International Journal of Orthopaedics Sciences

ISSN: 2395-1958
IJOS 2017; 3(1): 564-567

www.orthopaper.com
Received: 21-11-2016
Accepted: 22-12-2016

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Vitamin D status and bone turnover marker levels in Greek women with fragility hip fracture

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DOI: http://dx.doi.org/10.22271/ortho.2017.v3.i1i.81

Abstract
There is little data regarding Vitamin D deficiency in Greece. Also conflicting are the data regarding the bone turnover during the acute hip fracture interval and the situation is even more complex considering the recently developed bone turnover markers (BTMs). The aim of this study was to evaluate the parameters of bone mineral homeostasis of women with fragility hip fracture using the recently developed BTMs of carboxy-terminal collagen crosslinks (CTx) and the procollagen type 1 amino-terminal propeptide (PINP) and to determinate vitamin D (25-OHD) levels of elderly Greek women with acute hip fracture. 38 self-sufficient, community-living Greek women with acute hip fracture were included. A similar number of age- and sex-matched controls with no clinically evident fractures were included in the study meeting the same inclusion and exclusion criteria. To exclude the effect of trauma blood samples were drawn within 24 hours from the fracture. Hip fracture group had significantly lower serum 25-OHD levels and significantly higher intact parathormone (PTH) levels compared to the control group but no correlation between PTH and 25-OHD was found in both groups. CTx was significantly correlated with PINP in the total group and in both groups separately but there was no statistical difference of their levels between the two groups. There was high prevalence of severe Vitamin D deficiency within the postmenopausal Greek women with acute hip fracture and the fracture did not influence the above mentioned BTMs. The processes of bone resorption and bone production are in balance during the first 24 hours after the fracture.

Keywords: Hip fracture; bone turnover markers; CTX; PINP; vitamin D

1. Introduction
Fragility fractures of the hip are common among women with osteoporosis and contribute to an increase of morbidity and mortality in the elderly. Subclinical vitamin D deficiency is related to decreased bone strength and it is also a risk factor for osteoporotic hip fractures [1-3]. Inadequate sun exposure and poor nutrition in elderly people may cause vitamin D insufficiency [4, 5]. Several studies from Europe, Australia and Asia have revealed presence of variable vitamin D deficiency and secondary rise in parathormone (PTH) levels in women with hip fracture [6-10]. The prevalence of vitamin D deficiency though, is not well known in hip fracture patients from Greece.
Controversy persists whether osteoporotic fractures can be prevented with vitamin D supplementation [11, 12]. Furthermore, there is little and conflicting data regarding the bone turnover during the acute hip fracture interval and the situation is even more complex considering the recently developed bone turnover markers (BTMs), which are that is the carboxy-terminal collagen crosslinks (CTx) and the pro-collagen type 1 amino-terminal pro-peptide (PINP) [13-14]. Therefore, this matched control study was carried out to evaluate the parameters of bone mineral homeostasis including levels of serum 25-hydroxyvitamin D (25-OHD) and intact PTH in Greek postmenopausal women with acute hip fracture.

2. Material and Methods
Thirty-eight women with low energy hip fractures from the women admitted to our institution because of an acute hip fracture were included with the following inclusion criteria: self-sufficiency (independency), community-living.

Women with fracture following a road traffic accident and with tumor, primary hyperparathyroidism or secondary causes of osteoporosis (i.e. renal insufficiency, corticosteroid use) were excluded. Participants on treatment with calcium and vitamin D, anti-osteoporotic or other medications potentially influencing the bone metabolism were also excluded from the study. None of the patients had alcohol consumption of 3 or more units/day or smoked more than10 cigarettes/day

Control individuals were selected from women after the age of 65 referred to our outpatients department for a routine checkup for osteoporosis. From these women referred to our outpatients department 43 age-matched to the fracture group with no clinically evident osteoporotic fractures were included in the study meeting the same inclusion and exclusion criteria. All patients were informed of the nature of the study, and consent was obtained from each participant.

Blood samples were collected within 24 hours of admission. Fasting venous samples of all study subjects were drawn between 8 and 11 a.m. within 24 h from the time of the fracture to rule out the effect of the trauma or immobilization on 25-OHD levels and BBMs [15]. Serum was separated in a centrifuge and stored at -20°C for 25-OHD, CTx and PINP assays. Plasma was used for to measure the intact PTH. All tests were conducted under the supervision of a qualified biochemist.

Serum calcium and serum phosphate were determined with standard methods on the day of collection. Blood urea, creatinine, bilirubin, aspartate aminotransferase, and alanine aminotransferase levels were also determined to assess liver and renal function and exclude secondary causes of osteoporosis (i.e. renal insufficiency). The normal levels of serum calcium and phosphate were 8.7–10.2 and 2.5–4.5 mg/dl respectively. PINP was assayed as a bone formation marker (total procollagen type 1 amino-terminal propeptide kit, Roche Diagnostics Mannheim, Germany; reference values for post-menopausal women: 16-73 ng/ml). CTx was assayed as a bone resorption marker (β-CrossLaps/serum kit, ECLIA Roche Diagnostics Mannheim, Germany; reference values for post-menopausal women: <1.008 ng/ml). Plasma Parathyroid hormone (PTH) was measured by an enzyme-labeled immunometric assay, which detects intact PTH molecules (IMMULITE 2000 intact PTH immunoassay, Loss Angeles, U.S.A.; reference values: 16-87 pg/ml). Serum vitamin D was determined by measuring serum 25-OHD (Vitamin D total immunoassay, Roche Diagnostics Mannheim, Germany). Diagnosis of vitamin D deficiency was considered when levels of 25-OHD were <20 ng/ml [16].

Analysis was performed using computer software SPSS for Windows, version 14.0 (SPSS Inc., Chicago, IL, USA). Difference between the two means was performed using Student's t-test. Correlations were examined using Pearson's correlation test. Chi-square test was used to assess differences in the proportion between qualitative data. The confidence intervals for the correlation of the mean values were 95% and level of statistical significance was set at $p < 0.05$.

3. Results

The summary statistics of the study subjects is given in table 1. There was no significant difference in age between the hip fracture and the control group (p=0.601). Hip fracture group had significantly lower serum 25-OHD levels (p<0.001), significantly higher intact PTH levels (p<0.001) and significantly lower phosphate (P) levels (p<0.001) compared to the control group. None of the women had biochemical parameters suggestive of primary hyperparathyroidism. Compared to the 83% of the participants with hip fracture with serum 25-OHD levels less than 20 ng/ml, only 33% of the control group had 25-OHD less than 20 ng/ml. This difference is also statistically significant (p<0.001).

Correlations were also examined within the two groups. Among the hip fracture participants (table 2), there is positive correlation between CTx and PINP (r=0.612, p<0.001). The same is observed between CTx and PINP in the control group (table 3, r=0.654, p<0.01). Also, CTX and PINP are significantly correlated in the total group (r=0,555, p=0, 01).

In hip fracture participants with 25-OHD deficiency (<20 ng/ml), the positive correlation between CTx and PINP is even more significant (r=0.796, p<0.001). Also for the non-fracture group although there is significant correlation between Ca and PINP we don't think this is of clinical value (table 3).

Table 1: Baseline characteristics of the women with hip fracture and controls.

Parameters	Fracture (n=38)	Control (n=43)	p Value
Mean age (years)	81.2 ±10.3	79.9 ±11.4	p=0.601
25-OHD (ng/ml)	14.8 ±9,6	26 ±11	p<0.001
intact PTH (pg/ml)	118.8 ±69.9	81.4 ±40.7	p<0.001
P (mg/dl)	3.5 ±0,5	3.9 ±0.3	p<0.001
CTx (ng/ml)	0.40 ±0.21	0.34 ±0.17	p=0.149
PINP (ng/ml)	68.5±122	45.3 ±29.9	p=0.247
Ca (mg/dl)	8.6 ±0.44	8.8 ±0.33	p=0.094
Vitamin D deficiency, n (%)	31 (83)	13 (33)	p<0.001
Secondary Hyperparathyroidism, n (%)	24 (66)	19 (48)	P=0.052

Table 2: Correlation matrix for Ca, P, VITD, PTH, CTx and PINP (fracture group)

Variables	Ca	P	VITD	PTH	CTx	PINP
Ca		-0.182	-0.269	-0.015	0.004	-0.032
P			0.076	0.028	-0.017	-0.094
VITD				0.140	-0.233	-0.208
PTH					0.184	0.199
CTx						0.612**

**. Correlation is significant at the 0.01 level.
*. Correlation is significant at the 0.05 level.

Table 3: Correlation matrix for Ca, P, VITD, PTH, CTx and PINP (non fracture group)

Variables	Ca	P	VITD	PTH	CTx	PINP
Ca		-0,026	0,177	0,183	0,003	0,314*
P			-0,262	0,08	-0,057	0,132
VITD				-0,041	0,141	-0,062
PTH					-0,2	0,02
CTx						0,654**

*. Correlation is significant at the 0.05 level.

4. Discussion and Conclusion

The present study evaluates the parameters of bone mineral homeostasis in postmenopausal women with fragility hip fracture. According to the results 25-OHD was significantly lower and intact PTH was significantly higher in the hip fracture group compared to controls, consistent with previously reported results [7, 17, 18]. There are several studies reporting high prevalence of vitamin D deficiency in hip fracture women around the world. In a study on a British population (Baker *et al*.), from 98 women with neck of femur fracture, 40 % had 25-OHD level of <10 ng/ml, and mean serum 25-OHD of 13.8ng/ml. Similarly in another study from the US, 50% of women with osteoporotic hip fracture had a serum 25-OHD level less than 12 ng/ml [18]. The same vitamin

D deficiency is reported in a study from Italy as 21.6% of patients with hip fracture had serum 25-OHD less than 20 ng/ml [17]. In Spain, 67% of the patients with hip fracture had vitamin D levels less than 20 ng/ml, and 55 % had elevated intact PTH [9].

Our results show that 83% of the hip fracture participants had a serum 25-OHD less than 20 ng/ml and 51% had a level of less than 10ng/ml; these percentages were higher compared to the aforementioned studies. On the control group only 33% had a serum 25-OHD less than 20 ng/ml and 2.56% less than 10 ng/ml. These high percentages of vitamin D deficiency involve women from the Mediterranean country of Greece. As far as we are aware this is the first report in literature of high prevalence of Vitamin D deficiency in Greek women with fragility hip fracture. The cause for such a high prevalence of vitamin D deficiency in our study might be multifactorial and needs further investigation. According to other studies, inadequate dietary calcium and vitamin D intake, inadequate sun exposure, environmental pollution and lack of food fortification with vitamin D might contribute to hypovitaminosis D [9, 10, 17, 18]. Infact, the present study was conducted in the capital city of Athens with known problems of environmental pollution and with a common life style of limited sun exposure particularly for elderly people. Furthermore, it is our assumption that the economic crisis has negatively influenced diet and vitamin D intake as also life style and sun exposure.

Osteomalacia has been confirmed and levels of P are within or lower than normal limits in patients with hip fracture [20, 21, 22]. In our study 81% of the hip fracture women had evidence of biochemical osteomalacia comparing to the 30% of the control group. This may also explain the lower levels of mean serum P in the patients with hip fracture although within normal range.

Regarding the relationship between intact PTH and 25-OHD, although intact PTH is significantly higher in the fracture group (table 1), no correlation between PTH and 25-OHD was found in both groups. According to the literature when the 25-OHD level become insufficient, the intact PTH level generally rises [20]. Twenty six (59%) of the 44 women in the current study with a low 25-OHD had an elevated PTH level (>87 pg/ml). According to Chapuy *et al*. low serum 25-OHD does not always lead to an increase in serum PTH [20]. Furthermore, Sahota *et al* [23]. suggested that a slight decrease in serum calcium and a substantial reduction in 25-OHD may partly cause failure of the parathyroid gland to provide with an adequate PTH response. It may well be that the cutoff for definition of an elevated PTH level requires further examination but on the other side this would mean a dramatic rise in the diagnosis, and possible mis-diagnosis, of secondary hyperparathyroidism.

Concerning the role of the new BBMs, CTx and PINP, in bone fragility it has been suggested that hip-fracture patients have decreased bone production and increased bone resorption when compared to age-matched controls [13, 24, 25, 26, 27]. In most of these studies measurements have been performed after the incident. In our study bone resorption and production markers were found positively correlated in both groups ($p<0.001$) implying coupling between the two processes. This relation was even stronger in patients with 25-OHD less than 10ng/ml ($p<0.001$). In addition, no significant difference between the fracture and the control group was found for the BTMs (p=0.149 for CTx and p=0.247 for PINP). This result is very likely to indicate that the fracture did not have any influence on their levels and bone production is positively related to bone resorption during the immediate post fracture period also.

As a conclusion, Vitamin D tends to be low and PTH increased in Greek postmenopausal women with fragility fractures. This has been previously demonstrated (though not specifically reported for Greek patients). Also, in contrast with previous studies patients with hip fracture have balanced bone metabolism and this finding is reported for the first time in literature as far as we are aware. Replication of the results with larger, prospective studies using the recently developed BTMs would be of value.

5. Ethics Committee Approval

Ethics committee approval was received for this study from Asklipieion Athens General Hospital Ethics Committee.

6. Informed Consent

All patients were informed of the nature of the study, and consent was obtained from each participant.

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