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[1]Department of Biochemical Diagnostics, Chair of Laboratory Diagnostics,
[2]Chair and Department of Endocrinology,
[3]Department of Laboratory Diagnostics, Medical University of Lublin

BEATA WOJTYSIAK-DUMA[1], ARLETA MALECHA-JĘDRASZEK[1], AGATA BURSKA[1], HELENA DONICA[1], BEATA MATUSZEK[2], MONIKA LENART-LIPIŃSKA[3], ANDRZEJ NOWAKOWSKI[2]

Markers of bone metabolism in patients with type 2 diabetes mellitus

Markery kostne u pacjentów z cukrzycą typu 2

INTRODUCTION

Despite an enduring structure, bone is an active metabolic tissue which undergoes continuous turnover throughout its lifetime in response to changes in mechanical loading, altered serum calcium levels and many endocrine and paracrine factors. After reaching the maximal bone mass, resorption and formation are tightly coupled in a process called remodeling, where old bone is continuously removed by osteoclasts and replaced by new bone formed by osteoblast in a homeostatic equilibrium. The remodeling process is essential for maintaining bone strength and repairing microdamages [1, 2, 11].

It has been reported that diabetes (DM) and connected with the disease metabolic disturbances lead to important alterations in bone metabolism [1, 2, 7, 9, 10, 11]. In type 1 diabetes in the course of deficiency of insulin and insulin-like growth factors (IGFs), bone formation is impaired with the relative increase in bone resorption, which leads to the decrease in bone mass, disturbances in mineralization and bone microarchitecture [2, 9, 10].

Changes in bone metabolism in patients with type 2 diabetes have recently been reported in the literature [1, 4, 7, 9, 11]. Data concerning bone mineral density in type 2 diabetes are contradictory [10]. Increased bone mineral density (BMD), similarly as in obese subjects, have been observed in the majority of studies [4, 5]. Alterations in bone formations, impaired quality of bones and disturbances in bone micro- and macroarchitecture have also been observed, independently of differences in BMD in patients with type 2 diabetes. Patients with type 2 diabetes are also fall-prone and have an increased rate of bone fractures, which may result from the presence of chronic diabetic complications [5].

Recent research suggests that maintaining bone mass in type 2 diabetes is a coincidence of many factors which protectively influence bone metabolism, such as hyperinsulinism, androgens or obesity,

as well as factors which progress bone resorption [4,10]. An important meaning for maintaning bone mass is ascribed to good metabolic control in diabetes. It has been reported that poorly controlled type 2 diabetes may be a consequence of increased osteolysis and can lead to increased susceptibility to bone loss and development of bone changes such as osteopenia [1, 4, 6, 7, 11].

In the majority of recent studies it has been reported that bone turnover in patients with type 2 diabetes is decreased due to the decrease in the number of osteoclasts and delay in formation and mineralization of the osteoid [1, 2, 5, 7]. The aim of the study was evaluation of biochemical markers of bone metabolism in patients with type 2 diabetes.

MATERIAL AND METHODS

The study included 51 patients with type 2 diabetes. The examined group consisted of 26 women and 25 men at the mean age 62.3±9.3 years, treated at the Department of Endocrinology of the Medical University in Lublin. The mean duration of the disease was 11.1±17.0 years. In the examined group of patients in medical history the following were found: arterial hypertension (80.4%), coronary artery disease (53%), myocardial infarction (25.5%), heart failure (17.7%), stroke (9.8%), diabetic nephropathy (9.8%), polineuropathy (9.6%), diabetic foot syndrome (2%). The control group comprised 30 healthy persons of the mean age 55.1±13.2 years matched for age and gender to the study group, undergoing prophylactic examination at the Department of Laboratory Diagnostics of the Independent Public Clinical Hospital No. 1 in Lublin.

In every subject enrolled to the study determination of concentrations of C-terminal telopeptides of collagen type I (CTx), parathormone (PTH), total calcium (Ca), inorganic phosphates (P), glucose, HbA1c, creatinine and alkaline phosphatase activity (ALP) was performed. Studies were performed on peripheral venous blood samples withdrawn from the cubital vein in fasting condition between 8.00 am and 10.00 am. Blood samples were collected in the amount of 7 ml to the tubes containing K_3EDTA in order to determine HbA1c concentration or to the tubes with no anticoagulant in order to assess other biochemical parameters. Blood samples 20–30 minutes after collection were centrifuged for 10 minutes at 1000 rpm and the obtained serum was separated to the eppendorf tubes and stored at -20^{o}C until assayed.

CTX concentrations were measured using a solid phase enzyme-linked immunosorbent assay, based on the principle of competitive binding (CrossLaps ELISA, Nordic Bioscience Diagnostics A/S) according to the manufacturer's instructions. The concentration of total calcium, inorganic phosphate, glucose, HbA1C, creatinine and ALP activity was performed with the use of standard laboratory methods on Konelab biochemical analyzer (BioMerieux). The concentration of parathormone in the serum of blood was performed with the use of electrochemiluminescence method on Immulite 2000 analyzer (Siemens) using ready kits provided by Siemens.

Statistical analyses were performed using the Statistica version 8,0 programme (StatSoft). The results of the conducted studies were statistically analysed using basic parameters of descriptive statistics (the mean-X, standard deviation-SD, median-Me).The distribution of the examined parameters was tested with the use of Shapiro-Wilk test. Variables were compared by Student's t-test when normally distributed and in case of nonparametric variables, comparisons were made by Mann-Whitney U-test. Correlations between analyzed variables were assessed with the use of the Pearson's and Spearman's tests. $P<0.05$ was considered significant.

RESULTS

Biochemical characteristics of the study population are presented in Table 1.

Table 1. Biochemical characteristics of the study population

Parameters	Study group		Control group		p
	X±SD	Me	X±SD	Me	
CTx (ng/ml)	0.309±0.277	0.289	0.396±0.196	0.362	<0.05
PTH (pg/ml)	66.29±37.58	53.65	62.38±34.59	56.9	NS
ALP (IU/l)	74.9±24.1	74.0	72.1±21.1	71.0	NS
Ca (mmol/l)	2.46±0.15	2.47	2.38±0.19	2.34	<0.05
P (mmol/l)	1.26±0.30	1.28	1.01±0.22	0.98	<0.001
Glucose (mg/dl)	149.2±41.6	137.0	89.0±11.5	89.0	<0.001
HbA1c (%)	8.72±1.89	8.60	-	-	-
Creatinine (mg/dl)	1.10±0.28	1.05	0.85±0.20	0.81	<0.001

p – level of statistical significance ($p<0.05$), NS – statistically insignificant

The mean CTx concentration in the examined group was 0.309±0.277 and was significantly lower ($p<0.05$) in comparison to the healthy subjects (0.396±0.196), but no significant differences related to gender were observed. In the group of diabetic patients significantly elevated concentrations of total calcium, inorganic phosphorus, glucose and creatinine in comparison to the control group were found. PTH concentrations and alkaline phosphatase activity were not significantly different in the examined group of patients with type 2 diabetes in comparison to the control group. The mean HbA1c in diabetic subjects was 8.72±1.86 %.

In the group of patients with type 2 diabetes serum CTx concentrations were directly proportional to PTH ($r=0.48$; $p<0.01$) and creatinine ($r=0.37$; $p=<0.01$) concentrations.We did not observe any significant correlations between CTx concentrations and age, HbA1c and other biochemical parameters including markers of calcium and phosphate metabolism.

DISCUSSION

Biochemical markers of bone turnover are fragments of protein structural components of bones (or products of their biodegradation), enzymes and proteins released to circulation during metabolic activity of osteogenic cells – osteoblasts and osteolytic cells – osteoclasts. Bone metabolism can be monitored by measuring biochemical markers of bone turnover from serum or urine. Their concentrations in serum and urine result from the activity of all processes of bone remodeling in the whole skeleton. In recent years there has been considerable improvement in studies on biochemical markers of bone turnover. These non-invasive and relatively inexpensive tools can be very helpful in the quick assessment of physiological bone turnover and metabolic bone diseases. The role of bone turnover markers has especially emerged in monitoring the changes in bone mass in the course of treatment and predicting bone fractures, also in type 2 diabetes [1,2,11].

In our study we assessed one of the markers of bone turnover- CTx (C-terminal telopeptide of collagen type I) concentrations, as a marker of bone resorption created during breakdown of collagen type I fibres, reflecting decrease in bone mass [1, 9, 11]. We revealed a significant decrease in CTx concentration in serum of patients with type 2 diabetes compared to the control group. This observation indicates a mild decrease in osteolysis in the examined group of patients, which may be accompanied by normal or slightly increased bone mineral density.

Numerous studies have underlined the role of low bone turnover in patients with type 2 diabetes as a consequence of insufficient bone formation which results from disturbances in maturity and function of octeoclasts, and manifests as a decrease in osteogenic markers concentrations in serum [1, 2, 5, 7, 9]. Some authors reported opposite findings [3, 4, 11]. Research also suggests that the process of bone resorption in type 2 diabetes is in the majority of cases normal [1, 5] or slightly increased in relation to the decreased bone formation [7, 9] and only in a small number of cases it can be decreased [2, 6].

In the study conducted by Achemlal et al. [1] bone turnover markers were assessed in 35 patients with type 2 diabetes. Osteocalcin concentration as a marker of bone formation and normal concentration of bone resorption markers (CTx) in comparison to the control group was demonstrated significantly lower, which indicates a decrease in the bone formation process and unaffected bone resorption process during bone remodeling in the examined patients. Similar results were obtained by Leidig-Bruckner et al. [5]. The reduction in bone turnover in type 2 diabetes with the decreased bone formation and increased bone resorption, which manifested with low osteocalcin concentration and elevated Ctx urine concentration was shown by Oz et al. [7]. Others researchers also observed a significant decrease in bone fraction of alkaline phosphatase activity as a marker of bone formation, as well as total ALP in patients with type 2 diabetes [8]. In our study no significant differences in total ALP activity were observed.

The role of metabolic control of diabetes in maintaining bone mass should also be emphasized. It has been reported that disturbances in bone formation are mostly observed in patients with poorly controlled diabetes. Studies revealed that advanced glycation end products inhibit function of oseoblasts [1–3, 6, 11]. Okazaki et al. [6] demonstrated that restoration of good metabolic control of diabetes in a short time leads to inhibition of bone resorption and stabilization of bone mineral density. Other authors observed a negative correlation between CTx concentration and HbA1c, which may suggest increased bone turnover in patients with type 2 diabetes and improvement in metabolic control of the disease [1]. In our study we did not confirm this relationship.

CTx concentration in the examined group of patients was significantly associated with PTH ($r=0.48$; $p<0.01$) and serum creatinine concentration ($r=0.37$; $p<0.01$), which may indicate a relationship between the progression connected with diabetes renal insufficiency and an increase in bone resorption. This association has been noticed by Yendt et al. [12], who demonstrated a positive correlation between creatinine clearance and bone mineral density and bone mass.

CONCLUSIONS

On the basis of research conducted, it can be supposed that decreased CTx concentrations in patients with type 2 diabetes in comparison to the control group may suggest an increase in bone

mineral density or balance disturbances between bone formation and bone resorption processes. Further investigations are required to determine the clinical consequences of the observed changes in patients with type 2 diabetes.

REFERENCES

1. Achemlal L., Tellal S., Rkiouak F. et al.: *Bone metabolism in male patients with type 2 diabetes.* Clin. Rheumatol., 24, 493, 2005.
2. *Capoglu* I., Ozkan A., Ozkan B. et al.: *Bone* turnover markers in patients with type 2 diabetes and their correlation with glycosylated haemoglobin levels. J. Int. Med. Res., 36, 1392, 2008.
3. Cutrim D.M., Pereira F.A., Paula F.J.A. et al.: Lack of relationship between glycemic control and bone mineral density in type 2 diabetes mellitus. Braz. J. Med. Biol. Res., 40, 221, 2007.
4. Dennison E.M., Syddall H.E., Aihie Sayer A. et al.: Type 2 diabetes mellitus is associated with increased axial bone density in men and woman from the Hertfordshire Cohort Study: evidence for an indirect effect of insulin resisrance? Diabetologia, 47, 1963, 2004.
5. Leidig-Bruckner G., Ziegler R.: Diabetes mellitus a risk for osteoporosis? Exp. Clin. Endocrinol. Diabetes, 09, S493, 2001.
6. Okazaki R., Totsuka Y., Hamano K. et al.: Metabolic improvefment of poorly controlled non insulin dependent diabetes mellitus decreases bone turnover. J. Clin. Endocrinol. Metab., 82, 2915, 1997.
7. Oz S.G., Guven G.S., Kilicarslan A. et al.: Evaluation bone metabolism and bone mass in patients with type 2 diabetes mellitus. J. Natl. Med. Assoc., 98, 1598, 2006.
8. Rosato M.T., Schneider S.H., Shapses S.A.: Bone turnover and insulin-like growth factor-1 levels increase after improved glycemic control in non insulin-dependent diabetes mellitus. Calcif. Tissue Int., 63, 107, 1998.
9. Suzuki K., Kurose T., Takizawa M. et al.: Osteoclastic function is accelerated in male patients with type 2 diabetes mellitus. Diab. Res. Clin. Pract., 68, 117, 2005.
10. Thrailkill K.M., Lumpkin C.K., Bunn R.C. et al.: Is insulin an anabolic agent in bone? Dissecting the diabetic bone for clues. Am. J. Physiol. Endocrinol. Metab., 289, E735, 2005.
11. Vestergaard P.: Bone metabolism in type 2 diabetes and role of thiazolidinediones. Curr. Opin. Endocrinol. Diabetes Obes., 16, 125, 2009.
12. Yendt E.R., Cohanim M., Jarzylo S. et al.: Reduced creatinine clearance in primary osteoporosis in women. J. Bone Miner. Res., 8, 1045, 1993.

SUMMARY

There are only a few reports on bone metabolism in type 2 diabetes. Impaired bone turnover in type 2 diabetes appears to result from decreased bone formation. Some studies suggest that poor glycaemic control in type 2 diabetes may contribute to osteopaenia. The aim of our study was to investigate biochemical markers of bone turnover in patients with type 2 diabetes. In our study 51 type 2 diabetes mellitus patients and 30 healthy persons were enrolled. 30 of the patients had macrovascular complications and 19 of them had microangiopathies. The mean age of the

patients was 62.3 (9.3) years and the mean disease duration was 11.1 (7.0) years. Serum crosslaps (C-telopeptide, CTx), parathyroid hormone (PTH), calcium, inorganic phosphate, glucose, HbA1c, alkaline phosphatase and creatinine were measured. No differences between patients and controls were observed in serum PTH concentration and alkaline phosphatase activity. Patients had lower serum levels of CTX than controls [0.309 (0.277) vs 0.396 (0.196), $p< 0.05$]. In turn, serum levels of calcium and inorganic phosphates were higher in diabetic patients in comparison to the control group. We found a significant statistical correlation between CTx levels and PTH ($r=0.48$. $p< 0.05$). We also noticed a positive relationship between CTX, PTH and creatinine concentration. Our study suggests that decreased serum CTX levels in type 2 diabetes mellitus patients may be an effect of altered equilibrium in bone formation and resorption processes. A significant correlation between PTH and CTX and creatinine concentration indicate that renal function in diabetes patients may affect serum levels of bone metabolism markers.

Key words: bone markers, metabolism, bone, type 2 diabetes mellitus, CTx, PTH, ALP

STRESZCZENIE

W dostępnej literaturze istnieje niewiele prac opisujących metabolizm kostny u pacjentów z cukrzycą typu 2. Sugeruje się w nich, że zaburzony obrót kostny w tej grupie pacjentów związany jest głównie ze spadkiem procesów kościotworzenia. W niektórych badaniach wykazano również, że procesy osteopenii u chorych z cukrzycą mogą być zwiększone w efekcie słabej kontroli glikemii. Biorąc pod uwagę niewielką liczbę danych na temat metabolizmu kostnego u chorych z cukrzycą, celem naszych badań była ocena stężeń wybranych markerów kostnych w tej grupie chorych. Badaniami objęto 51 pacjentów z cukrzycą typu 2 oraz 30 osób zdrowych. U 30 pacjentów stwierdzono obecność powikłań makronaczyniowych, zaś u 19 obecność mikroangiopatii. Średni wiek chorych wynosił 62,3±9,3 lat, natomiast średni czas choroby 11,1±7,0 lat. W grupie osób chorych i w grupie kontrolnej oznaczono stężenia C-końcowego usieciowanego telopeptydu łańcucha alfa kolagenu typu I (CTx), parathormonu (PTH), wapnia, fosforanów nieorganicznych, glukozy, HbA1c, kreatyniny oraz aktywność fosfatazy alkalicznej (ALP). Nie stwierdzono występowania istotnych różnic w stężeniach PTH i aktywności ALP pomiędzy grupą badaną a grupą kontrolną. U pacjentów wykazano natomiast niższe stężenie CTx w surowicy w porównaniu z osobami zdrowymi (0,309±0,277 vs 0,396±0,196, $p< 0,05$), jak też wyższe stężenia wapnia i fosforanów nieorganicznych. Stwierdzono także występowanie istotnej zależności pomiędzy poziomami CTx a stężeniem PTH ($r=0,48$, $p< 0,05$) oraz tymi parametrami a stężeniem kreatyniny w surowicy krwi osób chorych na cukrzycę typu 2. Przeprowadzone badania sugerują, że obniżone stężenie CTx u osób z cukrzycą może być efektem zaburzonej równowagi w procesach tworzenia i resorpcji kości. Występowanie istotnych zależności pomiędzy stężeniami PTH oraz CTx a stężeniem kreatyniny wskazywać może, iż ważnym czynnikiem wpływającym na stężenia markerów charakteryzujących metabolizm kostny u pacjentów z cukrzycą może być upośledzona czynność nerek.

Key words: markery kostne, metabolizm, cukrzyca typu 2, CTx, PTH, ALP