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DOI:10.4158/EP151055.OR

Original Article EP151055.OR

TRABECULAR BONE SCORE IN PATIENTS WITH NORMOCALCEMIC HYPERPARATHYROIDISM

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Running title: TBS and hyperparathyroidism

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DOI:10.4158/EP151055.OR
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ABSTRACT

Objective. The effects of normocalcemic hyperparathyroidism (NHPT) on bone remain unclear. The objective of this study was to evaluate differences in the trabecular bone score (TBS) in NHPT patients and asymptomatic hypercalcemic hyperparathyroidism (HHPT) patients.

Methods. We performed a prospective study which enrolled consecutive patients with asymptomatic HPT (NHPT and HHPT) with a follow-up ≥ 1 year at the University Hospital of Valladolid, Spain. Metabolic phosphocalcium plasma and urine parameters were evaluated in ≥ 2 determinations during follow up to classify patients as NHPT patients or asymptomatic HHPT patients. A control group was enrolled during the same period. TBS and bone mineral density (BMD) were evaluated.

Results. 39 patients with asymptomatic HPT (24 with NHPT and 15 with HHPT) and 24 controls were recruited. NHPT patients and HHPT patients had a similar age, vitamin D level, TBS, and areal BMD (all sites). Asymptomatic HPT patients had significantly higher parathyroid hormone (PTH) and calcium levels and significantly lower TBS and areal BMD at all sites (all $p<0.05$) than controls. A significant negative relationship between TBS and PTH level was found in asymptomatic HPT patients ($r=-0.320$, $p= 0.043$), which remained significant after adjustment for age, sex and BMI.

Conclusions. There was no difference in the TBS between NHPT and HHPT patients. However, there was a reduction in the TBS in patients with asymptomatic HPT that was related to PTH levels but had no repercussion on bone mass. Higher levels of PTH seem to be responsible for this alteration in microarchitecture texture.

Count word: 249

DOI:10.4158/EP151055.OR

Key words: Normocalcemic hyperparathyroidism, trabecular bone score, parathyroid hormone, bone mineral density

Disclosure statement: All authors report no disclosures

Grant: SEIOMM-MEDIMAPS TBS

Abbreviations

aBMD = Areal bone mineral density; **BMD =** Bone mineral density ; **BMI =** Body mass index; **DXA =** Dual-energy X-ray absorptiometry; **HHPT =** hypercalcemic hyperparathyroidism; **HPT =** hyperparathyroidism; **HR-MRI =** High-resolution magnetic resonance; **HR-pQcT =** high-resolution peripheral quantitative computed tomography ; **NHPT =** Normocalcemic hyperparathyroidism; **PTH =** Parathyroid hormone; **TBS =** Trabecular bone score.

DOI:10.4158/EP151055.OR

INTRODUCTION

Normocalcemic hyperparathyroidism (NHPT) is a form of asymptomatic hyperparathyroidism (HPT) characterized by total and ionized calcium within normal limits, associated with consistently elevated parathyroid hormone (PTH) levels, and with secondary causes of elevated PTH ruled out (1,2). NHPT has been associated with a high prevalence of osteoporosis, both densitometric and as reflected by a higher number of fragility fractures, although these data may be subject to bias (3,4), since osteoporosis is on of the reasons why PTH is tested. NHPT patients show no overt skeletal disease but dual-energy X-ray absorptiometry may detect evidence of bone involvement. Despite the preferential involvement of cortical bone, an increased rate of vertebral fractures has been reported in patients with NHPT, but no increase in the risk of femur and femoral neck fractures (5). This is due to trabecular microarchitectural deficits (4). The trabecular number, thickness, connectivity, and bone geometry may be important determinants of bone strength (6)

TBS is a texture parameter derived from DXA imaging and correlates with 3D bone microarchitecture parameters, such as connectivity density and trabecular number and negatively with trabecular separation (7). Other methods of evaluating microarchitecture, such as high-resolution peripheral quantitative computed tomography (HR-pQcT) and high-resolution magnetic resonance (HR-MRI) are more expensive and difficult to perform in clinical practice (8). To our knowledge, no data are available on the clinical utility of TBS in assessing skeletal involvement in patients with NHPT. The objective of this study was to

DOI:10.4158/EP151055.OR

evaluate differences in the TBS in NHPT and asymptomatic hypercalcemic hyperparathyroidism (HHPT) patients.

PATIENTS AND METHODS

Patients and procedure

We performed a prospective study which enrolled consecutive patients with asymptomatic HPT (NHPT or HHPT). Patients were followed for ≥ 1 year at the University Hospital of Valladolid, Spain, during which intact plasma PTH, 25-hydroxyvitamin D (25vitD), and serum total calcium were analyzed in at least two determinations.

NHPT was defined as persistently normal total, albumin-corrected, and ionized serum calcium concentrations and consistently elevated PTH levels. HHPT was defined as consistently elevated or unsuppressed PTH and at least one determination of total, albumin-corrected, or ionized serum calcium above the upper reference limit, without clinical manifestations. Asymptomatic hyperparathyroidism includes NHPT and HHPT. Secondary causes of elevated PTH were ruled out, including renal disease (glomerular filtration rate < 60ml/min), vitamin D deficiency (25VitD < 30 ng/ml), genetic disorders such as familial hypocalciuric hypercalcemia, other metabolic bone diseases and thiazide diuretic, lithium or bisphosphonate treatment. Secondary elevation of PTH and symptomatic HHPT patients were excluded from the study. The control group was composed of men and postmenopausal women matched for age and

DOI:10.4158/EP151055.OR

body mass index (BMI) with asymptomatic HPT patients. Control subjects had normal levels of total serum calcium and PTH. Clinical fractures were evaluated

The study was carried out at the Endocrinology and Nutrition Research Centre (IEN), and University Hospital of Valladolid, Spain. It was performed according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the local Ethics Committee. Written informed consent was obtained from all patients. A standardized questionnaire was used to collect clinical and laboratory data. Clinical features were recorded after patients visited the hospital. The privacy and confidentiality of the data collected was ensured.

Methods

Biochemical studies were measured in fasting conditions and included: intact PTH (reference range, 10–65 pg/ml), total serum calcium (reference range, 8. 5–10. 5 mg/dl), total serum phosphorus, 25vitD (reference range 3–70 ng/ml). Calcium and creatinine were measured in urine 24 hours. Areal bone mineral density (aBMD) was assessed by dual-energy X-ray absorptiometry (Prodigy, IDXA, GE-Lunar, General Electrics Healthcare, USA) at the lumbar spine (L1-L4), proximal femur, and femoral neck. The aBMD T-score and Z-score were evaluated at the three sites. The TBS was evaluated at the lumbar spine (L1-L4) using TBSiNsight®v2.1 (Med-Imaps, Merignac, France).

Statistical Analysis

The results are expressed as means and standard deviation (SD). The distribution of variables was analyzed using the Kolmogorov-Smirnov test. Quantitative variables with a normal distribution were analyzed using the two-tailed Student's-t test. The Chi square test was used to evaluate qualitative variables. Multivariate multiple linear regression was used to evaluate the contribution of PTH, age, sex and BMI in predicting TBS levels. A p-value < 0.05 was considered statistically significant. All analyses were carried out using the

DOI:10.4158/EP151055.OR

Statistical Package for Social Sciences (SPSS) version 20. 0 (SPSS, Chicago, IL, USA)

RESULTS

We included 39 patients with asymptomatic HPT (24 NHPT and 15 HHPT). The control group was composed of 24 patients matched for age, sex, and BMI with asymptomatic HPT patients. All subjects were Caucasian. Table 1 shows the characteristics of the three groups. There were no clinical fractures.

NHTP and HHPT patients were similar in terms of age, BMI, and gender distribution. NHPT patients had significantly-lower PTH (p=0.003) and total serum calcium (p=0.001) levels but higher total serum phosphorus (p=0.04) and vitamin D (p=0.01). No significant differences were found in 24-hour urine calcium (218 ± 121 mg vs 252 ± 178 mg) or in the urine calcium/creatinine ratio (0.26 ± 0.13 vs 0.31 ± 0.15). No significant differences in TBS or aBMD (all sites) were found between NHTP and HHPT patients.

Asymptomatic HPT patients and control subjects were similar in terms of vitamin D levels and total serum phosphorus. Asymptomatic HPT patients had significantly-higher PTH and total serum calcium levels (p<0.0001 and 0.013, respectively). Asymptomatic HPT patients had significantly lower TBS values (p<0.0001) and aBMD values at all sites (p=0.04, 0.001 and 0.01 at the spine, femoral neck and total hip, respectively). Of asymptomatic HPT patients, 31% were classified as osteoporotic (T-score≤-2.5SD) compared with 0% of controls. Likewise, 41% of asymptomatic HPT patients had a TBS lower than 1.200, degraded microtexture, compared with 4% of controls (p= 0.043). A significant positive relationship between TBS and lumbar aDXA (r=0.529, p= 0.008) was found in controls but not in asymptomatic HPT patients (r=0.319, p=0.098). There was no correlation between hip DXA and TBS in either group.

A significant negative relationship between PTH and TBS was observed in asymptomatic HPT patients (r=-0. 329, p=0. 043) but not in the control group. There wasn´t a significant relationship between PTH and spine aDXA (r= -

DOI:10.4158/EP151055.OR

0.289, p= 0.136) and hip aDXA (r=0.097, p=0.642). After adjustment for age, sex and BMI, the correlation between TBS and the PTH level remained stable and significant (β = -0. 370, p = 0. 011).

DISCUSSION

Our results show there was no difference in the TBS between NHPT and HHPT patients. There was a reduction in the TBS in patients with asymptomatic HPT which was linked to the PTH level but had no repercussion on bone mass. There was no relationship between TBS and lumbar and hip aDXA.

PTH acts on osteoblasts by increasing the production of RANKL and inhibiting the synthesis of osteoprotegerin (3), increasing bone turnover and being responsible for the alterations in microarchitecture observed in patients with asymptomatic HPT. In patients with hyperparathyroidism, extirpation of the abnormal parathyroid gland reduces PTH, normalizes serum calcium and markers of bone turnover, and is associated with an increase in BMD and normalization of the microarchitecture (9). TBS has been proposed as an indirect index of bone microarchitecture that is simple and easy to perform in clinical practice (10). Studies with new HRpQcT techniques have shown a deterioration in the cortical and trabecular microstructure in patients with primary HPT that resulted in decresed whole-bone stiffness and trabecular stiffness (11). However, these techniques are expensive and not available in all hospitals. Therefore, a technique such as TBS, which is simple to perform using conventional DXA, identifies deterioration of bone microarchitecture. Our results showed no differences in the TBS in patients with NHPT and HHPT but a reduction in asymptomatic HPT patients (NHPT and HHPT) compared with controls. There are no previous studies of the TBS in patients with NHPT, and very few studies have analyzed the role of the TBS in patients with HHPT. A case-control study of 73 patients diagnosed with primary HPT by Romagnoli et al (12) found a reduction in TBS. TBS allowed the differentiation of patients

DOI:10.4158/EP151055.OR

without and with vertebral fracture, whose values were even lower. Similar results were obtained by Eller-Vainicher et al (13), who observed an improvement in TBS after surgery. Silva et al (14) evaluated the correlation between TBS and HRpQcT in 22 postmenopausal women with primary HPT. TBS correlated with whole bone stiffness and all HRpQCT indices, except for trabecular thickness and trabecular stiffness at the radius, and with all indices of trabecular microarchitecture, except trabecular thickness. Our results in patients with NHTP are similar to those described in these studies.

However, some studies have compared bone alterations between NHPT and HHPT patients. The used procedures were DXA and the prevalence of fractures. The prevalence of osteoporosis and osteopenia was similar, with no differences in DXA values (15-18). Only the study by Amaral et al (18) found that patients with NHPT had higher BMD values in the distal radius. All patients with HHPT had higher levels of PTH and serum calcium, in agreement with our results. There may be two possible explanations for these results: first, that NHPT is a mild initial form of HHPT, and second that there is resistance to PTH in the renal and bone target organs (19)

Globally, patients with asymptomatic HPT had lower TBS levels than the control group, even though there were no differences in lumbar DXA. The International Society for Clinical Densitometry (ISCD) has determined that this procedure is associated with the risk of vertebral and hip fracture and greater osteoporotic fracture, although there is no evidence on its therapeutic indication in solitary (20). Our patients had a reduction in aBMD and TBS, which was similar in NHTP and HHPT patients although the latter group had a significantly higher level of PTH. The increase in PTH might explain the reduction in aBMD and TBS due to the increase in bone remodeling.

Patients with HHPT are characterized by a lower bone mass at the cortical level with preservation of trabecular BMD (21), as confirmed by histomorphometric studies. Typically, bone loss is greater in the forearm, where there is a predominance of cortical bone, and is lower in the lumbar column, where trabecular bone predominates (21). However, our patients had a low BMD at

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both cortical and trabecular sites. Moreover, clinically, an increased risk of forearm and vertebral fractures was observed (22, 23). It may be that the TBS is more sensitive in the evaluation of bone damage in the asymptomatic forms of hyperparathyroidism. Our results showed no differences in lumbar DXA between patients and controls. However, the TBS was lower in patients with asymptomatic PTH. Studies with a larger sample size are necessary to determine the role of TBS in the management of these patients.

The main limitations of our study are the small sample size and the lack of data on fractures. However, no patient reported a history of osteoporosis-related (non-traumatic) or during follow clinical fractures. Moreover, BMD was not measured in the 33% radius. The main strength of the study is the diagnosis of NPHPT, with two determinations of calcium at a one year interval.

In conclusion, there was no difference in the TBS between NHPT and HHPT patients. However, there was a reduction in the TBS in patients with asymptomatic HPT that was possibly related to PTH levels but had repercussion on bone mass. Higher levels of PTH seem to be responsible for this alteration in microarchitecture texture.

Acknowledgements: This work received a grant from SEIOMM-MEDIMAPS. We thank Luis Del Río MD and Renaud Winzenneth MD for their review of the manuscript

DOI:10.4158/EP151055.OR

REFERENCES

1. **Díaz-Soto G, Julián MT, Puig-Domingo M.** Normocalcemic primary hyperparathyroidism: a newly emerging disease needing therapeutic intervention. *Hormones (Athens, Greece)*. 2012; 11:390-6.
2. **Eastell R, Brandi ML, Costa AG, D´Amour P, Shoback DM, Thakker RV.** Diagnosis of asymptomatic primary hyperparathyroidism: Proceedings of the Fourth International Workshop. *J Clin Endocrinol Metab* 2014; 99:3570-9
3. **Lewiecki EM, Miller PD.** Skeletal effects of primary hyperparathyroidism: Bone mineral density and fracture risk. *J Clin Densitom* 2013; 16: 28-32
4. **Rejnmark L, Vestergaard P, Brot C, Mosekilde L.** Increased fracture risk in normocalcemic postmenopausal women with high parathyroid hormone levels: a 16-year follow-up study. *Calcif Tissue Int* 2011; 88: 238-245
5. **Vestergaard P, Mollerup CL, Frokjaer VG et al.** Cohort study of risk of fracture before and after surgery for primary hyperparathyroidism. *BMJ* 2000; 321: 598-602
6. **Parisien M, Silverberg SJ, Shane E et al.** The histomorphometry of bone in primary hyperparatyroidism: preservation of cancellous bone structure. *J Clin Endocrinol Metab* 1990; 70: 930-938

DOI:10.4158/EP151055.OR

7. **Bousson V, Bergot C, Sutter B, Levitz P, Cortet B, the Scientific Committee of the GRIO (Groupe de Recherche et d´Information sur les Osteoporoses).** Trabecular Bone Score (TBS): available knowledge, clinical relevance and future prospects. *Osteoporos Int* 2012; 23: 1489-1501

8. **Link TM, Vieth V, Sthelling C et al.** High-resolution MRI vs multislice spiral CT: wich technique depicts the trabecular bone structure best ?. *Eur Radiol Apr* 2003; 13: 633-671

9. **Christiansen P, Gartenberg F, Jacobs TP et al.** Primary hyperparathyroidism: short-term changes in bone remodeling and bone mineral density following parathyroidectomy. *Bone* 1995; 25: 237-244

10. **Hans D, Barthe N, Boutroy S, Pothuaud L, Winzenrieth R, Krieg MA.** Correlation between trabecular bone score, measured using anteroposterior dual-energy x-ray absortiometry acquisition and 3-dimensional parameters of bone microarchitecture: an experimental study on human cadaveric vertebrae. *J Clin Densitom* 2011; 14: 302-312

11. **Stein EM, Silva BC, Boutroy S et al.** Primary Hyperparathyroidism is associated with abnormal cortical and trabecular microstructure and reduced bone stiffness in postmenopausal women.*J Bone Miner Res* 2013; 28: 1029-1040

12. **Romagnoli E, Cipriani C, Nofroni I et al.** Trabecular Bone Score (TBS): An indirect measure of bone micro-architecture in postmenopausal patients with primary hyperparathyroidism. *Bone* 2013; 53: 154-159

DOI:10.4158/EP151055.OR

13. **Eller-Vainicher C, Filopanti M, Palmieri S et al.** Bone quality as measured by trabecular bone score in patients with primary hyperparathyroidism. *Eur J Clin Endocrinol* 2013; 169: 155-162

14. **Silva BC, Boutroy S, Zhang C et al.** Trabecular bone score (TBS): a novel method to evaluate bone michroarchitectural texture in patients with primary hyperparathyroidism. *J Clin Endocrinol Metab* 2013; 98: 1963-1970

15. **García-Martín A, Reyes-García R, Muñoz-Torres M.** Normocalcemic primary hyperparathyroidism: one year follow-up in one hundred postmenopausal women. *Endocrine* 2012; 42: 764-766

16. **Tuna MM, Cahskan M, Unal M et al.** Normocalcemic hyperparathyroidism is asociated with complications similar to those of hypercalcemic hyperparathyroidism. *J Bone Miner Metab* 2015 DOI 10.1007/s00774-015-0673-2

17. **Spivacow FR, Sapag Duran A, Zanchetta MB.** Hiperparatiroidismo primario normocalcémico. *Medicina (Buenos Aires)* 2014; 74: 457-461

18. **Amaral LM, Queiroz DC, Marques TF, Mendes M, Bandeira F.** Normocalcemic versus hypercalcemic primary hyperparathyroidism: more stone than bone ? *J Osteoporosis* 2012 Article ID 128352

19. **Pawlowska M, Cisano NE.** An overview of normocalcemic primary hyperparathyroidism. *Curr Opin Endocrinol Diabetes Obes* 2015; 22: 413-421

20. **Silva BC, Broy SB, Boutroy S, Schousboe JT, Shepherd JA, Leslie WD.** Fracture risk prediction by non-BMD DXA measures: the 2015 ISCD

DOI:10.4158/EP151055.OR

Official Positions. Part 2: Trabecular Bone Score. *J Clin Densitom* 2015; 18: 309-330

21. **Silverberg SJ, Shane E, De la Cruz L et al.** Skeletal disease in primary hyperparathyroidism. *J Bone Miner Res* 1989; 4: 283-291

22. **Vignali E, Viccice G, Diacinti D et al.** Morphometric vertebral fractures in postmenopausal women with primary hyperparathyroidism. *J Clin Endocrinol Metab* 2009; 94: 2306-2312

23. **Khosla S, Melton LJ 3rd, Wermers RA, Crowson CS, O´Fallon WM, Riggs BL.** Primary hyperparathyroidism and the risk of fracture: a population-based study. *J Bone Miner Res* 1999; 14: 1700-1707

DOI:10.4158/EP151055.OR

Tabla 1. Characteristics of patients with normocalcemic hyperparathyroidism (NHPT), hypercalcemic hyperparathyroidism (HHPT) and control group

	NHPT (24)	HHPT (15)	Control (24)
Age (years)	66 ± 13	64 ± 12	63 ± 12
Gender Ratio (Female/Male)	20/4	12/3	21/3
BMI	26 ± 4	27 ± 5	26 ± 6.
PTHi (pg/ml)	98 ± 22[a]	134 ± 49	25 ± 20[b,c]
25-hidroxy vitamin D (ng/ml)	27 ± 11	25 ± 18	19 ± 14
serum calcium (mg/dl)	9.5 ± 0.4[d]	10.6 ± 0.6	9.4 ± 0.3[e]
Serum phosphorus (mg/dl)	3.4 ± 0.5[f]	3.02 ± 0.6	3.6 ± 0.5[g]
TBS	1.228 ± 0.1	1.221 ± 0.1	1.328 ± 0.1[h]
z-score TBS	-0.60 ± 0.47	-0.76 ± 1.14	-0.52 ± 1.18[i]
TBS < 1.200 (Degraded)	37 %	40 %	4 %[j]
L1-L4 DXA (g/cm^2)	1.004 ± 0.17	0.999 ± 0.2	1.100 ± 0.16
Femoral neck (g/cm^2)	0.754 ± 0.07	0.793 ± 0.01	0.841 ± 0.07[k]
Total hip (g/cm^2)	0.822 ± 0.11	0.850 ± 0.10	0.909 ± 0.09[l]
Osteoporosis %	29 %	33%	0%[m]

a) NHPT vs HHPT p= 0.003; b) NHPT vs control p= 0.0001; c) HHPT vs control p= 0.0001 d) NHPT vs HHPT p= 0.0001 e) HHPT vs control p= 0.0001
f) NHPT vs HHPT p= 0.026 g) HHPT vs control p= 0.026
h) AHPT vs control p= 0.001, i) AHPT vs control p= 0.001 j) AHPT vs control p= 0.0001 k) AHPT vs control p= 0.002; l) AHPT vs control p= 0.03; m) AHPT vs control p= 0.001