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Bone mineral density in patients of Graves disease pre- & post-treatment in a predominantly vitamin D deficient population

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***Background & objectives*: Hyperthyroidism causes bone loss, and its treatment may restore bone mass, however, concomitant vitamin D deficiency may prevent this. We undertook this study to measure the bone mineral density (BMD) 25 (OH) vitamin D levels in patients with Graves disease in our population which is predominently vitamin D deficient and how we change with when patients become euthyroid.**

***Methods*: The biochemical, thyroid functions, serum vitamin D levels and BMD were estimated in 80 consecutive patients with Graves and 80 euthyroid controls. Patients were treated and rendered euthyroid. Fifty four completed one year, and 27 completed two years of follow up.**

***Results*: Patients had significant reduced BMD during hyperthyroid state compared to normal healthy controls. The mean vitamin D levels at baseline were in the insufficient range both patients (12.67±6.24 ng/ml) and controls (10.99±7.05 ng/ml). The BMD improved at all sites with antithyroid treatment. But, the BMD adjusted for body mass index (BMI) and age at all sites showed significant decrease with time.**

***Interpretation & conclusions*: Age and body mass index positively correlated with BMD. There was improvement in absolute BMD of patients at one and two years of follow up. When the BMD was adjusted for age and BMI, there was a decrease in BMD at one year which was less in the second year including that the damage in BMD caused by thyroid hormone excess is not made up even after two years of patient being euthyroid. Whether vitamin D replacement would change this needs to be studied.**

Key words Bone mineral density - Graves'disease - hyperthyroidism - vitamin D deficiency

Untreated hyperthyroidism has been associated with a decreased bone mineral density (BMD)[1,2] and an increased fracture risk[3]. This may be the consequence of the stimulatory effect of thyroid hormones on bone turnover[4]. The increased level of thyroid hormones causes a reversible bone loss due to an expansion of the remodeling space and an irreversible loss due to a negative net bone balance and eventually an increased risk of trabecular perforations[4]. Thus, hyperthyroidism has been considered a major risk factor for osteoporosis[1-4]. Cross-sectional studies of subjects with hyperthyroidism have confirmed reduced bone density at various skeletal sites[5,6]. Patients with Graves disease have been shown to have either normal or low serum 25(OH) vit-D level[7,8]. Only a few longitudinal studies have looked into progression of bone mineral density after treatment of hyperthyroidism[9-15].

Thyroid hormone excess, whether due to hyperthyroidism or thyrotoxicosis is associated with increased bone turnover. Graves disease is the most common cause of hyperthyroidism. Studies report that vitamin D deficiency is common in India in all age groups[16,17]. Some studies have documented normal serum levels of 25(OH) vitamin D in thyrotoxicosis patients whereas others showed subnormal vitamin D levels[7,8].

Bone density assessed by dual-energy X-ray absorptiometry (DXA) in hyperthyroid patients on follow up in different studies have shown different results. Some showed improvement while others showed decrement in BMD despite antithyroid drug therapy[11-15].

None of the studies on patients with Graves disease in India have evaluated bone health in vitamin D deficient subjects. In a study on BMD follow up in Graves disease patients vitamin D deficiency has been ruled out[18].

The objectives of the present study were to determine the serum levels of calcium, phosphorus, alkaline phosphatase (ALP), parathyroid hormone (PTH), 25 (OH) vitamin D and BMD in hyperthyroid Graves disease patients with vitamin D deficiency and to see whether these differ from age and sex matched healthy controls, and also to assess the changes in the above parameters after the patients became and remained euthyroid.

Material & Methods

Eighty consecutive patients (62 female, 18 male) with Graves disease attending Endocrine Clinic in All India Institute of Medical Sciences (AIIMS), New Delhi during January 2006 to January 2008 were included. Of these, 54 completed one and 27 completed two years of follow up. The diagnosis was based upon clinical features of thyrotoxicosis, serum T4 and TSH levels in hyperthyroid range, radio-iodine uptake (RAIU) and diffuse thyromegaly. Healthy controls, age (±5 yr) and sex matched were taken in same month (±1 month) as the cases to avoid seasonal variation of 25 (OH) D level. The healthy controls taken were mainly hospital staff nurses, students, hospital employees where a low vitamin D status has been reported[16,17].

Pregnant and lactating women, patients with chronic liver disease, renal failure, taking steroids, antitubercular drugs, anti-epileptic drugs, ketoconazole, diuretics or calcium and vitamin D supplements were excluded from both cases and healthy control group of the study. All patients and controls gave written informed consent and study protocol was approved by the institute's ethical committee.

All patients were subjected to detailed history and clinical examination using a pre-designed proforma. Average duration of sun exposure was assessed in patients and controls. Carbimazole was started in a dose varying between 30-60 mg/day depending on the severity of Graves disease. T4, TSH were measured 2-monthly during the follow up. The maintenance dose varied from 20 to 5 mg depending on T4 levels and clinical symptoms, the aim being to keep the patients euthyroid. It took 2 to 4 months for patients to have T4 in normal range and T4 was then maintained in normal range throughout the study by altering the dose of carbimazole.

Fasting morning venous sample was taken in calcium free test tubes. For all patients serum T4, TSH were done at baseline and every 2 monthly, whereas 25(OH) D and PTH at baseline, one and two years. Serum calcium, phosphate, ALP and 24 h urinary calcium, phosphate and creatinine were estimated on the day of collection of samples. Urinary creatinine was estimated to know the adequacy of sample collection.

The normal ranges for T4, TSH, serum calcium, serum phosphate, alkaline phosphatase and PTH were 5.1-14.1µg/dl, 0.27-4.2 µIU/ml, 8.1-10.4 mg/dl, 2.5-4.5 mg/dl, 80-240 I.U and 15-65 pg/ml. Hypercalcemia was considered when the serum calcium was more than 10.4 mg/dl. For hypercalciurea cut-off used was 200 mg in 24 h.

Calcium, phosphorus and alkaline phosphatase were estimated by the colorimetric method (calcium, Fluitest CA CPC Kit Analyticon biotechnologies AG Germany, serum phosphorus, Randox kit, Randox Laboratories Limited, United Kingdom and serum alkaline phosphatase, Centronic GmbHAm, Germany). Serum 25(OH) D was estimated by radio immunoassay (RIA) using a kit from Dia Sorin Inc Stillwater, USA. Serum T4, TSH and PTH assays were done using patented electrochemiluminescent technology on an autoanalyser ELECSYS 2010 from Roche diagnostic (Germany).

Intra-assay coefficient of variation for serum T4 was 2 per cent at 14 µg/dl and 2.3 per cent at 8.7 µg/dl. Inter-assay coefficient of variation for serum T4 was 2.7 per cent at 14 µg/dl and 3.3 per cent at 8.7 µg/dl. Intra- and inter-assay coefficients of variation for

serum TSH were 1.5 and 1.8 per cent at 10.6 µU and 1.9 and 2.2 at 2.4 µu respectively. Intra- and inter-assay coefficients of variation for serum PTH was 4.1 and 6.2 per cent at 20.2 pg/ml and 1.9 and 26 per cent at 676 pg/ml respectively. Intra- and inter-assay coefficients of variation for serum vitamin D was 11 and 9 per cent at 8.6 ng/ml and 12 and 11 per cent at 49 ng/ml respectively.

BMD was assessed by DXA (Hologic DR 4500 A densitometer, USA) at lumbar spines (L1-L4), hip and forearm using A-P view. The hologic BMD machine had a clinically insignificant BMD drift (maximum of 0.20%), over the period of study.

The average duration of cloud-free sunshine during the study was 5.59 h/day in winter (November to February), 6.28 h/day in summer (March to June) and 6.47 h/day in rainy (July to October) seasons in Delhi.

Descriptive statistical methods such as mean, standard deviation were applied to summarise the continuous variables. Proportions and percentages were used to summarise categorical variables. Comparison of baseline continuous variables between groups was done by applying independent t-test. For the comparison of categorical variables between the groups, chi square or Fishers exact test has been applied. For baseline, to see the effect of different parameters on BMD, multiple linear regression was applied separately in patients and controls at baseline. Time-wise differences were assessed using repeated measures ANOVA.

To see the overall trend in BMD over time, adjusting for variables age, BMI and T4, generalised estimating equation (GEE) was applied. All statistical analyses were carried out by using Stata 9.2 software -Stata Corp. 4905 Lakeway Drive College station, Texas 77845, USA.

Results

The baseline clinical, biochemical and BMD data of patients and controls were given in Table I. The mean radioactive Iodine uptake at 2 h was 38.56±17.81 and at 24 h was 65.89±14.75 per cent. Age in both groups was comparable. Serum creatinine, BMI and TSH were significantly (P<0.001) lower in Graves patients compared to healthy controls. Serum T4, ALP, duration of sun exposure, serum calcium and corrected serum calcium were significantly (P<0.001) higher in hyperthyroid Graves patients compared to healthy euthyroid controls. The serum PTH levels were higher in patients than controls though this difference was not statistically significant (Table I).

Patients were treated with carbimazole and all became euthyroid. It took 2 to 4 months for patients to have T4 in normal range. The mean serum T4 levels at baseline was 20.94 ± 4.41 µg/dl (normal 4.5 to 14 µg /dl). At 6, 12, 18 and 24 months the mean T4 levels were 10.52 ± 2.86, 10.16 ± 2.55, 10.27 ± 2.40 and 10.96 ± 1.91, all in normal range. The TSH levels at baseline, 6, 12, 18 and 24 months was 0.08 ± 0.10, 1.29 ± 1.75, 1.24 ±1.16, 1.38 ± 1.28 and 1.59 ± 1.67 µIU/ml respectively.

BMI increased with therapy on achieving euthyroid state. The BMI at baseline was 20.24 ±3.36 kg/m^2, at 1 year it was 21.93 ± 2.95 and at 2 year 21.68 ± 2.85 kg/m^2. BMD corrected for age and BMI was found to be significantly lower in patients at hip, spine and forearm compared to controls. The mean vitamin D levels at baseline were in the insufficient range in both patients (12.67±6.24 ng/ml) and controls (10.99±7.05 ng/ml) (Table I).

Serum 25(OH) D levels < 20 ng/ml was found in 70 (87.5%) patients and 72 (90%) controls. Serum 25(OH) D correlated positively with BMD at spine, hip and forearm in controls and spine and forearm in

Table I. Baseline clinical and biochemical characteristics of patients and controls

Variables	Patients N=	Controls N=
Age (yr)	36.33±11.15**	36.42±10.40
BMI (kg/m²)	20.43±3.48**	23.68±4.03
Sun exposure (min/day)	87.73±68.46**	53.16±62.28
Creatnine (mg/dl)	0.71±0.16**	0.82±0.16
Calcium (mg/dl)	9.78±0.71**	9.45±0.46
Corrected Calcium (mg/dl)	9.48±0.78**	9.12±0.40
Phosphate (mg/dl)	4.03±0.69	4.18±0.67
Alk. Phosphatase IU	297.5±137.56**	188.45±51.49
Serum T4 (µg/dl)	20.94±4.41**	8.91±1.49
Serum TSH (µIU/ml)	0.08±0.10**	2.23±1.31
PTH (pg/ml)	44.6±40.81	37.75±27.35
25(OH)Vit D (ng/ml)	12.67±6.24*	10.99±7.05*
BMD at Spine (g/cm^2)	0.86±0.141	0.91±0.20
BMD at Hip (g/cm^2)	0.74±0.14**	0.84±0.19
BMD at Forearm (g/cm^2)	0.45±0.09**	0.52±0.13
BMI and age adjusted BMD spine	0.86 ± 0.005**	0.909 ± 0.083
BMI and age adjusted BMD hip	0.742 ± 0.006**	0.821 ±0.080
BMI and age adjusted BMD forearm	0.46 ± 0.003**	0.523 ± 0.05

Values are mean ± SD
*P<0.05, **<0.001 compared to controls

Table II. On linear regression analysis showing factors affecting BMD at baseline

Dependable variable	Predictors	Patients	
		P value	Reg.Co
BMD- spine	Age	<0.05	-.003
	T4	<0.01	-0.009
	BMI	<0.05	0.011
	PTH	0.21	0.000
	Vit D	0.336	0.002
BMD- hip	Age	<0.05	-0.003
	T4	<0.01	-0.011
	BMI	<0.01	0.013
	PTH	0.34	0.000
	Vit D	0.44	0.002
BMD- forearm	Age	<0.01	-0.002
	T4	<0.01	-0.006
	BMI	<0.05	0.006
	PTH	0.12	0.000
	Vit D	0.41	0.001

patients. This correlation was significant only at the forearm in healthy controls.

On linear regression analysis, age, T4 and BMI had significant relationship with BMD at all sites (spine, hip and forearm) among patients (Table II).

All the patients were rendered euthyroid by medical treatment by 4 to 6 months. There was significant decrease in mean serum corrected Ca and serum phosphate with treatment (Table III). Though the BMD at all sites increased, this increase was not statistically significant. When the BMD was adjusted for age and BMI, there was significant (P<0.001) decrease in the spine and hip BMD over a period of two years. But the BMD decrease (adjusted for age and BMI) at the forearm was not significant (Table III). When the BMD was corrected for BMI and age, (factors found to significantly affect BMD in patients), there was decrement in BMD at spine and hip. This decrement was more in the first year after starting antithyroid treatment and stabilized in the second year of patients remaining euthyroid (Table III).

Serum alkaline phosphatase was significantly higher in patients compared to controls (Table I). In 49 (61.25%) patients and 12 (15%) controls, serum alkaline phosphatase was above upper limit of normal *i.e.* > 240 IU/l. After one and two years of treatment with antithyroid drugs, though the serum alkaline phosphatase levels decreased compared to baseline, but still remained above normal range.

There was no significant change in the alkaline phosphatase, 24 h urinary calcium excretion, serum vitamin D levels and PTH levels after one and two years of treatment when compared to their baseline values (Table III). Among patients, there was a significant negative correlation between serum 25 (OH) vitamin D levels and PTH (n = 80, r = - 0.28, P=0.01).

Discussion

Mean serum calcium and corrected calcium among Graves patients were in normocalcemic range.

Table III. Comparing the baseline data with 1 and 2 year follow up data

Variables	Pre-treatment (n=80)	Post-treatment at 12 month (n=54)	Post-treatment at 24 months (n=27)	*P* value
Ca (mg/dl)	9.78±0.714	9.65±0.560	9.74±0.594	0.087
Corrected Ca (mg/dl)	9.48±0.784	9.05±0.582	8.936±0.692	0.007
PO4 (mg/dl)	4.03±0.697	3.83±0.603	3.568±0.705	<0.001
Alk. Phosphatase (IU/l)	297.50±137.560	294.76±124.342	274.526±86.822	0.137
24 h Urinary Ca (mg/day)	0.572±0.927*	0.12±0.105	0.602±1.799	0.298
24 h Urinary PO4 (mg/day)	0.253±0.300*	0.51±0.186	0.774±0.282	0.093
PTH (pg/ml)	44.60±40.819	48.49±41.372	48.421±61.290	0.384
Vit-D (ng/ml)	12.67±6.241	13.88±9.694	11.138±5.539	0.880
BMD spine (g/cm^2)	0.86±0.141	0.919±0.123	0.896±0.144	0.284
BMD Hip (g/cm^2)	0.74±0.146	0.795±0.124	0.808±0.154	0.155
BMD Forearm (g/cm^2)	0.456±0.092	0.472±0.080	0.465±0.064	0.418
BMD spine adjusted for BMI and age	0.86 ±0.005	0.72 ± 0.08	0.71 ± 0.08	<0.001
BMD Hip adjusted for BMI and age	0.742 ± 0.006	0.57 ± 0.08	0.59 ± 0.08	<0.001
BMD Forearm adjusted for BMI and age	0.46 ± 0.003	0.37 ± 0.05	0.37 ± 0.05	0.328

Values are mean ± SD; *n=50

Hypercalcemia was found in 7.5 per cent. These findings are lower from those reported from the west (23-51%)[19,20].

In our study, at baseline, 15 of 80 (18%) patients had hypercalciurea (mean 24 h urinary Ca= 0.13 ± 0.12 mg/day). At 12 months, four out of 50 (only 8 %) were hypercalciuric (24 h Urinary Ca (mg/day) = 0.12 ± 0.105). Thus, there was a significant reduction in number of hypercalciurea cases at 12 months. Of the 12 initially hypercalciuric patients in whom urine calcium status was known at baseline and one year, eight became normocalciuric. This change was statistically significant (P<0.01). At 24 months, 5 of 17 (29.4%) were hypercalceuric with a mean value of 0.19 ± 0.18 mg/dl. The increase seen at 2 years may be due to small sample size.

Presence of normocalcemia in majority of patients and much reduced frequency of hypercalciuria in the present group of thyrotoxic patients could be due to vitamin D deficiency which was present in majority of our patients. Hyperthyroid state is associated with increase in bone turnover, possibly a direct effect of T4 on osteoclast, thereby resulting in subtle hypercalcemia with consequently suppressed PTH. There was no significant difference in serum PTH levels in patients and controls. Both patients and controls were vitamin D insufficient. The relatively high PTH levels in patients could possibly be due to vitamin D deficiency associated secondary hyperparathyroidism overwhelming T4 mediated PTH suppression.

In a study of 34 untreated hyperthyroid patients[7], a mean 25(OH)D level of 23.4±7.8 ng/ml was found as compared to the normal controls levels of 28±9.6 ng/ml. In another study[8] in 208 Graves patients, serum 25(OH)D value < 10 ng/dl was found in 40 per cent of female and in 18 per cent of male patients. In this study, euthyroid healthy controls were not taken for estimation of serum 25(OH)D levels. Hence it is difficult to comment whether the high prevalence of vitamin D deficiency in Graves patients was due to high prevalence of vitamin D deficiency in general population or due to hyperthyroidism itself.

When the BMD was adjusted for the BMI and age there was a significant decrease in BMD at spine, hip and forearm in the first year which stabilized in the second year of patient remaining euthyroid. This implies that the decrease in BMD due to hyperthyroidism did not recover after two years of patient being treated for hyperthyroidism and remaining euthyroid. None of these patients received vitamin D replacement.

In the present study, serum alkaline phosphatase was significantly higher in patients compared to controls and did not normalize even after two years of antithyroid therapy. This finding was similar to previous studies[21,22]. A study showed that all thyrotoxic patients became euthyroid 4-8 wk after antithyroid treatment, however, bone specific alkaline phosphatase remained elevated till 52 wk indicating continuing bone formation[21]. Other studies have also shown persistent elevation of alkaline phosphatase at more than 1 year[22]. Hence persistent elevation of alkaline phosphatase for several months suggests increased osteoblastic activity and bone turnover continued even after restoration of a normal metabolic rate.

A strong positive correlation between BMI and BMD at all sites was found in our study. Others have also shown similar correlation among normal healthy adults[28]. Lack of substantial increase in BMD after 2 yr of treatment with attainment of euthyroid state could occur because the bone turnover may not be normalized for a sufficient length of time. Some previous studies have shown paradoxical deterioration of bone density despite antithyroid drug treatment[13,14].

In another study of patients with thyrotoxicosis, a trend of continuous decline of bone density at forearm was demonstrated despite improvement at hip and spine after two years of carbimazole therapy[15]. The reason for delayed recovery or failure to show recovery at forearm has been attributed to predominance of cortical bone at forearm, which has slower activation frequency time. Increased fracture risk at forearm in post-menopausal women with hyperthyroidism treated with different modalities even after 5 years of euthyroidism has been reported[24].

There are several limitations to the study. Firstly, the area of sunlight exposure has not been recorded making it difficult to correlate the sun exposure with the vitamin D status. Secondly, Clinical scoring (Wynes index) was not done nor was the history of menopause recorded. Thirdly, dietary calcium intakes of the subjects and their socio-economic status were not recorded. Fourthly, urinary creatinine was not recorded and hence calcium creatinine ratio or phosphate excretion index could not be derived. Lastly, the latest definition of vitamin D deficiency has not been used in this study.

In this study vitamin D was not given to patients though they were deficient. Vitamin D was not replaced in these patients as vitamin D status was known only at the end of the study when vitamin D kits were

procured and test done as part of the research protocol. In the standard treatment of Graves disease vitamin D replacement is not given.

In conclusion, in hyperthyroid Graves patients, BMD was found to be significantly lower at hip, spine and forearm compared to healthy, euthyroid controls. When treated for hyperthyroidism, the absolute BMD improved at all sites. But the BMD corrected for BMI showed a decrease which stabilized after one year. This indicates that damage in BMD caused by thyroid hormone excess is not made up even after two years of patient being euthyroid. Our patients were vitamin D deficient. This raises the question whether BMD would have been more had the patients been supplemented with vitamin D. Future research is needed to answer this question.

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