Metabolic bone markers can be related to preserved insulin secretion in children with newly diagnosed type 1 diabetes

Markery metabolizmu kostnego mogą być związane z przetrwałą insulinosekrecją u dzieci z nowo rozpoznaną cukrzycą typu 1

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Abstract

Introduction: Type 1 diabetes (T1D) may be associated with numerous complications including bone metabolism disorders. The aim of the study was to evaluate the bone metabolism markers twice in children with a newly diagnosed T1D and after an average of seven months of its duration in relation to parameters of the clinical course of diabetes.

Material and methods: In 100 T1D patients and 52 control subjects, the following bone turnover markers were evaluated: osteocalcin – OC, osteoprotegerin – OPG, sRANKL, and deoxypyridoline in urine – DPD and DXA examination was also performed.

Results: Lower OC concentration at T1D onset in comparison to controls (p < 0.001) and its increase during follow-up (p < 0.001) was observed. The OPG concentration was elevated at T1D onset as compared to the control group (p = 0.024) and decreased thereafter (p < 0.001). The s-RANKL level increased during follow-up (p < 0.001) and was lower than in controls (p < 0.001). Urine DPD concentration also increased during follow-up in the T1D patient group (p < 0.001) and was higher in comparison to the control group (p = 0.021). BMD-TBLH was higher in the control group as compared to patients both at T1D onset (p = 0.025) and in follow-up observation (p = 0.034). Moreover, OPG correlated positively with glycated haemoglobin (HbA1c) (p = 0.004) and negatively with fasting C-peptide level (p = 0.046) and BMI Z-score (p = 0.003), whereas s-RANKL correlated positively with both fasting (p < 0.001) and stimulated C-peptide levels (p < 0.001).

Conclusions: Bone metabolism disorders observed at T1D onset in children and modified after reaching the metabolic control of the disease seem to be most strongly associated with preserved insulin secretion.

Key words: type 1 diabetes, osteocalcin, osteoprotegerin, s-RANKL, densitometry.
w grupie pacjentów z T1D (p < 0,001) i było większe w porównaniu z grupą kontrolną (p = 0,021). BMD-TBLH był wyższy w grupie kontrolnej w porównaniu z pacjentami, zarówno w momencie wystąpienia T1D (p = 0,025), jak i w dalszej obserwacji (p = 0,034). Ponadto OPG korelowało dodatnio z HbA₁c (p = 0,004) i ujemnie ze stężeniem peptydu C na czczo (p = 0,046) i BMI Z-score (p = 0,003), natomiast s-RANKL korelowało dodatnio ze stężeniem peptydu C zarówno na czczo (p < 0,001), jak i po stymulacji (p < 0,001).

Wnioski: Zaburzenia metabolizmu kostnego obserwowane w momencie wystąpienia T1D u dzieci i ulegające modyfikacji po osiągnięciu kontroli metabolicznej choroby wydają się najsilniej związane z przetwarzalną insulinosekrecją.

Słowa kluczowe: cukrzyca typu 1, osteokalcyna, osteoprotegeryna, s-RANKL, densytometria.

Introduction

Type 1 diabetes (T1D) is an autoimmune disease characterised by insulin deficiency resulting in hyperglycaemia [1]. The new definition of T1D which includes dysglycaemia already present in patients at prediabetes stage also draws attention to the chronic duration of the autoimmune process that precedes the clinical onset of the disease [2]. In the course of clinical T1D both acute and chronic complications may develop. Many observations are focused on the occurrence of bone metabolism disorders in children and adolescents with T1D.

The first reports of bone metabolism concerned the presence of delayed bone growth and bone mass reduction in T1D children [3]. Nowadays, many papers argue that both osteopaenia and osteoporosis may be important complications of T1D occurring not only in adult patients but also in children with T1D. Even in more than 50% of paediatric patients, after several years of disease duration, the bone mineral density (BMD) deficit may be present, which suggests a relatively rapid influence of diabetes on bone metabolism [3, 4].

The influence of glucose metabolism on the regulation of bone metabolism seems to be complex and not yet fully known. The most important pathogenetic factors of BMD development in T1D are: insulin deficiency, chronic hyperglycaemia, metabolic acidosis, vitamin D deficiency, and coexistence of celiac disease or other autoimmune diseases [3]. Moreover, the role of the osteoprotegerin/RANKL/RANKL pathway in the assessment of bone metabolism disorders is still being enhanced.

Osteoprotegerin (OPG) belongs to the group of tumour necrosis factor receptors and is an element of the pathway playing a key role in the differentiation and functioning of osteoclasts. By preventing the binding of the RANK (NF-κB activating receptor) to the RANKL (ligand of RANK) it inhibits osteoclast maturation at its initial stage [5, 6]. Osteocalcin (OC) is a hormone of osteoblastic origin, which takes part in the regulation of bone formation as well as in the function of β cells, body adiposity, and glucose metabolism [7].

Furthermore, pyridoline and deoxypyridoline (DPD) are amino acids that stabilise the structure of collagen fibres [8, 9]. This is important during the bone resorption process, when cross-linked collagen undergoes proteolytic decomposition and cross-linked components are released into circulation and urine [10].

However, the dual-energy X-ray absorptiometry (DXA) examination with AP spine and total body projection is still considered the gold standard among quantitative BMD tests [11].

The aim of the study was to evaluate the markers of bone metabolism disorders twice in children at clinical onset of T1D and after several months of disease duration, after the metabolic disorders of diabetes stopped and the insulin demand was definitively established, and additionally in relation to parameters of the clinical course of diabetes.

Material and methods

The study group consisted of 100 patients with T1D diagnosed according to WHO definition, including 40 girls and 60 boys, aged from 5.1 to 17.7 years.

Glycated haemoglobin (HbA₁c) was determined by high-performance liquid chromatography (HPLC) using the Bio-Rad VARIANT™ Hemoglobin A1c Program (Bio-Rad Laboratories Inc., Hercules, CA, US) with its values represented as percentages.

Fasting C-peptide levels as well as the concentrations of C-peptide after six minutes of the glucagon test, in order to evaluate the preserved β cells function, were measured using the electrochemiluminescence (ECLIA) method (Roche Diagnostics GmbH, Germany), and their values are presented as ng/ml.

Body mass index Z-scores of relative weight were measured, adjusted for the child’s age and sex [12]. Partial clinical remission was assessed after a mean of seven months of disease duration and defined as insulin requirement < 0.5 IU/kg and HbA₁c < 7% [13]. During a follow-up observation, this was recognised in 57% of T1D patients, which is similar to previous reports [14].

The control group consisted of 52 patients, including 33 girls and 19 boys, aged 7.2–18.0 years, without glucose tolerance disorders.

Detailed clinical characteristics of the study and control groups are shown in Table I.

Patients with autoimmune thyroid disease and/or coeliac disease, obesity, short stature, cancer, active inflammatory process, or receiving therapeutic doses of vitamin D were excluded from the study and control groups.

Before initiating the study, the Bioethics Committee of the Medical University of Lodz approved the study protocol (RNN/218/15/KE) and all patients or parents of underage participants gave informed, written consent to participate in the study.

In both groups the markers of bone turnover (OC, OPG, s-RANKL, and DPD) and BMD were measured. All analyses in the study group were performed at two time points: at the onset
Serum osteocalcin levels were evaluated by the electrochemiluminescence method (ECLIA) (Roche Diagnostics GmbH, Germany), whereas urine DPD levels were assessed using the chemiluminescence method (Siemens, UK). Serum OPG and s-RANKL levels were assessed by the ELISA method (respectively, BI-20403, BIOMEDICA, Austria and BI-20462, BIOMEDICA, Austria).

The body mineral density was evaluated by DXA using a Prodigy device (GE Lunar, US) in total body projection (TBLH) and lumbar projection (L1–L4). BMDs results were interpreted as the Z-score index referring to the mean and standard deviation for age and gender [15].

Results of metabolic bone markers were also related to parameters of the clinical course of diabetes, including: pH at T1D onset, HbA1c level at onset and at follow-up (%), BMI-Z-score at onset and at follow-up, and both fasting and stimulated C-peptide levels (ng/ml) at two time points.

Statistical analysis
Comparisons between the study group and the control group using the Mann-Whitney test were performed. Comparisons between groups of patients with T1D at clinical diagnosis and during follow-up observation were made using the Wilcoxon signed-rank test. Spearman’s correlation was calculated for evaluation of the relations between clinical parameters and the bone turnover markers. For all analyses STATISTICA 13.0 software (Statsoft, Tulsa, OK, USA) was used. Results with p levels < 0.05 were considered as statistically significant.

Results
At onset of T1D, autoantibodies and decreased C-peptide level were detected in all patients.

In 31/100 (31%) of patients at onset of T1D, diabetic ketoacidosis (DKA) was recognised according to pH and BE values.

Lower concentrations of osteocalcin in patients at T1D onset were observed in comparison to the control group (p < 0.001) and its increase in the follow-up observation (p < 0.001) was noted (Table II).

OPG concentration was elevated in the study group at T1D onset as compared to the control group (p = 0.024) and then decreased during the seven-month follow-up (p < 0.001) to show a tendency towards a lower level in the study group in the follow-up in relation to the control group (p = 0.105) (Table II).

In the study group the level of s-RANKL increased during the follow-up observation (p < 0.001) and decreased at T1D onset in the study group as compared to the control group (p < 0.001).

The urine DPD concentration also increased during follow-up in the T1D patients from the study group (p < 0.001) and was higher in relation to the control group (p = 0.021) (Table II).

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BMD-TBLH was higher in the control group than in the study group both at T1D onset (p = 0.025) and during follow-up (p = 0.034) (Table II). No significant differences were observed for lumbar BMD (Table II).

Analysing the correlations between the bone turnover markers and parameters of clinical course of T1D in patients from the study group, it was observed that osteocalcin at T1D onset negatively correlated with HbA1c (R = −0.409, p < 0.001) and positively both with fasting (R = 0.351, p < 0.001) and stimulated C-peptide levels (R = 0.434, p < 0.001) as well as with pH (R = 0.423, p < 0.001). A positive correlation between fasting

Table I. Clinical characteristics of the study and control groups

| Parameters                        | Study group     | Control group |
|-----------------------------------|-----------------|---------------|
|                                    | Percentages or median | Percentages or median |
| N (F/M)                           | 100 (40%/60%)    | 52 (63.5%/36.5%) |
| Age (years)                       | 5.1–17.7        | 7.2–18.0      |
| BMI Z-score at onset              | −2.01 (from −3.60 to −0.59) | −0.71 (−1.80–0.58) |
| BMI Z-score at follow-up          | −1.57 (from −2.92 to −0.37) | −0.71 (−1.80–0.58) |
| HbA1c at onset (%)                | 11.60 (10.20–13.70) | 0.66 (0.46–0.90) |
| HbA1c at follow-up (%)            | 6.50 (6.00–6.95) | 0.46 (0.28–0.61) |
| C-peptide in 0 minute at onset (ng/ml) | 0.29 (0.20–0.55) | 0.55 (0.33–0.81) |
| C-peptide after 6 minutes at onset (ng/ml) | 0.64 (0.36–1.06) | 0.97 (0.66–1.36) |
| C-peptide in 0 minute at follow-up (ng/ml) | 0.55 (0.33–0.81) | 0.71 (−1.80–0.58) |
| DKA at onset (%)                  | 31%             |               |
| Daily insulin intake at onset (units/kg) | 0.66 (0.46–0.90) | 0.46 (0.28–0.61) |
| Daily insulin intake at follow-up (units/kg) | 0.55 (0.33–0.81) | 0.71 (−1.80–0.58) |
In T1D patients a positive correlation between the OPG concentration at disease onset and HbA1c (R = 0.288, p < 0.004) and negative correlations between OPG and fasting C-peptide (R = −0.204, p = 0.046), as well as C-peptide level after six minutes of glucagon test (R = −0.275, p = 0.007), pH (R = −0.420, p < 0.001), and BMI Z-score (R = −0.300, p = 0.003) (Fig. 1) were observed. A negative correlation between OPG level and BMI Z-score was also maintained at the second time point (R = −0.244, p = 0.009).

In T1D patients there were also a negative correlation between s-RANKL concentration and HbA1c at disease onset (R = −0.377, p < 0.001) and positive correlations between s-RANKL level and both fasting C-peptide concentrations (R = 0.409, p < 0.001) (Fig. 2) and after six minutes of the glucagon test (R = 0.499, p < 0.001) (Fig. 3), BMI Z-score (R = 0.330, p < 0.001), and pH (R = 0.565, p < 0.001). Interestingly, after seven months of observation positive correlations between s-RANKL concentration and both fasting and stimulated C-peptide levels (respectively, R = 0.330, p < 0.003 and R = 0.269, p < 0.007) were still observed.

A negative correlation between urine DPD level and HbA1c (R = −0.315, p = 0.002) was also found in patients at onset of T1D. Moreover, in children at T1D onset the BMDs parameters – TBLH Z-score and lumbar Z-score – correlated positively with BMI Z-score (R = 0.392, p = 0.001 and R = 0.316, p = 0.012, respectively). For lumbar BMD the positive correlation with BMI Z-score was also noted after seven-month observation (R = 0.286, p = 0.046).

Other parameters of clinical course of T1D did not correlate with markers of bone turnover and BMD (p ≥ 0.051).

Taking into account the occurrence of clinical partial remission of T1D, a significantly lower urine DPD level in patients

### Table II. Comparison of serum osteocalcin, osteoprotegerin, s-RANKL, and urine DPD concentrations, and TBLH Z-score and lumbar Z-score BMD parameters between the study group at 2 time points and the control group. Data are presented as medians with 25th percentile and 75th percentile values

| Parameter                  | Study group at onset of T1D | Study group at follow-up of T1D | Control group | p value (at onset vs. controls) | p value (at onset vs. follow-up) | p value (at follow-up vs. controls) |
|----------------------------|-----------------------------|---------------------------------|---------------|---------------------------------|----------------------------------|------------------------------------|
| OC (ng/ml)                 | 39.13 (28.145–58.115)       | 92.39 (66.05–124)               | 98.49 (51.38–132.3) | < 0.001                         | < 0.001                          | 0.951                              |
| OPG (pmol/l)               | 3.89 (3.25–5.14)            | 3.32 (2.63–4.03)                | 3.63 (2.95–4.33) | 0.024                           | < 0.001                          | 0.105                              |
| S-RANKL (pmol/l)           | 0.33 (0.18–0.55)            | 0.61 (0.44–0.75)                | 0.56 (0.43–0.68) | < 0.001                         | < 0.001                          | 0.815                              |
| Urine DPD (nmol/mmol creatinine) | 17.51 (14.60–22.54)     | 23.63 (19.43–27.96)             | 19.66 (11.29–26.05) | 0.638                           | < 0.001                          | 0.021                              |
| BMD-TBLH Z-score           | −0.20 (−0.80–−0.27)         | −0.20 (−0.80–−0.30)             | 0.10 (−0.45–0.90) | 0.025                           | 0.336                            | 0.034                              |
| BMD-Lumbar Z-score         | 0.00 (−0.90–0.80)           | −0.10 (−0.80–0.50)              | 0.00 (−0.70–0.90) | 0.446                           | 0.146                            | 0.148                              |

T1D – type 1 diabetes; OC – osteocalcin; OPG – osteoprotegerin; s-RANKL – receptor activator of nuclear factor κ – B ligand; DPD – deoxypyridinolone; TBLH – total body less head; BMD – bone mineral density

*p values < 0.05 are indicated as bold

C-peptide level and OC concentration was also noted after seven months of observation (R = 0.243, p = 0.014).

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A tendency towards higher TBLH BMD Z-score values (respectively, remission 0.000 [–0.700–0.500], no remission –0.550 [–1.000–0.150], \( p = 0.050 \)) was also noted.

**Discussion**

In this study, bone metabolism disturbances in paediatric patients with T1D were evaluated for the first time at two time points: at clinical diagnosis of T1D and after seven months of follow-up. Differences in osteocalcin, OPG, s-RANKL, and DPD levels between the study and control groups were observed, especially strongly expressed at T1D onset and modified after several months of observation after obtaining metabolic control of diabetes and determination of insulin demand. Moreover, densitometry showed lower TBLH BMD Z-score values in T1D patients both at T1D onset and after seven months of observation as compared to the control group, whereas the results of the lumbar BMD did not differ between the groups. However, the results of TBLH BMD measurements were similar at both time points in the study group, which may indicate the lack of bone remodelling in children at the early stage of clinical T1D and is not surprising after only seven months of observation. Contrary to the results obtained, several studies showed decreased BMD lumbar values in children with T1D even a few months after the clinical diagnosis of diabetes [4, 16].

Interestingly, in the group of patients with partial clinical remission the TBLH BMD Z-score values tended to be higher in comparison to patients without remission. Less severe bone resorption may also be reflected in significantly lower urine DPD in the subgroup of patients with partial clinical remission.

According to other authors, bone metabolism disorders predisposing to more frequent bone fractures are particularly severe at the moment of clinical diagnosis of T1D [17]. This seems understandable considering the presence of ketoacidosis increasing osteoclast activity and the transient catabolic state caused by insulin deficiency leading to lower production of bone matrix proteins and decreased bone mineralisation. Chronic hyperglycaemia and metabolic acidosis at the cellular level cause oxidative stress and promote non-enzymatic glycation of proteins, as well as collagen, and reduce osteoblast activity [18].

In the presented study, the intensity of ketoacidosis assessed by pH value correlated negatively with osteocalcin and
OPG concentrations, whereas HbA1c correlated negatively with osteocalcin, s-RANKL, and urine DPD and positively with OPG level. Also, BMI Z-score values correlated negatively with OPG and urine DPD and positively with s-RANKL level.

Chrysis et al., in a group of T1D patients after several years of diabetes duration, also found a positive correlation between HbA1c and OPG level and negative correlation between OPG concentration and BMI as well as higher osteoprotegerin concentration in children with T1D, in comparison to a control group. There were no differences in RANKL concentration between the study group and the control group [19]. Similarly, Galluzzi et al. noted significantly higher levels of osteoprotegerin in children with several-year course of T1D compared to the controls [20]. Loureiro et al. observed reduced osteoprotegerin levels in children with T1D and their strong correlation with metabolic control of diabetes [21]. However, in another study focused on metabolic control of diabetes in patients with an average three-year duration of T1D, higher osteocalcin levels as compared to the control group were observed, while the highest levels in patients with good metabolic control were noticed. No significant difference was observed in osteoprotegerin and RANKL levels [22]. Abd El Dayem et al. noted lower levels of osteoprotegerin and osteocalcin and higher levels of urine DPD in T1D patients compared to the control group, suggesting diagnostic superiority of bone resorption markers over bone formation markers [23].

However, in several studies no correlation was found between bone metabolism in T1D patients and duration of diabetes, glycaemic control, insulin administration pattern, or other chronic complications [24–27].

However, there are only a few studies relating to the level of preserved insulin secretion in children with a newly diagnosed T1D in the context of bone metabolism disorders. Our results indicated a negative correlation between both fasting and stimulated by glucagon insulin secretion and OPG levels at the time of clinical diagnosis of T1D. At the same time, s-RANKL levels correlated positively with fasting and stimulated C-peptide levels, also after seven months of its duration. Therefore, it may be speculated that the decreased insulin secretion in patients at the time of clinical T1D diagnosis results in decreased insulin binding to the osteoblast receptor, which may lead to a temporary increase of OPG level observed in children with T1D at the time of diabetes diagnosis. The increase of OPG can affect the change of the OPG/RANKL ratio [28], which was also confirmed in the presented results of our study. This situation may be reversed after implementation of exogenous insulin therapy, which was assessed after several months of follow-up. During a partial clinical remission, the ability to produce the preserved endogenous insulin is partially restored. This results in a decrease in the demand for exogenous insulin in children, which reaches its peak after about 6-7 months of diabetes duration. This is consistent with our observations on the tendency for a temporary increase in TBLH in our diabetic patients with partial clinical remission.

The hypothesis about the relationship between bone turnover markers and insulin secretion in diabetic patients seems to be confirmed by the observations of other authors suggesting that an increase of OPG concentration may be a defensive response of the skeletal system against increased bone resorption [29]. The decrease in s-RANKL levels in diabetic patients is probably associated with the blocking of s-RANKL signal by OPG increase on the OPG/RANKL/RANK pathway [19]. Therefore, it seems that the evaluation of bone markers, especially OPG and s-RANKL as sensitive and specific indicators of bone metabolism disorders in children with T1D, is fully justified and indicated already at the moment of clinical T1D diagnosis, before BMD changes observed in densitometry and leading to osteopaenia and osteoporosis.

A limitation of our work may be the relatively small size of the study group, even assuming that the tests were performed twice during the first year of clinical T1D. In addition, in the control group the bone turnover markers were evaluated only once. However, the follow-up of 6–8 months is relatively short to observe significant changes in bone mineral density in healthy children. This may be influenced by puberty, which would be interesting to evaluate if the study was continued. Moreover, an interesting addition to these studies could be the evaluation of bone metabolism markers carried out at prediabetes stage, even before the occurrence of clinical T1D. However, this seems to be possible only in the siblings of T1D patients. Another limitation may be the lack of consideration of the status of puberty of T1D patients.

Conclusions

Summarising, early diagnosis of bone disorders in children with T1D is important for early detection of abnormalities and additionally enables greater motivation to improve the glycaemic control, intensify treatment, and thus avoid the development of osteoporosis in the further clinical course of type 1 diabetes in children. The bone metabolism disorders observed at onset of type 1 diabetes in children are modified once the metabolic control of the disease has been achieved and seem to be the most strongly associated with preserved insulin secretion in the patients.
