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Original article:

Influence of hypothyroidism on biochemical markers of liver function test: a cross sectional study

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Abstract:

Introduction: Normal level of thyroid hormone is important for normal hepatic function as it maintain the metabolism of bilirubin by playing a role in the enzymatic activity of glucuronyltransferase and by regulating the level of ligandin. The liver in turn glucuronidates and sulphates the thyroid hormone, excretes into bile and regulates their systemic endocrine effects. Therefore hepatic dysfunction is commonly observed in patients with thyroid disease. Aim was to determine the biochemical markers of Liver Function Test (LFT) in patients with hypothyroidism and their possible correlation with thyroid profile.

Methods: Thyroid profile and liver function test (LFT) were evaluated in 40 patients with subclinical hypothyroidism **(TSH 6.0-9.9mIU/L)**, 40 patients with overt hypothyroidism **(TSH ≥10.0 mIU/L)** between 20-50 years of age and were compared with 40 age matched normal euthyroid controls after applying exclusion criteria. Thyroid profile and LFT were estimated using fully autoanalyser VITROS 5600 considering p value **<0.05** as significant.

Results and observations: Subjects with both subclinical hypothyroidism and overt hypothyroidism had significantly raised serum AST, ALT, ALP **(P<0.0001)** and total protein levels **(P<0.01)** compared to controls. Further, TSH showed significant positive correlation with AST, ALT and ALP (P<0.05) in both subclinical and overt hypothyroidism whereas FT3 and FT4 had a significant negative correlation with AST, ALT and ALP **(P<0.05)** in overt hypothyroidism.

Conclusion: It might be necessary to monitor liver enzymes frequently in hypothyroid patients as declining liver function may be missed by single assessment and deranged biochemical parameters of LFT might indicate underlying altered thyroid status.

Key words- LFT, FT3, FT4, TSH

Introduction:

The thyroid gland synthesizes and releases triiodothyronine (T3) and thyroxine (T4), which represent the only iodine containing hormones in the vertebrates. T3 is the biologically active thyroid hormone[(1)] .The major secretory product of the thyroid is a prohormone (T4), which is activated in peripheral tissues by outer ring deiodination to T3. There are three homologous iodothyronine deiodinases which catalyses these reactions [(2),(3)] . Type I deiodinase is located in liver, kidney, and thyroid. In addition to that, the liver has an important role in thyroid hormone transport and metabolism. These hormones are required for the normal growth, development and function of nearly all tissues, with major effects on oxygen consumption and metabolic rate [(4)]. Thyroid hormone synthesis and secretion is regulated by a negative feedback system that involves the hypothalamus, pituitary, and the thyroid gland [(5)].

The free, unbound component of thyroid hormone within plasma is in equilibrium with the protein-bound hormone and accounts for it's biological activities. Though tissues are exposed to the same plasma concentrations of free T4 and T3 free hormone, their concentrations in different tissues vary according to the transport and deiodinase activity within specific tissues[6] . Thyroid hormones regulate the basal metabolic rate of all cells including hepatocytes. The liver in turn metabolizes the thyroid hormones and regulates their systemic endocrine effects[7] . Thyroid hormones are glucuronidated and sulphated within the liver and subsequently excreted into bile; in addition, these hormones maintain the metabolism of bilirubin by playing a role in the enzymatic activity of glucuronyltransferase and by regulating the level of ligandin, a major organic anion-binding protein[8] .In fact, there are several clinical and laboratory associations between thyroid and liver diseases namely-

(i) Liver damage secondary to the systemic effect of thyroid hormone excess or direct toxic effects and subclinical physiological effects of thyroid hormone on liver functions.

(ii) Some patients with chronic liver diseases may have thyroiditis, hyperthyroidism or hypothyroidism through autoimmune mechanisms.

(iii) Alterations of thyroid hormone metabolism or tests secondary to liver disease, and

(iv) Liver or thyroid disorders related to the therapy of thyroid or liver disease.

Therefore, it is not surprising that hepatic dysfunction is commonly observed in patients with thyroid disease. Thus, the interpretation of thyroid function tests in patients with liver disease as well as the interpretation of liver bio-chemical tests in patients with thyroid disease must take these facts into account if errors in patients care are to be avoided[9-12].

The thyroid frequently is a common target of disease or dysfunction[13] .Thyroid disorders are commonly separated into two major categories, hyperthyroidism (caused by an overactive thyroid gland) and hypothyroidism (due to a poorly functioning thyroid gland), depending on whether serum thyroid hormone levels (T4 and T3) are increased or decreased, respectively. Both hypothyroidism and hyperthyroidism have potentially fatal systemic manifestations [14].

Thyroid hormones are essential for normal organ growth, development, function and regulation of the basal metabolic rate of all cells and therefore, its alteration can affects the entire metabolism [15]**.** Most affected organs include liver and heart. So, it alters the liver enzymes like ALP, AST, ALT, GGT and cardiac enzymes like CPK, LDH and AST [16, 17, 18]. ALT may also be elevated occasionally and cholesterol elevation is as a rule due to hypometabolism. The later may result in fatty liver causing mild but prolonged AST and/or ALT elevation, and therefore, be erroneously considered chronic hepatitis, particularly before the advent of hepatitis C virus asseys. These biochemical changes, usually mild, are also reversible with adequate thyroid replacement therapy[19].

There is also evidence that hypothyroidism may directly affect the liver structure or function. Hypothyroidism has been associated in a few case reports with cholestatic jaundice attributed to reduced bilirubin and bile excretion. In experimental hypothyroidism, the activity of bilirubin UDP-glucuronyltransferase is decreased, resulting in a reduction in bilirubin excretion[20]. The reduction in bile flow may be in part due to an increase in membrane cholesterol-phospholipid ratio and diminished membrane fluidity[20] which may affect a number of canalicular membrane

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transporters and enzymes, including the Na^+-K^+-ATPase.

The triad of reduced bilirubin excretion, hypercholesterolaemia and hypotonia of the gall bladder seen in hypothyroidism increases the incidence of gallstones[21]. Recent studies have shown that the hepatic abnormalities associated with hypothyroidism can be reversible over a matter of weeks with thyroxine replacement, with no residual liver damage[22,23] . So, it is evident that thyroid dysfunction may affect liver function and liver disease modulates thyroid hormone metabolism, and a variety of systemic diseases affect both organs.

Aims and objectives:

Present study was done to evaluate the biochemical parameters of thyroid function test (FT3, FT4, TSH) and liver function test among known hypothyroid patient and healthy control as well as to find any possible correlation among the measured parameters under study.

Materials and methods:

An analytical cross-sectional study was conducted on subjects of age group between 20-50 yrs attending out patient department (OPD) of Gauhati Medical College & Hospital after taking approval from institutional ethics committee. Total no of 40 patients with diagnosed subclinical hypothyroidism and 40 patients with diagnosed overt hypothyroidism each, coming for thyroid function test were enrolled in the study and compared with 40 age matched normal euthyroid controls. Informed consent duly signed by each of the participants was taken. Thyroid profile tests (FT3, FT4 and TSH) were estimated to categorize subclinical hypothyroidism and overt hypothyroidism. Subjects were divided into two groups, test group1 and test group 2.

1) **Test group 1-** consists of 40 patients with subclinical hypothyroidism **(TSH 6.0-9.9 mIU/L)**
2) **Test group 2-** consists of 40 patients with overt hypothyroidism **(TSH ≥10.0 mIU/L)**

Biochemical parameters of liver function test (total bilirubin, AST, ALT, ALP, total protein, albumin) were estimated in subclinical hypothyroids, overt hypothyroids and in healthy controls. Patients with history of diabetes mellitus, renal disorders, active infection or a recent infection, liver disease, bone and muscle disease, cardiac disease, pancreatic disease, hypertension, malignancy, taking oral contraceptive pills (OCP), pregnancy, alcoholics, and drug abusers were excluded from the study group.

Taking aseptic and antiseptic precautions, about 5 ml of fasting blood was collected by venous puncture and transferred to appropriate sterile vial. Serum obtained after centrifugation was separated into two aliquots- one for estimation of Liver Function test and other for estimation of thyroid function test and were analysed immediately after separation using fully automated analyzer (VITROS-5600). FT3, FT4, TSH were estimated by Chemiluminescence method[24], total bilirubin (TBil) was estimated by Jendrassik-Grof method[25], aspertate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were estimated using IFCC recommended methods[26,27,28], total protein(TP) was estimated by biuret method[29] and albumin was estimated by modified bromocresol green method[30].

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Statistical analysis

Data were expressed as **mean ± SD**. ANOVA tests were used to analyze differences in baseline characteristics and biochemical parameters between the control and the test groups. Correlations were observed by using Pearson's correlation coefficient and probability (p value) **< 0.05** was considered significant. Statistical analysis was done using GraphPad InStat version 3.00. All the statistical graphs were prepared using Microsoft Excel 2007.

Observations and results

In the control group, comprising of 40 individuals, the mean±SD of age was **34.2 ±6.68** years. In test group1, comprising of 40 individuals, the mean±SD of age of the subjects was **35.05±7.15** years while in overt hypothyroidism (test group2), the mean±SD of age of the subjects was **33.45 ±4.92** years **(table1, fig 2).**

On comparing the baseline characteristics, we did not find any significant difference in mean level of age **(p=0.726)** and sex ratio **(p=0.648)** between control group and the test groups **(table 3)**. However, mean of FT3, FT4 and TSH levels decreased in both test group1 and test group2 when compared with controls with p value found to be extremely significantly **(p<0.0001) (table 3, fig 4-6)** We also found that the mean of AST, ALT and ALP levels increased in both test group1 and test group2 when compared with healthy controls with p value again found to be extremely significantly **(p<0.0001)(table 3, fig 7-9)**. Similarly, the mean of total protein levels of both test group 1 and test group 2 were found to be increased significantly when compared with controls **(p=0.0011)(table 3, fig 10)**. However, we did not find any significant difference of mean total bilirubin and albumin levels **(p=0.093 and p=0.074**) when both test group 1 and test group 2 were compared with normal healthy controls.

On observing correlation using Pearson's correlation coefficient, we found that TSH levels showed highly significant positive correlation with AST **(p<0.0001),** ALT **(p<0.0001)** and significant positive correlation with ALP levels **(P<0.05)** in subjects with subclinical hypothyroidism **(table 11, fig 13,14)**. TSH levels also showed highly significant positive correlation with AST **(p=0.0002),** ALT **(p<0.0001)** and ALP levels **(P<0.0001)** in subjects with overt hypothyroidism **(table 12, fig 15,16).** Moreover, FT3 and FT4 levels had a significant negative correlation with AST, ALT and ALP levels **(P<0.05)** in overt hypothyroidism **(table 11,12)**.

STATISTICS	CONTROL	TEST GROUP1	TEST GROUP2
N	40	40	40
MEAN	34.2	35.05	33.45
MEDIAN	33.5	35	34
SD	6.68	7.15	4.92
RANGE	20-50	20-50	20-50

Table1: Showing comparison of the statistics of ages in the study groups

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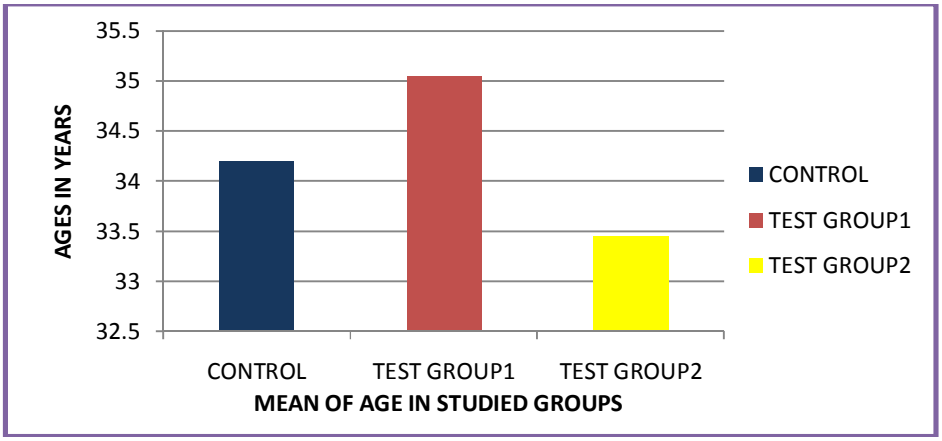
Fig 2: Showing mean of ages in the studied groups



PARAMETERS (NORMAL RANGE)	CONTROL (n=40) Mean± SD	TEST GROUP1 (n=40) Mean± SD	TEST GROUP2 (n=40) Mean± SD	P value
AGE (Years)	34.2±6.68	35.05±7.15	33.45±4.92	0.726
SEX (M/F)	22/18	19/21	18/22	0.648
FT3 (4.26-8.10pmol/L)	5.56±0.756	4.38±0.447	3.75±0.558	<0.0001**
FT4 (10-28.2pmol/L)	14.01±1.805	10.81±0.985	8.91±0.46	<0.0001**
TSH (0.465- 4.68mIU/L)	2.39±1.20	7.72±1.56	20.09±10.24	<0.0001**
T.BILIRUBIN(0.2-1.3mg/dl)	0.68±0.159	0.69±0.177	0.76±0.171	0.093
AST (17-59U/L)	42.58±11.99	60.87±11.62	90.43±24.84	<0.0001**
ALT (21-72U/L)	45.99±11.86	65.92±5.71	90.38±24.01	<0.0001**
ALP (38-126U/L)	95.18±19.27	151.13±34.26	165.13±25.51	<0.0001**
T. PROTEIN(6.4-8.3g/dl)	7.22±0.523	7.52±0.542	7.56±0.591	0.0011*
ALBUMIN (3.5-5.0g/dl)	4.51±0.373	4.54±0.284	4.65±0.291	0.074

Table 3: shows comparison between the baseline and biochemical characteristics of the studied groups. Anova test used for comparison of means between the three groups. **denotes extremely significant and * denotes highly significant p values when test groups were compared with controls.

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Fig 4: Showing means of FT3 in the studied groups

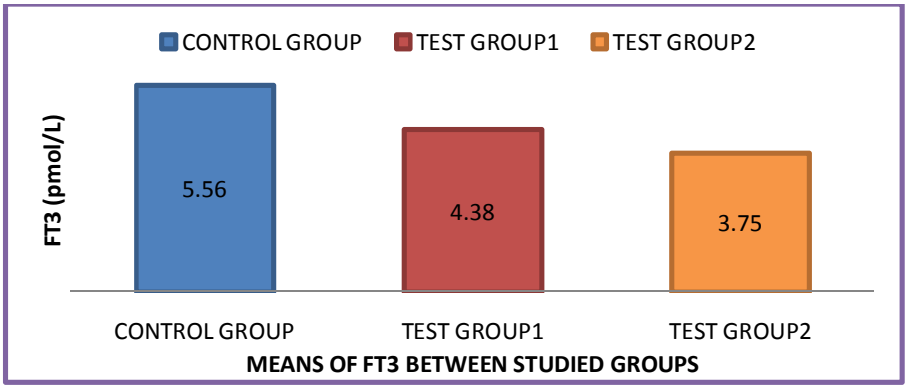


Fig 5: Showing means of FT4 in the studied groups

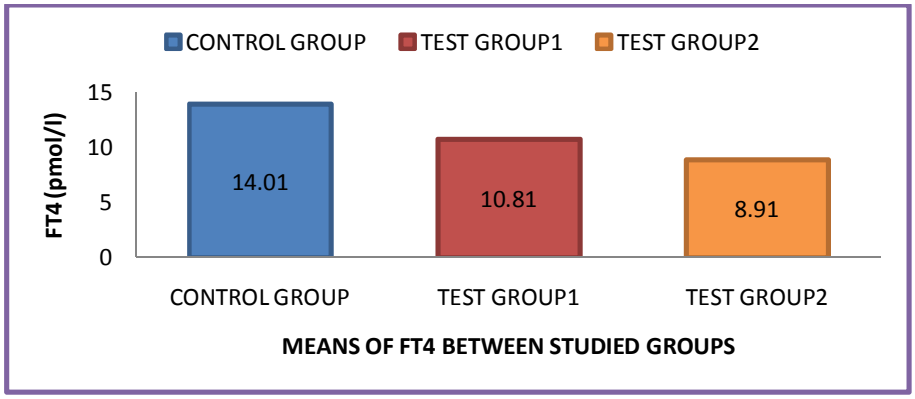


Fig 6: Showing means of TSH in the studied groups

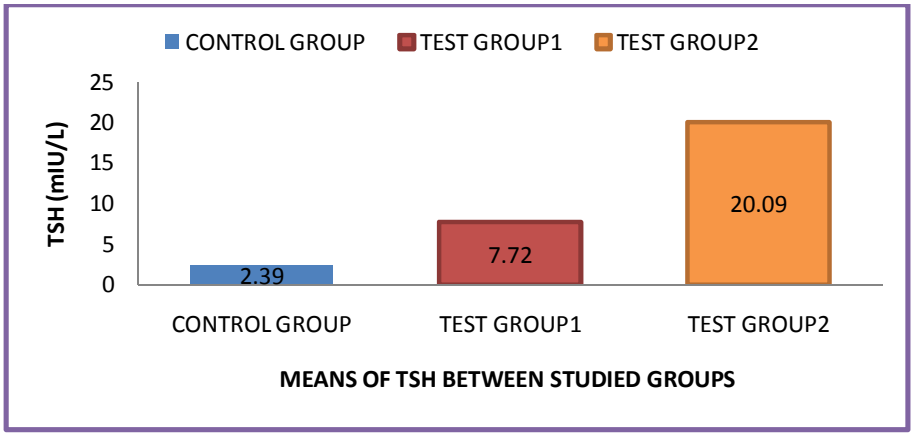


Fig 7: Showing means of AST in the studied groups

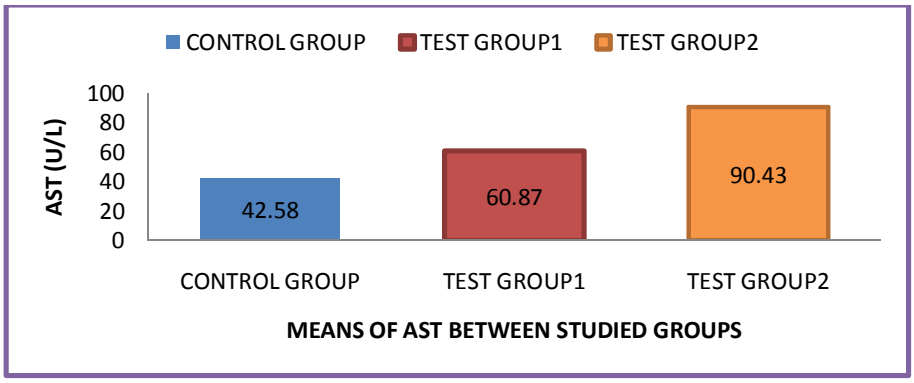


Fig 8: Showing means of ALT in the studied groups

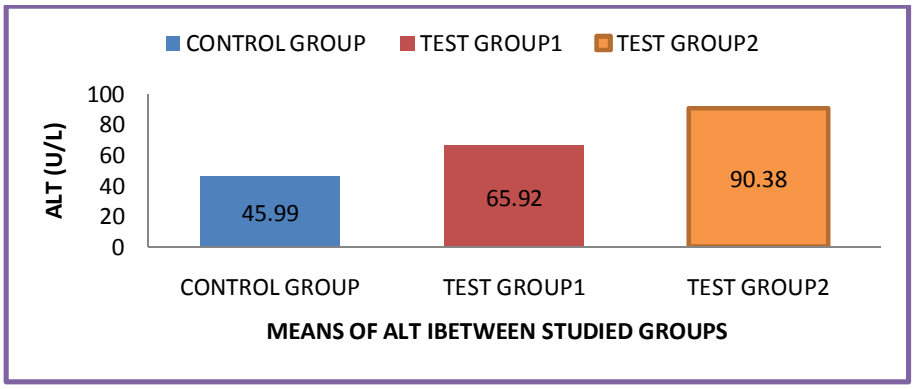


Fig 9: Showing means of ALP in the studied groups

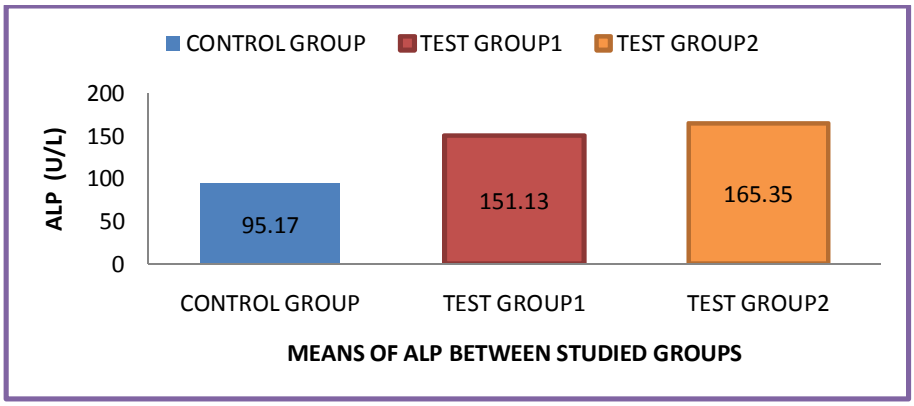
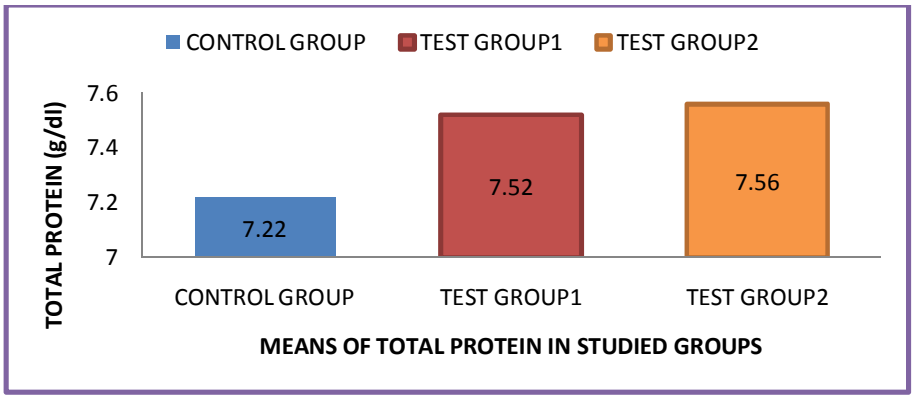


Fig 10: Showing means of Total protein in the studied groups



PARAMETERS	TOTAL BILIRUBIN	AST	ALT	ALP	TOTAL PROTEIN	ALBUMIN
FT3	r=-0.044 p=0.812	r=-0.044 p=0.789	r=-0.0493 p=0.765	r=-0.079 p=0.629	r=-0.0386 p=0.815	r=-0.125 P=0.444
FT4	r=0.208 p=0.260	r=0.0121 p=0.941	r=0.140 p=0.392	r=0.092 p=0.576	r=0.0695 p=0.674	r=0.0673 p=0.683
TSH	r=-0.092 p= 0.514	r=0.805** p<0.0001**	r=0.354** p<0.0001**	r=0.651** P<0.05*	r=-0.0043 p=0.891	r=0.2875 P=0.245

Table 11: Showing correlations between thyroid profile and LFT in subclinical hypothyroidism. * denotes significant and ** denotes highly significant p values.

PARAMETERS	TOTAL BILIRUBIN	AST	ALT	ALP	TOTAL PROTEIN	ALBUMIN
FT3	r=0.310 P=0.061	-0.344* P=0.031*	r= -0.415* p=0.007*	r= -0.323* p=0.04*	0.083	0.226
FT4	r=0.0133 P=0.941	-0.547* P<0.05*	-0.485* P<0.05*	-0.247* P<0.05*	0.095	0.253
TSH	r=-0.0036 p=0.983	r=0.557** p=0.0002**	r=0.612** p<0.0001**	r= 0.579** p<0.0001**	r= 0.0878	r= -0.1066

Table 12: Showing correlations between thyroid profile and LFT in overt hypothyroidism * denotes significant and ** denotes highly significant p value.

Fig 13: Showing Pearson's correlation between TSH and AST in test group1.

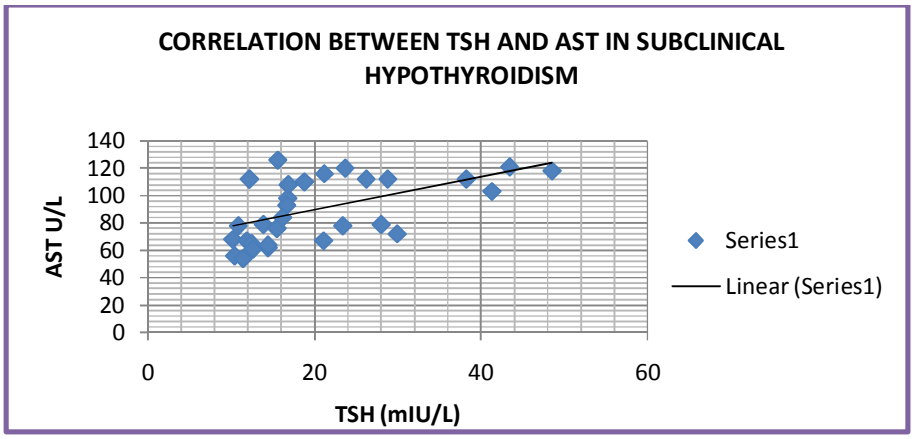


Fig 14: Showing Pearson's correlation between TSH and ALT in test group1.

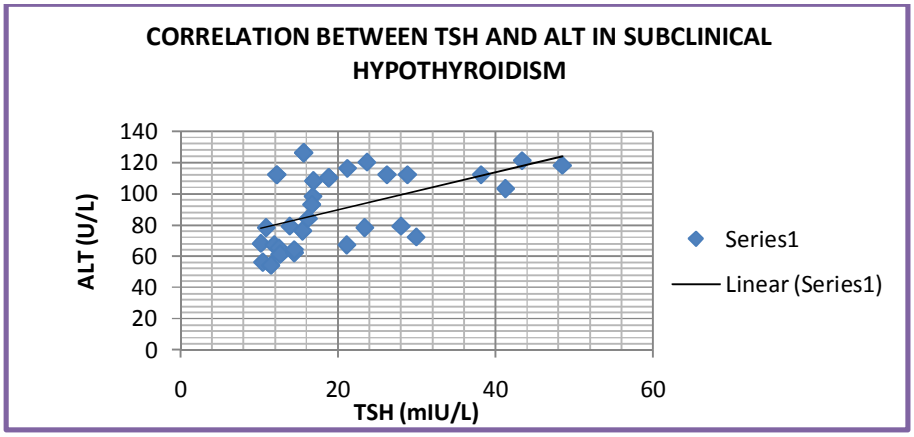


Fig 15: Showing Pearson's correlation between TSH and ALT in test group2.

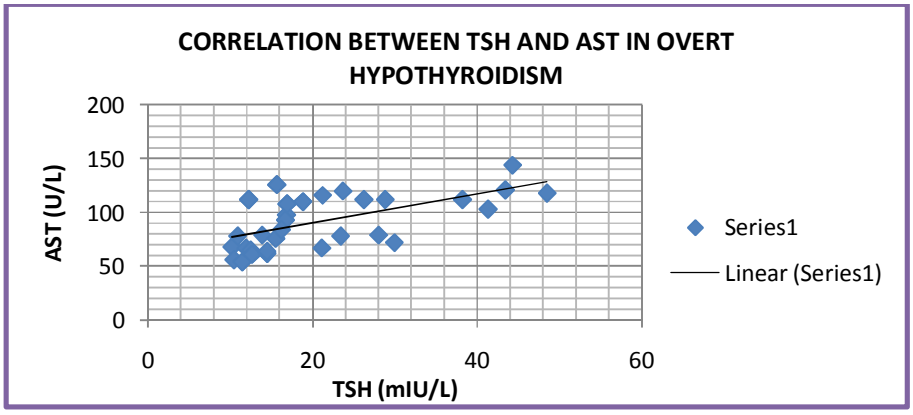


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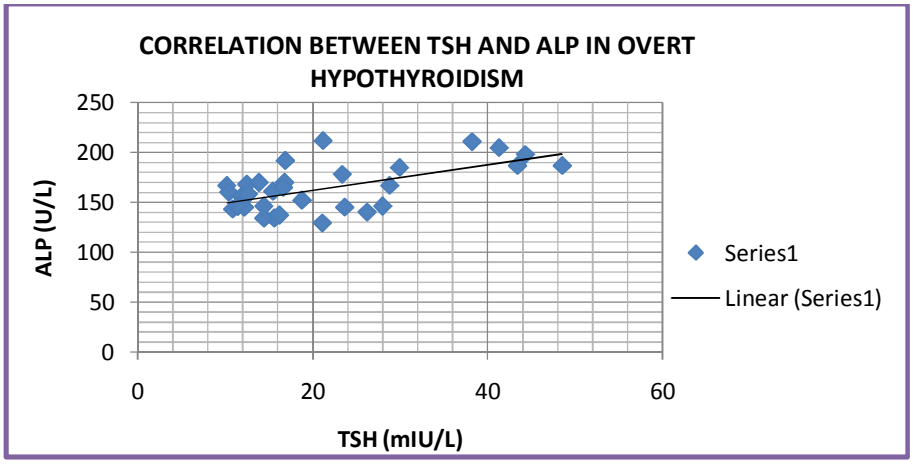
Fig 16– Showing Pearson's correlation between TSH and ALP in test group2.



Discussion

The data presented here shows that there is a significant increase in biochemical parameters of liver function test in hypothyroid patients when compared to normal controls. All these findings clearly suggest that biochemical markers of liver may be affected by alteration in the thyroid hormone levels in the body. Our data showed a significant increase in AST, ALT, ALP and total protein levels in hypothyroid patients when compared to healthy controls and this increase was also significant when overt hypothyroid patients were compared with subclinical hypothyroid patients. The findings of our study is in corroboration with findings of the study by **Yadav A.** ***et al.***[31] and **Pandey R.** ***et al***[32]. **Malik and Hodgson** ***et al*** also mentioned that thyroid hormones T3 and T4 regulate BMR of hepatocytes and modulate all the organ functions. The liver, muscle and kidney in turn metabolizes thyroid hormones and regulates their systemic endocrine effects. Therefore, thyroid dysfunction may disturb liver, muscle and other organ functions and vice versa[7].

In our study TSH level showed significant positive correlation with AST, ALT and ALP and total protein levels in both subclinical and overt hypothyroidism whereas FT3 and FT4 levels had a significant negative correlation with AST, ALT and ALP levels in overt hypothyroidism.

The significant positive correlation of TSH levels with levels of ALT in both patients with subclinical hypothyroidism (test group 1) **(p<0.0001)** and over hypothyroidism (test group 2) **(p<0.0001)** in our study may be explained by the observations made by **Targhar G.** ***et al.***[16]**, Khan T.** ***et al.***[17] and **Prakash A.** ***et al.***[18] that thyroid alteration effects the liver enzymes like ALP, AST and ALT. **Couzigou P** ***et al.***[19] also found that ALT may also be elevated occasionally and cholesterol elevation is as a rule due to hypometabolism. The later may result in fatty liver causing mild but prolonged AST and/or ALT elevation.The significant positive correlation of TSH levels with AST levels in both subclinical and overt hypothyroid subjects **(p<0.0001 and p=0.0002** respectively) may be because of myopathy associated with hypothyroidism. The significant positive correlation of serum TSH levels with ALP in both

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sublinical **(p<0.05)** and overt hypothyroidism **(p<0.0001)** may be explained on the basis observations of **Klion F *et al.*** that in hypothyroidism there is an increase in membrane cholesterol phospholipid ratio and diminished membrane fluidity, which affect a number of canalicular membrane transporters and enzymes, including the Na^+, K^+-ATPase resulting in the change of ALP enzymes[20].

Serum total protein also demonstrated a statistically significant increase in hypothyroid subjects when compared with normal controls. However correlation of serum albumin with thyroid profile was not found to be statistically significant. This indicates that probably in hypothyroidism, proteins other than albumin may be synthesized by the liver including a number of plasma proteins that bind the lipophilic thyroid hormones. Added to this, low-grade inflammation associated with even mild degrees of hypothyroidism may lead to a resultant increase in inflammatory proteins and immunoglobulins[33].

In few case reports, hypothyroidism has been associated with cholestatic jaundice attributed to reduced bile excretion[21]. But our study did find any significant increase in total bilirubin level when hypothyroid subjects were compared with normal healthy controls may be due to selective data of referral cases from OPD.

Limitations of the study : Small sample size was the limitation of our study.

Conclusion

To conclude, the present study indicates that thyroid disorder might cause significant effect on metabolism of various cells including hepatocytes reflected by increase in biochemical parameters of liver function test and it's significant correlation with components of thyroid profile test (FT3, FT4 and TSH) in both subclinical and overt hypothyroid subjects. Therefore, might be essential to measure liver function test in hypothyroid patients to know the association between thyroid alteration and deranged biochemical parameters of liver function. It might be necessary to monitor liver enzymes frequently in hypothyroid patients as declining liver function may be missed by single assessment and deranged biochemical parameters of liver function test (LFT) might indicate underlying altered thyroid status. However, further studies are required to be carried out in large sample size to confirm our findings.

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