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MARKERS OF BONE TURNOVER AND 25-HYDROXY VITAMIN D IN WOMEN WITH TYPE 2 DIABETES AND NEWLY DIAGNOSED OSTEOPOROSIS

Raluca Nan [1], ***Adrian Cursaru*** [2], ***Daniel Grigorie*** [1,3], ***Alina Șucaliuc*** [1,3], ***Ramona M. Drăguț*** [1], ***Emilia Rusu*** [1,✉], ***Mădălina D. Mușat*** [1,3], ***Gabriela Radulian*** [1,4]

[1] „Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2] Bucharest Emergency University Hospital, Bucharest, Romania
[3] National Institute of Endocrinology „C.I. Parhon", Bucharest, Romania
[4] „Prof. N.C. Paulescu" National Institute of Diabetes, Nutrition and Metabolic Diseases, Bucharest, Romania

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Abstract

Background and Aims: *The aim of the present study was to evaluate the characteristics of bone turnover markers and 25-hydroxy vitamin D levels in women with type 2 diabetes and recently diagnosed postmenopausal osteoporosis.* ***Material and Methods:*** *We performed a retrospective study that included 80 women with newly diagnosed postmenopausal osteoporosis: 40 women with type 2 diabetes (diabetic group) and 40 women without diabetes (non-diabetic group). For data collection we used the patients database of the National Institute of Endocrinology "C.I. Parhon", Bucharest, Romania.* ***Results:*** *Women with type 2 diabetes had significantly reduced levels of osteocalcin and crosslaps than non-diabetic women. In the diabetic group, alkaline phosphatase and 25-hydroxy vitamin D were lower than in non-diabetic women but without statistical significance. There was not statistically significant difference of bone mineral density (BMD) between diabetic and non-diabetic groups. Magnesium was significantly lower in diabetic women compared with non-diabetic women.* ***Conclusions:*** *Our results demonstrated a low bone turnover in type 2 diabetic women and it was shown an insufficiency of 25-hydroxy vitamin D in both studied groups. These findings should be taken into account when screening these patients for diabetes complications.*

key words: *type 2 diabetes, osteocalcin, crosslaps, bone mineral density.*

Background and Aims

Diabetes is a major public health issue worldwide with a prevalence of 7.87% in Europe and 9.28% in Romania [1]. The relationship between diabetes and bone tissue has been investigated for many years. In 1948, Albright and Reifenstein showed a decrease in bone mineral density (BMD) and a major risk of fracture in patients with diabetes [2]. Currently, the mechanisms of bone changes in type 2 diabetes (T2D) appear to be different from those occurring in type 1 diabetes. In T2D hyperinsulinemia might contribute to a high BMD and insulin resistance may also occur in bone cells [3].

✉ Dionisie Lupu Street, No. 37, District 2, Bucharest, Romania; Phone: +40-742.959.946
corresponding author e-mail: emiliarusumd@yahoo.com

Early studies that have investigated bone turnover in diabetes used non-specific markers like urinary calcium and hydroxyproline [4,5]. In recent studies, osteocalcin, alkaline phosphatase (ALP) and bone specific alkaline phosphatase are used as markers of bone formation, while urinary or serum collagen type 1 cross-linked C-telopeptide (CTX), crosslaps (serum beta CTX) and urinary free deoxypyridinoline are used as markers of bone resorption [6]. In a genetically modified mouse model, osteocalcin signals increased insulin secretion and beta cell proliferation and regulated body fat mass and adiponectin gene expression [7].

Vitamin D status seems to play a key role for general health and particularly for the bone, even if it is variable and depends on sunlight and latitude. On the other hand, levels of vitamin D seem to be lower in obese individuals and its role in diabetes is still debated [8,9].

The aim of this study was to assess the characteristics of bone turnover using biochemical bone markers (osteocalcin, ALP and crosslaps) and 25-hidroxy vitamin D (25(OH)D) in women with T2D and recently diagnosed postmenopausal osteoporosis.

Material and Methods

Study design and control groups

We performed a retrospective study using the patients database of the National Institute of Endocrinology ”C.I. Parhon”, Bucharest, Romania, from June 2011 to June 2014. Inclusion criteria were: women aged between 50 and 80 years with newly diagnosed postmenopausal osteoporosis. Exclusion criteria were: thyroid gland disease, early onset hypogonadism, primary hyperparathyroidism, growth hormone deficiency, cirrhosis, reduced glomerular filtration rate (GFR <60 ml/min/1.73 m²), corticosteroid therapy, neoplasms, total or partial hysterectomy, rheumathoid artritis, systemic lupus erythematosus, malabsorbtion or any bone-active medication (e.g. bisphosphonates), menopause hormone replacement therapy at the time of bone assessment.

Treatments for diabetes were diet or oral agents (other than thiazolidinediones). Body mass index (BMI) was calculated according to World Health Organization criteria (WHO) [10].

Study groups comprised 40 women with postmenopausal osteoporosis and T2D (diabetic group) and 40 non-diabetic women with postmenopausal osteoporosis (non-diabetic group). Women were matched in terms of age. The study has been approved by the Institute of Endocrinology ”C.I. Parhon” Ethical Committee.

Biochemical parameters

The measurements of fasting plasma glucose (FPG), creatinine, total calcium, magnesium, ALP, phosphorus (photometric method) and glycosylated hemoglobin (HbA1c) (imunoturbidimetric method) concentrations were performed using the standard laboratory methods applied on Roche Cobas C501 (Roche, Hitachi Corporation Japan). Bone turnover markers (osteocalcin and crosslaps), parathyroid hormone (PTH) and 25(OH)D were measured by electrochemiluminescence immunoassay (ECLIA) on a Roche Cobas e 601 (Roche Hitachi Corporation Japan). HbA1c was measured only for the diabetic group.

Bone mineral density assessment

Postmenopausal osteoporosis was diagnosed according to WHO criteria at a value for BMD lower than -2.5 SD below the reference mean [11]. Each subject underwent measurement of BMD at lumbar and femoral neck using GE-iDXA machine (General Electric Company - Lunar iDXA, United States).

Statistical analysis

Data analysis was performed using Statistical Package for the Social Sciences

(SPSS). All variables in study group and controls are shown as elements of descriptive statistics (means, standard deviation). Comparisons among groups were made by use of ANOVA for quantitative variables and the $\chi 2$ test of independence for categorical variables. Correlation analysis was performed using Spearman's correlation coefficient. Two-tailed, p-values < 0.05 were considered statistically significant.

Results

In diabetic group was observed a higher BMI than in the non-diabetic group (Table 1) as well as higher FPG values as compared to the non-diabetic women. Appart BMI and FPG (not surprisingly higher in diabetic women), only magnesium levels were significantly lower ($p<0.001$) in the diabetic group (Table 1) while other biochemical parameters like calcium, phosphorus, creatinine did not show significant differences between the two groups (Table 1).

Table 1. The anthropometric and biochemical characteristics of the study populations.

Parameters	**Diabetic group (n=40)**	**Non-diabetic group (n=40)**	**p-value**
	Mean±SD	**Mean±SD**	
Age (years)	64.80±8.99	63.65±8.67	0.562
BMI (kg/m^2)	29.83±4.10	25.07±3.71	<0.001
Calcium (mg/dl)	9.59±0.45	9.60±0.48	0.887
Magnesium (mg/dl)	1.87±0.22	2.07±0.19	<0.001
Phosphorus (mg/dl)	3.63±0.45	3.54±0.51	0.431
FPG (mg/dl)	135.94±51.54	91.16±11.71	<0.001
HbA1c (%)	6.99±0.90	-	-
Creatinine (mg/dl)	0.72±0.17	0.69±0.13	0.405

BMI, body mass index; FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin

Table 2. Markers of bone turnover, 25(OH)D and BMD in diabetic and non-diabetic women.

Parameters	**Diabetic group (n=40)**	**Non-diabetic group (n=40)**	**p-value**
	Mean±SD	**Mean±SD**	
Osteocalcin (ng/ml)	19.74±11.08	31.47±14.68	<0.001
Crosslaps (ng/ml)	0.30±0.16	0.56±0.28	<0.001
ALP (UI/l)	74.72±21.79	90.56±55.76	0.102
25(OH)D (ng/ml)	17.33±9.16	18.77±8.62	0.477
PTH (pg/ml)	47.35±17.13	44.88±16.80	0.545
BMD femoral neck (T score)	-2.27±0.80	-2.38±0.66	0.585
BMD lumbar (T score)	-3.02±0.52	-3.23±0.72	0.147

ALP, alkaline phosphatase; PTH, parathyroid hormone; BMD, bone mineral density

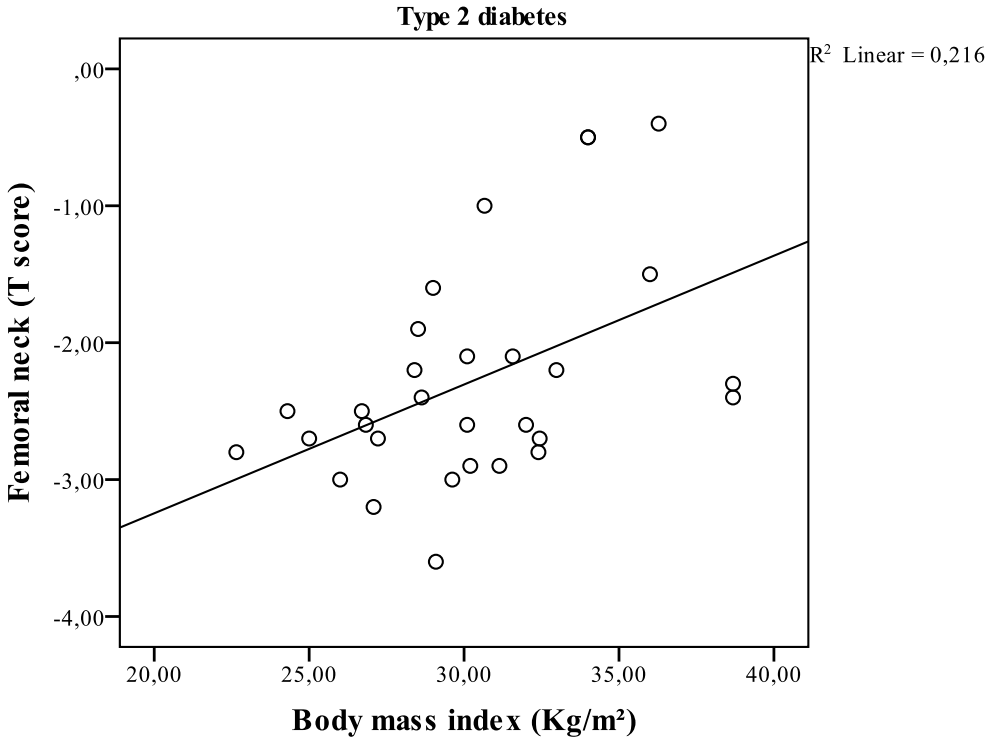

Figure 1. *Correlation between bone mineral density and body mass index.*

Concentrations of osteocalcin and crosslaps in women with T2D were significantly lower than in women without diabetes (Table 2). Another marker of bone formation, ALP was lower in diabetic group compared with control-group, but without statistical significance (Table 2).

The levels of 25(OH)D were not statistical significant between the groups while concentration of PTH tended to be higher in diabetic group but without statistical significance (Table 2).

Compared to controls, a positive correlation between BMI and BMD - femoral neck was observed in the T2D group (Figure 1), correlation that was not found in the non-T2D group. BMD – lumbar was not correlated with BMI in both studied groups.

There was not statistically significant difference between BMD in women with T2D than non-T2D (Table 2).

Discussions

A strong relationship was observed between osteoporosis and increased risk of fracture in patients with T2D, although the mechanisms of bone changes in T2D are still poorly understood. Osteoporosis leads to less bone strength and is accompanied with increase of fracture risk [12,13]. In our study BMD (T score) was decreased in both groups (women with recently diagnosed and not treated osteoporosis). Data regarding the impact of T2D on the BMD are controversial. Thus, different studies have found that people with T2D have increased [13,14], similar [15,16] or decreased [17,18] bone mass in comparison to healthy control subjects. According to these studies BMD seems to underestimate the diagnosis of osteoporosis in this group of patients and markers of bone turnover could be potentially better candidates for the evaluation of osteoporosis. Lower osteocalcin and crosslaps levels may suggest slower bone metabolism with reduced bone

formation and bone resorption in T2D compared to non-T2D. Similar findings have been reported by Farr et al. [14] and Oz et al. [19]. Lower serum levels of crosslaps in women with diabetes suggest a mild decrease in osteolysis as a result of insufficient formation process due to defects in osteoblasts maturation. It is unclear whether or not using bone turnover markers could predict the occurrence of new fractures in T2D patients, but they seem to provide more insights [12,20].

Previous studies have shown that both osteoporosis and diabetes are associated with low levels of vitamin D. Vitamin D is likely to have an important role in glycemic control, probably by the associated beta cell dysfunction and insulin resistance in cases with vitamin D deficiency [8]. In our whole study group, 57.5% of women with recently diagnosed osteoporosis had levels of 25(OH)D below 20 ng/ml, suggesting vitamin D insufficiency according to the guidelines of the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) [21]. The percentage was similar in both study groups. This is in accordance with other studies showing that 25(OH)D levels did not differ between patients with T2D and non-T2D, indicating that they were equally deficient [8,14]. Another study conducted by Grigorie et al. has shown a very high prevalence of vitamin D deficiency (22.23%) and insufficiency (61.26%) in 1048 Romanian women with postmenopausal osteoporosis [22].

Magnesium deficiency appears to play an important role in osteoporosis and this was observed in patients with diabetes in our study. Hypomagnesemia can reduce insulin secretion, alter cellular glucose transport and insulin–insulin receptor interactions [23]. Lower magnesium levels were also observed in our diabetic group. The same result was obtained by Diwan et al. [24] and by Kauser et al. [25] in their studies.

When women enter menopause, there is a decline in circulating estrogens which determines alterations in energy homeostasis and makes the body cells less responsive to insulin or predispose to insulin resistance [26]. Therefore, menopausal women are three times more likely to develop obesity and metabolic syndrome abnormalities than premenopausal women. The overweight and obesity are protective factors on bone health, respectively of BMD while a low BMI is associated with decrease BMD and thus, with a higher risk fracture [12,27]. In a meta-analysis, Vestergaard [28] has shown that BMI was significantly associated with BMD in T2D but not in T1D. Similar results were also reached in our study for T2D women. On contrary, hyperglycemia represents a factor which accelerates bone resorption process [12].

It should be acknowledged that our study has several limitations, including its retrospective design and the small number of patients.

Conclusions

In the present study, women with type 2 diabetes and recently diagnosed postmenopausal osteoporosis have significantly reduced levels of osteocalcin and serum crosslaps. Lower serum levels of 25(OH)D were observed both in diabetic and non-diabetic groups. These data suggest a low bone turnover in diabetic women and an insufficiency of 25(OH)D in the population studied. These findings should be taken into consideration when talking about diabetes complications.

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Conflicts of interest: The authors have no conflicts of interest to declare in relation to this article.

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