increase the rate of synthesis of liver enzymes. Due to this complex inter play between these vital organs of the body and thyroid hormone, multi system approach should be adopted in the diagnosis of clinical conditions affecting either organs.

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