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ASSESSMENT OF SOME BIOCHEMICAL MARKERS OF BONE TURNOVER IN THYROID DYSFUNCTION STATE.

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ABSTRACT

Thyroid hormones affect bone metabolism by altering normal bone remodeling processes. This study involved assessment of biochemical markers of bone turnover in 94 patients with thyroid dysfunction. Clear hypocalcaemia was observed in hypothyroidism while in hyperthyroid patients there was frank hypercalcaemia. In hyperthyroidism there is increased activity of osteoclast which leads to increased bone turnover (increased resorption/demineralization). Serum magnesium levels were lower in hypothyroid patients, which is the result of impaired magnesium homeostasis. Serum alkaline phosphatase levels were highest in hyperthyroid patients. The biochemical profile of elevated bone specific alkaline phosphatase, total calcium and ionised calcium together with normal liver function tests strongly indicate that bone resorption occurs at higher rate and speeded up by the thyroid hormones in hyperthyroid patients. Bone turnover is increased in favor of resorption and the rate is associated with the levels of thyroid hormones in hyperthyroidism. Opposite changes occur in hypothyroid patient. If other reasons for increased bone turnover are ruled out then elevated bone resorption markers point to subclinical hyperthyroidism.

KEY WORDS : Hyperthyroidism, Hypothyroidism, Calcium, Ionised calcium, Alkaline phosphatase, Magnesium

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INTRODUCTION

Thyroid hormones affect bone metabolism by stimulating bone resorption and formation that are critical for maintenance of normal bone remodeling[1,2,3]. One of the leading causes of secondary osteoporosis is thyroid diseases[3]. The effect is mediated through several biochemical mechanisms. Biochemical bone markers show smaller yet important changes in bone mineral metabolism associated with thyroid function disorders. Hypothyroidism retards formation and destruction of bone. Hypothyroidism for more than a year will lead to retardation of bone age[4]. Adult hypothyroid patients tend to exhibit higher than normal bone density. It is usually associated with normal or low alkaline phosphatase (ALP) together with normal serum calcium and phosphorus concentrations[5]. Hypothyroid patients have a reduced tolerance to oral calcium and may develop hypercalcaemia when given calcium. The defect appears to be retarded disposition of calcium, rather than excess absorption[6]. Majority of patients with hyperthyroidism have elevated serum levels of calcium and ionised Calcium (ICa)[7,8,9]. Hyperthyroidism is associated with reduced bone mineral density[10,11]. Osteoporosis is a systemic skeletal disease characterized by micro-architectural deterioration of bone tissue leading to enhanced bone fragility and consequent increase in fracture risk[12]. Serum ALP level is well known to be abnormal in thyroid disorders. Measurement of bone specific isoenzyme of ALP is more reliable and cost effective biomarker than osteocalcein[13]. Bone specific ALP is elevated in metabolic diseases including hyperthyroidism[9]. In experimental model, it is demonstrated that bone loss that occurs in thyrotoxicosis, is independent of circulating thyrotropin levels and mediated predominantly by thyroid hormone receptor-α[14]. It is reported that plasma magnesium value is lowered in hypothyroidism and elevated in hyperthyroidism and that hyperthyroid patients excrete significantly larger amounts of ingested magnesium in the urine than hypothyroid patients[7]. In view of these, this study was undertaken to reassess the association of biochemical bone markers with thyroid dysfunction states.

MATERIAL AND METHODS

This study was carried out jointly with the Department of Biochemistry at Government Medical College, Miraj (Maharashtra), and at R. D. Gardi Medical College, Ujjain (MP). The study involved 165 subjects. All of the subjects were examined thoroughly and their detailed medical histories were recorded. Of these subjects, 94 were patients with thyroid disorders and 71 were euthyroid normal healthy controls. The patient group comprised of 45 hyperthyroid and 49 hypothyroid patients attending out-patient clinics of ENT and Medicine departments. These patients were on regular treatment. The clinical diagnosis was confirmed after detail laboratory examinations. The control subjects were carefully selected with strict criteria of no previous family history of cretinism, myxoedema, Graves' disease, thyrotoxicosis and other thyroid abnormalities or diseases. The age ranged from 6 to 75 years. The study protocol was approved by the institutional ethical committee and informed consent was taken from the subjects selected for the study.

10 ml of fasting venous whole blood was collected aseptically in plain bulbs. Following specific biochemical tests were carried out on the serum: The thyroid profile comprised of serum levels of free triiodothyronine (T_3), thyroxin (T_4) and thyroid stimulating hormone (TSH). It was done by IMx assay which is based on the micro-particle enzyme immuno-assay (MEIA) technology[15]. These assays were carried out on IMx analyzer using reagent manufactured by Abbott Laboratories, Abbott Park, IL 60064, USA. The bone related profile included serum levels of total calcium (TCa), ionised calcium (ICa), magnesium and heat labile ALP (HL-ALP). The TCa was measured using photometric Arsenazo-III dye binding method[16]. The serum magnesium was assayed based on titan yellow method[17]. Serum total protein (biuret reaction), albumin

(BCG dye binding) and bilirubin (diazo reaction) and ALP were determined by end-point colorimetric methods, using reagent kits obtained from Reckon Diagnostics Pvt. Ltd., Vadodara, India[18]. The bone isoenzyme is heat labile. It was estimated after heating one aliquot of serum to 56˚C for 10 minutes (Bone isoenzyme = Total ALP activity – Residual heat resistant ALP activity). The enzyme activities are expressed in IU/L. The KA units of ALP are converted to IU/L by 7.1 as multiplying factor[19]. ICa was calculated by Maclean-Hasting equation (Zeister's formula) using serum total protein and albumin values[20]. The assays were carried out strictly as per the guidelines and instructions of the manufacturers. Statistical analysis include student-'t' test.

OBSERVATION AND RESULTS

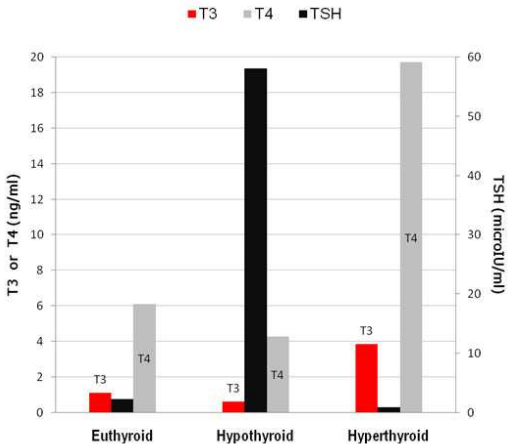
The clinical diagnosis was confirmed by findings of laboratory results on thyroid profile (Table-1). It shows lower normal T_3, T_4 and very high TSH in hypothyroid group. The mean value was almost 25 times higher than that of euthyroid group. In the hyperthyroids, T_3 and T_4 were high while TSH was suppressed. The mean values of T_3, T_4 in hyperthyroids were higher by 3.4 and 3.2 times of the value of the euthyroid group, whereas mean TSH level was almost 62% lower than that of the euthyroid group.

TABLE 1
The thyroid profile in the subjects.

	Subject	N	T_3 (ng/ml)	T_4 (µg/dl)	TSH (µIU/ml)
1	**Reference range**		0.50 – 1.65	4.50 – 12.0	0.47 – 5.01
2	**Euthyroid**	71	1.12 ± 0.46	6.11 ± 2.11	2.31 ± 1.39
3	**Hypothyroid**	49	0.62 ± 1.10*	4.26 ± 9.38	58.04 ± 38.36*
4	**Hyperthyroid**	45	3.854 ± 2.70^	19.72 ± 14.85*	0.88 ± 0.31*

a Figures indicate Mean ± SD.
b * P<0.001; ^ P<0.005

Figure 1.
Thyroid profile in the subjects



Bars indicate mean values; T3 = Triiodothyronine, T4 = Thyroxine; TSH = Thyrotropin

The mean value of TCa in hypothyroidism was significantly lower while in hyperthyroid patients it was higher than the reference limit when compared with the euthyroid group This was statistically significant (P<0.001). Mean levels of ICa in hypothyroid group was significantly lower than that of euthyroid (P<0.001), while in hyperthyroid group it was in the upper normal limit, however, it was statistically significant (P<0.05).

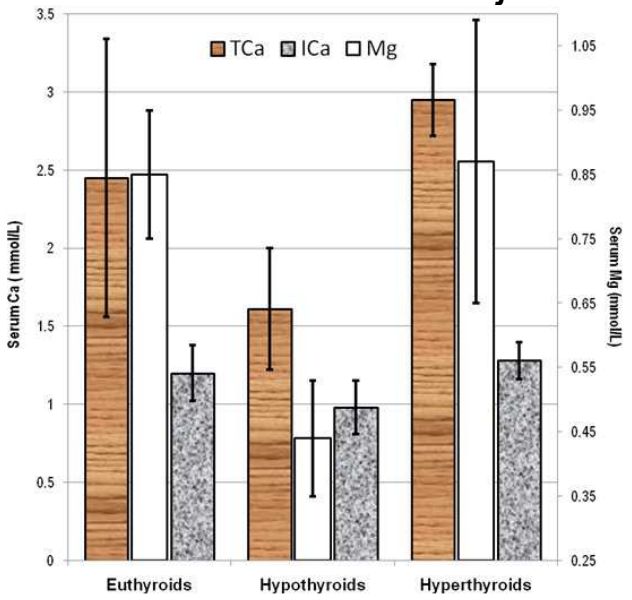
In the present study the mean serum magnesium in hypothyroid patients was significantly low (P<0.001) while no change was found in hyperthyroid patients as compared with euthyroid control group. (Table 2, Fig 2).

TABLE 2
The biochemistry parameters in the subjects.

	Biochemical analyte (units)	Reference Range	Euthyroids (n=71)	Hypothyroids (n=49)	Hyperthyroids (n=45)
1	**Total Proteins (gm/dl)**	6.3 – 8.8	7.07 ± 0.52	7.89 ± 0.56*	5.52 ± 0.46*
2	**Serum albumin (gm/dl)**	3.6 – 5.4	3.67 ± 0.38	2.79 ± 0.39	3.72 ± 0.46*
3	**Total bilirubin (µmol/L)**	5 – 21	12.5 ± 0.32	13.8 ± 0.48	13.0 ± 0.29
4	**Total ALP (IU/L)**	20 – 90	30.5 ± 6.8	27.0 ± 6.6^	95.5 ± 7.9*
5	**HL-ALP (IU/L)**	15 – 70	24.4 ± 6.2	21.5 ± 5.2^	78.6 ± 8.8*
6	**Total Calcium (mmol/L)**	2.15 – 2.5	2.45 ± 0.89	1.61 ± 0.39*	2.95 ± 0.23*
7	**Ionised Calcium (mmol/L)**	1.16 – 1.32	1.20 ± 0.18	0.98 ± 0.17*	1.28 ± 0.12•
8	**Magnesium (mmol/L)**	0.66 – 1.07	0.85 ± 0.10	0.44 ± 0.22*	0.87 ± 0.22

***a** *P<0.001; ^P<0.01; •P<0.05 cf euthyroid group.*
***b** HL-ALP = Heat labile alkaline phosphatase*

Figure 2.
Serum mineral levels in the subjects.

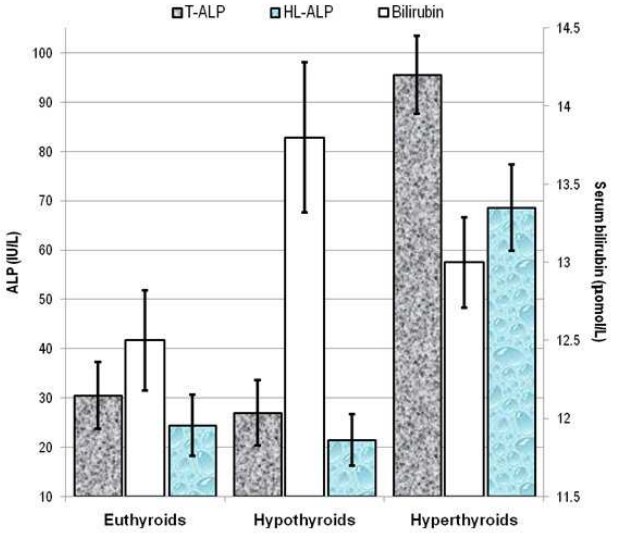


Column bars indicate mean values and error bars as standard deviation.
TCa = Total calcium; ICa = Ionised calcium; Mg = Magnesium

The mean values of Total ALP as well as HL-ALP fraction in hyperthyroid group was almost 3.2 times that of hypothyroid group and it was significantly higher than that of euthyroid group. The

values in hypothyroids were significantly (P<0.001) lower compared to euthyroids, however, it was within normal reference limit (Table 2, Fig 3). Total bilirubin values do not show any significant deviation from the normal group.

Figure 3.
Serum bilirubin and ALP level in subjects



Column bars indicate mean values and error bars as standard deviation.
T-ALP = Total Alkaline phosphatase,HL-ALP = Heat labile alkaline phosphatase

DISCUSSION

Majority of thyroid function disorders affect bone metabolism. In population studies, hyperthyroidism and hypothyroidism have both been associated with an increased risk of fractures. This study attempts to assess changes in bone turnover markers in thyroid dysfunction. The markers selected for the study are simple, inexpensive and suitable for small laboratories. The battery of markers is relatively specific for assessing bone remodeling status.

In the present study serum TCa was higher in hyperthyroid patients whereas it was significantly lower in patients of hypothyroidism as compared to euthyroid controls. The mean value of serum TCa in hypothyroid patients was significantly much less than that in hyperthyroid patients (P<0.001). The mean value of ICa was high in hyperthyroid patient and is significantly lower in hypothyroid patients as compared with euthyroid controls. These results are similar to the earlier reports[5,6,7].

The mean value of ~~total~~ serum TCa in hyperthyroidism was 2.95 mmol/L, which was above the upper normal limits indicating hypercalcaemia. On the other hand the Calcium level in hypothyroid patients was 1.61 mmol/L, which was below the lower normal limits showing hypocalcaemia. In fact, patients with hypothyroidism show marked hypocalcaemia. High levels of thyroid hormones, as found in hyperthyroid state, accelerate the process of bone resorption and subsequently bring about increase in the rate of bone loss which is observed as high serum calcium values in hyperthyroid subjects.

Calcium level was low in hypothyroidism, because the dynamics of bone and mineral metabolism slow down in hypothyroidism. Even though hypocalcemia is not the rule, exchangeable calcium pool, urinary excretion of calcium and absorption of calcium from the gut are all reduced[5,21]. This explains the low calcium level in hypothyroidism.

It is reported that plasma magnesium value is lowered in hypothyroidism and

elevated in hyperthyroidism[5]. It is also reported that hyperthyroid patients excrete

significantly larger amounts of ingested magnesium in the urine than hypothyroid patients. Accordingly serum magnesium level in hyperthyroid patients is expected to be lower than the euthyroids. However, present finding is contradictory to the earlier reports. It is well known that homeostasis of magnesium is regulated most efficiently at intestinal absorption and by reabsorption in kidneys. It is likely that low serum levels of magnesium in hypothyroid patients must have resulted either due to impaired intestinal magnesium absorption or by increased renal loss. Since serum ICa was low, it is likely that renal regulation is affected in the hypothyroid patients. Other possible reason could be attributed to poor dietary magnesium intake by the patients with hyperthyroidism, included in the study.

Although thyroid hormones stimulates both osteoblast and osteoclast, stimulation of the latter appears to be indirect. The exact mechanism by which thyroid hormones increase osteoclast activity remains unknown. *In vitro* experiments indicate that the presence of activated osteoclast is a prerequisite for stimulation of osteoblasts[21]. Unlike Osteoclasts, osteoblast passes nuclear receptors for T_3 as shown by biochemical binding studies and detectable mRNA levels for thyroid hormone receptors. It has been suggested that the effect is medicated by a soluble factor termed "osteoclast resorption stimulating activity" which appears to be associated with cell surface or the extra cellular matrix of osteoblasts[14].

Thus a probable mechanism for hypocalcemia in hypothyroidism may be explained. The deficiency of thyroid hormones, (especially T_3) or extremely low or undetectable levels of thyroid hormones in serum suppress the activation of osteoclasts. This results in deceleration of bone turnover process. Subsequently the rate of bone loss is decreased thereby serum level of TCa as well as ICa is reduced. In hyperthyroidism, all process those occur in hypothyroidism, take place in opposite or reverse fashion.

Total ALP level in serum has low specificity and give only a gross idea of deranged bone metabolism. The HL-ALP has high specificity as it originates from osteoblasts in bone. Therefore, serum levels of this isoenzyme fraction reflect osteoblastic activity. Measurement of the bone specific ALP has several advantages over other markers such as osteocalcein. It is more useful in impaired renal function, 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[13].

As seen from the table 2 & fig 3, the serum ALP activity was found to be highest in patients with hyperthyroidism as compared to that in patients with hypothyroidism and the euthyroid controls. However in hypothyroid group the mean total ALP as well as HL-ALP activity was found to be in normal range. The changes in ALP levels in thyroid disorders are not due to altered liver function. Since other LFTs such as serum bilirubin, ALT, AST are not much different from the euthyroid controls[5,22]. 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 resorption and formation. This indicates high degree of bone turnover or remodeling activity. These observations are consistent with the other studies[9].

It is reported that most markers of bone turnover are decreased in patients with hypothyroidism[8,9,10,11]. In the present study it was found that serum TCa as well as ICa levels were lower in hypothyroid than hyperthyroid patients, which indicate that in bone resorption was accelerated much more in hyperthyroid patients and correlated with thyroid hormone excess state.

CONCLUSION

In hypothyroid patients, clear hypocalcaemia was observed while in hyperthyroid patients there was frank hypercalcaemia. This is mediated through suppression of osteoclast activation in absence of thyroid hormones. In hyperthyroidism there is increased activity of osteoclast which leads to increased bone

turnover (increased resorption / demineralization). Serum magnesium levels were lower in hypothyroid patients, which is the result of impaired magnesium homeostasis. Serum ALP levels were highest in hyperthyroid patients. The biochemical profile of elevated HL-ALP, TCa and ICa together with normal LFT strongly indicate that bone resorption occurs at higher rate and speeded up by the thyroid hormones in hyperthyroid patients. Bone turnover is increased in favor of resorption and the rate of resorption is associated with the levels of thyroid hormones in hyperthyroidism. Opposite changes occur in hypothyroid patient. If other reasons for increased bone turnover are ruled out then elevated bone resorption markers point to subclinical hyperthyroidism.

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