Serum β-CrossLaps as a predictor for osteoporosis in postmenopausal women with early diabetic nephropathy

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Background
Osteoporosis is a progressive bone disease with an increased risk of fracture.
Objective
To estimate bone turnover through an early marker, β-CrossLaps (β-CTx), in postmenopausal women with type 2 diabetes mellitus (DM) and early diabetic nephropathy (DN).
Patients and methods
A case–control study comprised 80 Egyptian postmenopausal nonsmoker women: 26 women without DM (group 1) as a control group, 28 women had type 2 DM without DN (group 2), and 26 woman were type 2 DM with early DN (group 3). All demographic and clinical data were collected. Laboratory tests including serum calcium, phosphorus, alkaline phosphatase (ALP), bone ALP, β-CTx, glycated hemoglobin, microalbuminuria, serum creatinine, and blood sugar levels were estimated.
Results
Regarding bone minerals, there was a significant lower serum level of calcium and phosphorus in group 3 compared with groups 1 and 2 (P=0.029 and <0.0001, respectively). Groups 2 and 3 had significantly lower serum levels of total ALP (P=0.011 and 0.006, respectively) and bone ALP (with lowest level in group 3) (P<0.0001) compared with group 1. Groups 2 and 3 had significantly higher serum levels of β-CTx (with highest level in group 3) compared with group 1 (P=0.016 and 0.003, respectively). Dual-energy X-ray absorptiometry scan revealed a significant elevation in osteoporosis percent in group 3 (63.4%) in comparison with group 1 (26.9%) (P=0.001), whereas osteopenia percent was significantly higher in group 2 (P=0.004) compared with group 3.
Conclusion
In diabetic postmenopausal women, serum β-CTx and microalbuminuria are potentially useful tools for assessing bone resorption state.

Introduction

Osteoporosis is a bone metabolic disease that is considered as an inevitable aging consequence [1]. Osteoporosis is characterized by reduced bone mass and changed bone quality, with microarchitectural defects, leading to reduced bone strength with high fracture risk [2]. Old age patients with diabetes mellitus (DM) were reported to have a higher fractures risk, with 50–80% increased extremity fracture risk [3]. Women with type 2 DM have a three-fold higher vertebral fracture risk in comparison with nondiabetic women [4]. Osteoporosis is a common disease that women face after their menopause and is caused by both environmental and genetic factors [5]. Although the relationship between DM and osteoporosis has been widely investigated, it remains controversial. DM could influence bone and increase fracture risk through several mechanisms, mainly mechanical loading and hormonal factors; the absence of estrogen in postmenopausal women may be one of the major factors for accelerated bone loss [6]. Diabetic nephropathy (DN) is a common complication of diabetes and the leading cause of chronic kidney disease and associated with osteoporosis and osteodystrophy [7]. Chronic nephropathy is reported to affect bone metabolism [8]. Bone markers are subdivided into bone formation and bone resorption markers. Bone formation markers consist of osteocalcin, bone-specific alkaline phosphatase (ALP), etc., whereas resorptive markers consist of N-terminal cross-linked telopeptide of type-1 collagen (NTX) and C-terminal cross-linked telopeptide of type-1 collagen [9]. Many

laboratory tests were used for diagnosis and follow-up of bone loss. β-CrossLaps (β-CTx) are the C-terminal telopeptide of type I collagen, the chief element (∼90%) of the protein matrix of bone. β-CTx is released into the blood during bone resorption and is excreted mainly by the kidneys. Its estimation serves as a specific marker for the degradation of mature type I collagen from bone [10]. Therefore, in this work, we aimed to estimate bone turnover through an early marker β-CTx in postmenopausal women with DM and early DN.

Patients and methods

The present cross-sectional study included 80 postmenopausal patients from Mansoura University Hospital, Diabetes and Endocrinology Unit. The study was approved by Institutional Research Board in Mansoura Faculty of Medicine, and a written informed consent was obtained from all participants. The study was carried over a 6-month duration. Any unexpected risks appeared during the course of the research were cleared to the participants and the ethical committee on time. Our cohort included three groups: group 1 (control individuals) consisted of 26 apparently healthy nondiabetic postmenopausal women of matched age and BMI with the patients and not on hormonal therapy or drug therapy that affected the scope of the study, group 2 (diabetic postmenopausal women without DN) comprised 28 patients aged from 45 to 55 years, and group 3 (diabetic postmenopausal women with early DN) comprised 26 patients aged from 45 to 55 years. None of the patients were treated with drugs or had a disorder that would interfere with metabolism of phosphate or calcium and/or bone structure. Exclusion criteria included patients receiving hormone replacement therapy, patients on calcium or vitamin D supplementation, smokers, alcohol intake, obese patients, patients with hypertension, diabetic patient receiving insulin or TZDS (glitazone), any patients with estimated Glomerular Filteration Rate eGFR less than 60 ml/min/1.73 m^2, and patients with chronic liver disease, any organ failure, and malignancy. All patients included in the study were subjected to full history taking; complete clinical examination; and laboratory investigations including bone ALP, serum creatinine, random blood sugar, glycated hemoglobin (HbA1c), serum calcium, serum phosphorus, β-CTx serum assay, and urinary albumin/creatinine ratio (UACR). Renal function was estimated by estimated glomerular filteration rate eGFR with a Modification of Diet in Renal Disease equation [11] and the UACR [12]. Albuminuria was defined as UACR 30–300 mg/g as

microalbuminuria and UACR more than 300 mg/g as macroalbuminuria [13]. In addition, dual X-ray absorptiometry at the lumbar and hip region was done for all participants. It is interpreted as normal if the lowest T-score is higher than –1.0, osteopenia if between –1.0 and –2.5, and osteoporosis if it is less than –2.5.

Blood sampling

Five milliliter of venous blood sample under complete aseptic condition was withdrawn from every participant, of which 1 ml was delivered into EDTA tubes for HbA1c assay using fast cation exchange resin supplied by Stanbio (Germany) and 4 ml was delivered into plain tube and left to clot, and then, clear nonhemolized sera were separated by centrifugation at 3000 rpm for 10 min. These were re-divided into two aliquots: one of them was kept at 0–20°C for analysis of β-CTx using Elecsys 2010, supplied by Roche Diagnostics GmbH, Germany. The other aliquot was used for other laboratory investigations, such as random glucose, calcium, phosphorous, ALP, and creatinine. After assay for the total ALP, the sera was heated for 10 min at 56°C (water bath). The ALP was assayed. Bone ALP=total ALP$_{before\ heating}$–total ALP$_{after\ heating}$. Morning urine sample was obtained for urine analysis using available kits supplied by Sletche (France) and albumin/creatinine ratio. Urine albumin was assayed by turbidimetry method supplied by Spin react (Spain).

Statistical methods

Continuous variables were expressed as mean±SD, and one-way analysis of variance was used to estimate the significant difference between groups. A χ^2 was used to estimate the significant difference between categorical data, and Kruskal–Wallis test was used to estimate the significant difference. The correlation between all estimated parameters and β-CTx was assessed by the Pearson's rank correlation analysis, and the results were expressed as Pearson's correlation coefficient. The cutoff for significances is P value less than 0.05.

Results

Sociodemographic data of all participants who achieved the study and inclusion criteria are shown in Table 1. Age, residence distribution, and family history of hypertension or diabetes mellitus were adjusted, and there was no significant difference ($P>0.05$) between postmenopausal women groups. Laboratory data showed significant high serum levels of uric acid, HbA1c, and random blood glucose in groups 2 and 3 compared with group 1 ($P<0.0001$).

Table 1 Sociodemographic data of studied groups

Variables	Controls	Diabetic	Diabetic nephropathy	P value
N	26	28	26	
Age	48±5.28	48.67±3.82	47.84±3.75	0.753
Residence [n (%)]				
Rural	12 (46.2)	7 (25.0)	14 (53.8)	0.082
Urban	14 (53.8)	21 (75.0)	12 (46.2)	
Family Hx hypertension [n (%)]				
Positive	9 (34.6)	9 (32.1)	8 (30.8)	0.956
Negative	17 (65.4)	19 (67.9)	18 (69.2)	
Family Hx diabetes mellitus [n (%)]				
Positive	12 (46.2)	13 (46.4)	12 (46.2)	1.00
Negative	14 (53.8)	15 (53.6)	14 (53.8)	

$P < 0.05$, significant.

Table 2 Some biochemical parameters of studied groups

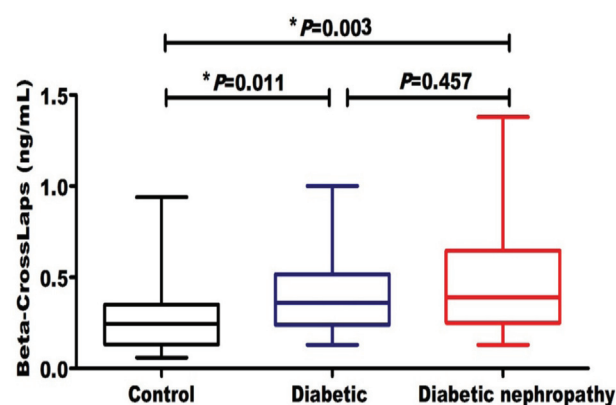| Variables	Controls	Diabetic	Diabetic nephropathy	P value
N	26	28	26	
Uric acid (mg/dl)	3.8±1.03	5.25±1.99	5.89±2.25	<0.0001
Creatinine (mg/dl)	0.75±0.16	0.80±0.28	0.91±0.19	0.872
Glycated hemoglobin (%)	4.45±0.95	8.14±3.41	8.37±2.65	<0.0001
RBS (mg/dl)	97.00±16.00	253.50±206.55	222.44±159.80	<0.0001
Microalbuminuria (mg/l)	4.56±5.47	10.10±11.87	234.5±68.94	<0.0001

$P < 0.05$, significant.

Table 3 Bone biomarkers and radiological characteristics

Variables	Controls	Diabetic	Diabetic nephropathy	P value
N	26	28	26	
Calcium (mg/dl)	9.06±0.90	9.10±0.78	8.49±1.013	0.029
Phosphorus (mg/dl)	5.53±0.60	4.6±0.86	3.9±1.0	<0.0001
Bone alkaline phosphatase (μ/l)	96.65±2.60	62.8±17.3	59.11±9.54	<0.0001
Total alkaline phosphatase (μ/l)	372.50±243.73	282.61±129.81	245.50±116.93	0.006
β-CrossLaps (ng/ml)	0.22±0.39	0.33±0.67	0.46±0.48	0.003
Dual X-ray absorptiometry scan [n (%)]				
Normal	8 (30.8)	4 (14.3)	4 (15.4)	0.001
Osteoporosis	7 (26.9)	11 (39.3)	17 (63.4)	0.004
Osteopenia	11 (42.3)	13 (46.4)	5 (19.2)	

$P < 0.05$, significant.

UACR, as expected, was significantly ($P < 0.0001$) high in group 3 compared with groups 1 and 2 (Table 2). Some bone biomarkers were compared between the studied groups (Table 3). Regarding bone minerals, in group 3, there was a significant lower serum calcium and phosphorus compared with groups 1 and 2 ($P=0.029$ and <0.0001, respectively). Regarding total ALP, group 3 had a significant lower level compared group 1 ($P=0.006$), and group 2 had an important lower level compared with group 1 ($P=0.011$). However, groups 2 and 3 had a significant lower bone ALP level than group 1 ($P < 0.0001$). Groups 2 and 3 had significantly higher serum levels of β-CTx ($P=0.016$ and 0.003, respectively) compared with group 1 (Fig. 1). Dual-

Figure 1

Serum levels of β-CrossLaps in the studied groups.

Table 4 Correlation between β-CrossLaps and estimated parameters in each studied group

Variables	Controls		Diabetic		Diabetic nephropathy	
	r	P	r	P	r	P
Age	−0.004	0.984	−0.155	0.430	0.291	0.127
Serum creatinine (mg/dl)	−0.224	0.271	−0.003	0.989	0.261	0.199
Glycated hemoglobin%	−0.260	00.342	−0.228	0.243	−0.198	0.331
Random blood glucose (mg/dl)	−0.078	0.706	−0.294	0.129	−0.165	0.421
Alkaline phosphatase (U/l)	0.333	0.096	0.065	0.754	0.111	0.591
Calcium (mg/dl)	0.379	0.056	0.038	0.848	−0.270	0.181
Phosphorus (mg/dl)	−0.061	0.766	0.027	0.893	0.367	0.065
Uric acid (mg/dl)	−0.129	0.528	**0.556**	**0.020**	−0.149	0.468
Bone alkaline-phosphatase (U/l)	−0.063	0.761	−0.131	0.508	−0.119	0.563
Microalbuminuria (mg/l)	−0.059	0.774	−0.176	0.371	**0.543**	**0.013**
Dual X-ray absorptiometry scan	0.225	0.269	−0.075	0.730	0.060	0.770

energy X-ray absorptiometry scan revealed a significant elevation (P=0.001) in osteoporosis percent in group 3 (63.4%) in comparison with group 1 (26.9%) and while in group 2 was (39.3%). However, osteopenia percent was significantly higher in group 2 (46.4%) (P=0.004) in comparison with group 3 (19.2%) and in group 1 was (42.3%) (Table 3). β-CTx was significantly and positively correlated with uric acid in group 2 (P=0.020) and with UACR in group 3 (P=0.013; Table 4). There was no significant correlation between UACR and the results of bone mineral density (BMD) in groups 1 and 2.

Discussion

Even though several studies have stated a strong relationship between DM and the high-risk of pathological fractures, the link between DM and osteoporosis remains controversial [14]. Regarding the effect of DM on the BMD; there are controversial data. Previous studies have found that T2DM patients have elevated [15], similar [16], or decreased [17] bone mass compared with healthy control. Moreover, type 2 DM in postmenopausal women was linked to lower levels of BMD and higher osteoporosis rate than control participants [18]. The current study showed that the development of DN was associated with a significant decrease in serum calcium and phosphorus, which is supported by a study by Pushpa and Anandan [19], which stated that, in diabetic patient with and without nephropathy, changes in phosphate and calcium levels reflect disorders of their metabolism, and the overall consequences would manifest as loss of bone calcium and phosphate, accelerated calcium mobilization, and development of bone loss and lead to osteopenia and even osteoporosis, particularly in patients with DN. The kidney is an important organ for controlling calcium and phosphorous metabolism, not only as a

site for parathyroid hormone action but also a main site for calcitriol (1,25-dihydroxy-vitamin D) production [20]. The link between chronic kidney disease and reduced BMD and the resulting increased risk of fracture is well recognized [21,22]. However, the effect of early DN with eGFR more than 60 ml/min1.73 m^2 was not fully studied. Here, in our work – as expected – UACR was significantly higher in DN (group 3) compared with the other two groups, whereas there was no significance in creatinine serum levels and eGFR between the groups. Herein, the mean serum levels of bone-specific ALP were lower in diabetic groups 2 and 3 compared with the control group 1. Similar findings have been reported in previous studies [23]. Moreover, DN group 3 had lower mean serum ALP levels than the control group. β-CTx is used as markers for bone resorption and also for prediction of fracture risk, independent of BMD, and monitoring of osteoporosis treatment [24,25]. The current study shows significant higher levels of β-CTx in the diabetic groups 2 and 3 than the control group 1 with significant higher level in group 3 (P=0.003), indicating high resorptive bone state. Zhao et al. [26] found statistically significantly higher serum β-CTx levels in patients with a high risk of osteoporotic or vertebral fracture, and others concluded that assessment of β-CTx was more accurate than BMD in the early evaluation of osteoporosis and follow-up treatment.

Rapid reduction in BMD is correlated with estrogen deficiency. Consequently, women who have had shorter than average exposure to estrogen during their life (including early menopause) are at higher osteoporosis risk. In this study, dual X-ray absorptiometry based assessment of BMD revealed a significant elevation in osteoporosis percent in group 3 (63.4%) in comparison with control group 1 and high prevalence of osteopenia in group 2, which matched

with others who reported similar findings [27]. Albuminuria and BMD appear to have a strong relationship when combined, and they contribute to the development of osteoporosis. We found a high prevalence of osteoporosis in group 3 and also a strong positive correlation between UACR and β-CTx in group 3 (DN), which was in line with a study which stated that the increase in UACR was related to high risk of osteoporosis in postmenopausal women with type 2 DM [28]. We found a positive correlation between serum β-CTx and UACR in group 3 and no correlation between serum β-CTx and BMD, whereas another study reported a negative correlation between serum β-CTx and BMD [26]. Finally, we can concluded that serum β-CTx and UACR are potentially useful biomarkers for bone resorption state and increased osteoporosis risk in postmenopausal women with early DN.

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Conflicts of interest

There are no conflicts of interest.

References

1 Stenevi Lundgren S, Rosengren BE, Dencker M, Nilsson JÅ, Karlsson C, Karlsson MK. Low physical activity is related to clustering of risk factors for fracture – a 2-year prospective study in children. Osteoporos Int 2017; 28:3373–3378.

2 NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis and Therapy. Osteoporosis prevention, diagnosis and therapy. JAMA 2001; 285:785–795.

3 Vestergaard P, Rejnmark L, Mosekilde L. Diabetes and its complications and their relationship with risk of fractures in type 1 and 2 diabetes. Calcif Tissue Int 2009; 84:45–55.

4 Holmberg AH, Johnell O, Nilsson PM, Nilsson J, Berglund G, Akesson K. Risk factors for fragility fracture in middle age. A prospective population-based study of 33,000 men and women. Osteoporos Int 2006; 17:1065–1077.

5 Kormi SMA, Ardehkhani S, Kerachian MA. The effect of Islamic fasting in Ramadan on osteoporosis. J Fast Health 2017; 5:74–77.

6 Yamaguchi T, Kanazawa I, Yamamoto M. Associations between components of the metabolic syndrome versus bone mineral density and vertebral fractures in patients with type 2 diabetes. Bone 2009; 452:174–179.

7 Starup-Linde J. Diabetes, biochemical markers of bone turnover, diabetes control, and bone. Front Endocrinol (Lausanne) 2013; 4:21.

8 Kazama JJ, Matsuo K, Iwasaki Y. Chronic kidney disease and bone metabolism. J Bone Miner Metab 2015; 33:245–252.

9 Vasikaran S, Eastell R, Bruy'ere O. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. Osteoporos Int 2011; 22:391–420.

10 Peichl P, Griesmacherb A, Marteau R. Serum cross laps in comparison to serum osteocalcin and urinary bone resorption markers. Clin Biochem 2001; 34:131–139.

11 Levey AS, Inker LA, Coresh J. GFR estimation: from physiology to public health. Am J Kidney Dis 2014; 63:820–834.

12 Ellam T, Fotheringham J, Wilkie ME. Bone mineral metabolism parameters and urinary albumin excretion in a representative US population sample. PLoS ONE 2014; 9:e88388.

13 Molitch ME, DeFronzo RA, Franz MJ. Nephropathy in diabetes. Diabetes Care 2004; 27(Suppl 1):S79–S83.

14 Montagnani A, Gonnelli S, Alessandri M. Osteoporosis and risk of fracture in patients with diabetes: an update. Aging Clin Exp Res 2011; 23:84–90.

15 Sosa M, Dominguez M, Navarro MC. Bone mineral metabolism is normal in non-insulin-dependent diabetes mellitus. J Diabetes Complications 1996; 104:201–205.

16 Nicodemus KK, Folsom AR. Type 1 and type 2 diabetes and incident hip fractures in postmenopausal women. Diabetes Care 2001; 247:1192–1197.

17 Wang LX, Wang N, Xu QL, Yan W, Dong L, Li BL. Effects of vitamin D combined with pioglitazone hydrochloride on bone mineral density and bone metabolism in type 2 diabetic nephropathy. Biosci Rep 2017;37:372.

18 Lee YY, Kim HB, Lee JW. The association between urine albumin to creatinine ratio and osteoporosis in postmenopausal women with type 2 diabetes. J Bone Metab 2016; 231:1–7.

19 Pushpa Rani D, Anandan S. A clinical study of serum alkaline phosphatase and calcium level in type 2 diabetes mellitus with periodontitis among the south Indian population. J Res Dent Scis 2012; 33:175–179.

20 Kim HL, Park IY, Choi JM. A decline in renal function is associated with loss of bone mass in Korean postmenopausal women with mild renal dysfunction. J Korean Med Sci 2011; 26:392–398.

21 Nickolas TL, Leonard MB, Shane E. Chronic kidney disease and bone fracture: a growing concern. Kidney Int 2008; 74:721–731.

22 Alem AM, Sherrard DJ, Gillen DL. Increased risk of hip fracture among patients with end-stage renal disease. Kidney Int 2000; 58:396–399.

23 Zulauf N, Marzi I, Oremek GM. Prognostic value of bone marker beta-cross-laps in patients with breast carcinoma. J Mol Biomark Diagn 2014; 5193:2.

24 Garnero P. New biochemical markers of bone turnover. IBMS Bone Key 2008; 5:84–102.

25 Vasikaran S, Eastell R, Bruyère O, Foldes AJ, Garnero P, Griesmacher A, et al. IOF-IFCC Bone Marker Standards Working Group. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: a need for international reference standards. Osteoporos Int 2011; 22:391–420.

26 Zhao J, Xia W, Nie M, Zheng X, Wang Q, Wang X, et al. The levels of bone turnover markers in Chinese postmenopausal women: Peking Vertebral Fracture study. Menopause 2011; 18:1237–1243.

27 Anaforoglu I, Nar-Demirer A, Bascil-Tutuncu N, Ertorer ME. Prevalence of osteoporosis and factors affecting bone mineral density among postmenopausal Turkish women with type 2 diabetes. J Diabetes Complications 2009; 231:12–17.

28 Lee YY, Kim HB, Lee JW, Lee GM, Kim SY, Hur JA, et al. Association between urine albumin to creatinine ratio and osteoporosis in postmenopausal women with type 2 diabetes. J Bone Metab 2016; 23:1–7.