

ORIGINAL ARTICLE

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Evaluation of the effect of metformin treatment on markers of bone formation and resorption in patients newly diagnosed with Type 2 diabetes

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Abstract

Type 2 Diabetes Mellitus can affect bone metabolism by many mechanisms and increase the risk of osteoporosis. It has been reported that glucose-lowering treatments, including metformin also have an effect on bone turnover. The aim of this study is to evaluate the effect of metformin treatment on bone formation and resorption in patients with newly diagnosed type 2 Diabetes Mellitus. There were 37 patients in the study population who were newly diagnosed with type 2 Diabetes Mellitus. Fasting plasma glucose level and postprandial plasma glucose level as well as plasma Hemoglobin A1C, calcium, phosphorus, parathyroid hormone, osteocalcin and C-Telopeptide levels were measured before initiation of metformin treatment. The tests were repeated 3 months after the treatment. The levels of fasting plasma glucose and postprandial plasma glucose decreased after 3 months of metformin treatment as expected and the difference was statistically significant. However there was no significant change in calcium, phosphorus and parathyroid hormone levels. There was also a significant decrease due to Metformin treatment in osteocalcin levels, which is a bone formation marker. Metformin treatment has decreased C-Telopeptide levels as well which is also a bone formation marker. Our results have shown that 3 months of metformin treatment may suppress the bone turnover. Another important finding of our study was the reduction in levels of bone formation markers in female patients. Controlled long-term studies are needed to be conducted in order to manifest the clinical implications of these findings.

Keywords: Type 2 diabetes, Metformin, bone formation, bone resorption

Introduction

Diabetes Mellitus (DM) is a metabolic disease that occurs with an absolute or relative deficiency of insulin secretion and/or insulin resistance, manifests itself with hyperglycemia, and is characterized by disorders of carbohydrate, fat, and protein metabolism [1,2].

It has been known for a long time that skeletal and bone metabolism are affected in diabetic patients. Although localized bone changes in the foot skeleton are associated with the coexistence of angiopathic and neuropathic changes, it is still unclear whether there is a bone disease called diabetic osteopenia and its relationship with the clinical findings of diabetes. The relationship

between diabetes and osteopenia has not been fully elucidated [1].

With a better understanding of the mechanisms leading to Type 1 and Type 2 DM, it has become clear that changes in bone metabolism are not the result of a single pathogenetic event, but that changes in bone metabolism are a multifactorial event that can occur in different clinical situations [3].

Factors involved in the pathogenesis of diabetic osteopenia are listed as hyperglycemic state, loss of calcium-phosphate from the kidneys, decrease in the effect of insulin-like growth factors (IGF-1), formation of advanced glycation product (AGE) complications such as neuropathy and nephropathy, changes in vitamin D metabolism, and a decrease in osteoblast functions. It is thought that the osteoblastic deficit plays a major role in the development of diabetic osteopenia [3].

In this study we aimed to evaluate bone formation and resorption parameters before and at least 3 months after metformin treatment in newly diagnosed Type 2 DM patients.

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Materials and Methods

Our study was carried out with the approval number 15.05.2012-2012/51 from İnönü university. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or the national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

A total of 54 patients who admitted to the Endocrinology and Metabolic Diseases outpatient clinic of İnönü University Faculty of Medicine and accepted the study, who were diagnosed with Type 2 diabetes and were prescribed metformin treatment, were included in the study. Seventeen patients were excluded from the study. Seven of them used their medication irregularly and the remaining 10 patients stopped taking their medication. A total of 37 patients completed the study. A diabetes diagnosis was confirmed by performing OGTT on patients with a high risk of Type 2 DM in the study. Patients who used 2x1000mg metformin for at least 3 months were included in the study.

Study Protocol

A systemic examination of all patients was performed at the beginning of the study. OGTT was administered to most of the patients with Type 2 DM after 10 hours of fasting. At least three days before the OGTT, patients should be included in a nutrition program containing at least 200g of carbohydrates per day. It was noted that the patient did not have any problems that could affect the OGTT, such as severe stress, acute cerebral and cardiac events, long-term inactivity, or infection. Blood was drawn from patients diagnosed with Type 2 DM by OGTT to measure the levels of serum hemogram, Fasting Plasma Glucose (FPG), Postprandial Plasma Glucose (PPG), A1C, Phosphorus (P), Calcium (Ca), Parathormone (PTH), osteocalcin, and human C-telopeptide of type I collagen (CTX) following a 10-hour night fasting in serum between 08:00 and 10:00. After their blood was collected for 1 month, a maximum of 2 grams of metformin treatment was administered. The first parameters were repeated after the patients had received treatment for at least three months.

During the study, the patients came for a monthly visit and were followed up in terms of FPG, PPG, and whether they took the drug. The vital functions of the patients and the side effects of the drug were evaluated. Patients used metformin for at least 3 months.

In routine biochemical examinations, serum Ca and P levels were determined by the spectrophotometric method in the Abbott brand, Architect C16000 model device, and serum osteocalcin levels by the chemiluminescence method in the PTH Siemens brand Immulite 2000 device, and CTX levels by the electrochemiluminescent method. CTX levels were determined by the ELISA (One Enzyme-Linked Immunosorbent Assay) method. A1C was studied in a TOSOH brand, G8 model device using the HPLC (high-performance liquid chromatography) method.

Statistics

The data were analyzed with the SPSS 15.0 program, which is compatible with Windows.

Two Simple Kolmogorov-Smirnov test was used for the distribution

between the start and end parameters. The Wilcoxon Signed Ranks Test was used for homogeneously distributed parameters. A Paired T-Test was used to compare the pre-treatment and post-treatment values. The Wilcoxon Signed Ranks Test was used to evaluate the levels of pre-treatment and post-treatment of CTX and osteocalcin in men and women. The relationship between CTX and osteocalcin in pre and post-treatment biochemical parameters of type 2 DM patients was evaluated using the Pearson correlation method. The results were presented as a mean standard deviation. The $P < 0.05$ value was accepted as statistically significant.

Results

A total of 37 patients with newly diagnosed diabetes who applied to the İnönü University Faculty of Medicine Endocrinology Outpatient Clinic in 2013 were included in the study. Although the ages of the patients ranged from 29 to 66, the mean age was 50.89 ± 10.84 . Of the patients, 59.5% (n:22) were female and 40.5% (n:15) were male.

The diabetes duration of the patients was between 1 month and 12 months, with a median of 142 ± 48.2 days. Of the patients, 2.7% had hypertension, 5.4% had cardiovascular disease, 27% had other diseases that doesn't effect bone turnover, and 23% had no disease beside DM.

Microvascular complications of the patients were evaluated. Of the patients, 7.4% had nephropathy, 16.2% had neuropathy, and 24% had retinopathy.

A total of 37 patients who were newly diagnosed with diabetes and completed the study were included in our study. Bone formation and resorption parameters, FPG, PPG, A1C, Ca, P, and PTH were studied before and after metformin treatment was initiated. Mean FPG was 143.75 ± 48.59 mg/dl before treatment and decreased to 126.6 ± 45.8 mg/dl after treatment, and PPG decreased from 233 ± 87.0 mg/dl to 166.4 ± 72.4 mg/dl in 37 patients who were administered metformin treatment. This change was found to be statistically significant ($p < 0.05$). While the mean A1C level was 6.83 ± 1.11 before the treatment, it decreased to 6.63 ± 0.87 after the treatment, but this regression was not statistically significant ($p > 0.05$). While the PTH level was 50.9 ± 26.0 before the treatment, it increased to 57.3 ± 24.1 after the treatment. This difference was not statistically significant ($p > 0.05$) (Table 1).

Table 1. Pre-Treatment and Post-Treatment Evaluation of Biochemical Parameters

Variables	Before treatment	After treatment	P-value
FPG (mg/dl)	143.7 ± 48.5	126.6 ± 45.8	< 0.05
PPG (mg/dL)	233.9 ± 87.0	166.4 ± 72.4	< 0.05
A1C %	6.8 ± 1.11	6.6 ± 0.87	0.35
Calcium (mg/dl)	9.32 ± 0.33	9.40 ± 0.31	0.24
Phosphor (mg/dl)	3.42 ± 0.51	3.30 ± 0.46	0.22
Parathormone (pg/ml)	50.9 ± 26.0	57.3 ± 24.1	0.13

While the bone resorption parameter CTX was 297 ± 187 before treatment, it decreased to 272 ± 196 after treatment. This difference was not statistically significant ($p > 0.05$) (Table 2). Bone formation parameter osteocalcin averaged 6.23 ± 5.52 pre-treatment and it decreased to 4.70 ± 3.04 post-treatment. This difference was statistically significant ($p < 0.05$).

Table 2. Pre and Post Treatment Evaluation of Bone Formation and Resorption

Variables	Before treatment	After treatment	P-value
CTX (ng / mL)	297±187.9	272.0±196.8	0.23
Osteocalcin (ng / mL)	6.23±5.52	4.70±3.04	<0.05

When CTX measurements in male and female cases were evaluated separately, the regression in the 3rd month compared to the baseline was not found to be statistically significant in both gro-ups (p>0.05) (Table 3).

Table 3. Pre- and Post-Treatment Evaluation of Bone Formation and Resorption Parameters by Gender

Variables	Before treatment	After treatment	P-value
Male patients			
CTX (ng / mL)	230.5±170.14	201.1±171.07	0.62
Osteocalcin (ng / mL)	7.275±6.561	4.870±3.250	P<0.05
Female patients			
CTX (ng / mL)	394.5±174.04	375.96±190.41	0.91
Osteocalcin (ng / mL)	4.717±3.098	4.458±2.796	0.60

When osteocalcin measurements in male and female subjects were evaluated separately, the decrease observed in the 3rd month compared to the baseline was found to be statistically significant in fe-males (p<0.05) (Table 3).

According to the correlation analysis with CTX, there was a negative correlation between FPG, P, PTH, and CTX pre- and post- treatment. Although there was a positive correlation between PPG and CTX after treatment, there was no statistical significance (p>0.05). There is a negative correlation between PPG and CTX after treatment (Table 4).

Table 4. Correlation Between CTX and Biochemical Parameters

Variables	Before treatment		After treatment	
	R	P	r	P
FPG(mg/dl)	-0.08	0.63	0.26	0.12
PPG(mg/dl)	0.21	0.26	0.24	0.17
A1C %	0.09	0.96	-0.72	0.69
Calcium(mg/dl)	0.15	0.37	0.33	0.45
Phosphor(mg/dl)	0.50	0.77	-0.8	0.56
Parathormone(pg/dl)	-0.17	0.31	-0.13	0.43
Procalcitonin (ng/mL)	-0.48	0.02	-0.25	0.13

While there is a positive correlation between Ca and CTX before treatment, there is a negative correlation after treatment. Although there was a negative correlation between osteocalcin and CTX before treatment, it was determined to be statistically significant (p<0.05) (Table 4).

Although there was a negative correlation between osteocalcin and CTX before treatment, it was found to be statistically significant (p<0.05). A negative correlation was observed between osteocalcin and CTX after treatment.

In the correlation analysis performed with osteocalcin, there was a negative correlation between PPBG, A1C, Ca, and osteocalcin before and after treatment. While there was a positive correlation with PPG before treatment, there was a negative correlation after

treatment.

There was a positive correlation between PTH and osteocalcin before treatment. (p<0.01). Although there was a positive correlation between PTH and osteocalcin after treatment, it was not found to be statistically significant (p>0.05) (Table 5).

Table 5. Correlation Between Osteocalcin and Biochemical Parameters

Variables	Before treatment		After treatment	
	R	P	r	P
FPG(mg/dl)	-0.92	0.60	-0.21	0.21
PPG(mg/dl)	0.42	0.81	-0.17	0.36
A1C %	-0.38	0.03	-0.36	0.05
Calcium(mg/dl)	-0.03	0.83	-0.03	0.82
Phosphor(mg/dl)	0.05	0.74	0.21	0.19
Parathormone(pg/dl)	0.55	0.00	0.05	0.73
CTX (ng / mL)	-0.17	0.31	-0.13	0.43

Discussion

Diabetes mellitus is a disease that occurs with an absolute or relative deficiency of insulin secretion or insulin resistance, manifests itself with hyperglycemia, and is characterized by disorders of carbohydrate, fat, and protein metabolism. It is an important metabolic disease with a rapidly increasing incidence worldwide [4].

It is a health problem that concerns very large masses with its increasing frequency and complications, and epidemics have begun to be mentioned. According to WHO, the number of diabetic patients is around 250 million and is expected to reach 500 million by the end of the next 10 years, according to WHO. This increase is attributed to a sedentary lifestyle, changing eating habits, and an increase in life expectancy [5].

Diabetes is a common disease with a very high economic cost, which seriously affects public health and causes negative consequences [6].

It has been shown that the end products of glycolysis and their receptors, which occur as a result of impaired glucose metabolism in type 2 DM, have negative effects on bone metabolism and bone strength. It is known that the increase in AGE causes a decrease in the collagen that provides the hardness of the bone [7].

Other metabolic changes that can reduce bone strength in type 2 DM include vitamin D disorders, calcium, parathyroid hormone, and changes related to glucose-lowering therapy [8].

Factors contributing to the pathogenesis of diabetic osteopenia can be listed as chronic hyperglycemic state, renal calcium-phosphate loss, decrease in insulin-like growth factor, formation of glycosylation end products and neuropathy, diabetic complications like nephropathy, changes in vitamin D metabolism, and a decrease in osteoblast functions [3]. It has been shown that hyperglycemia suppresses osteoblast proliferation [4]. Although IGF-1 is anabolic for bone, it may be low in DM [9]. An increase in inflammation and related cytokines accelerates bone turnover and causes bone loss [10,11]. In addition to prolonged hyperglycemia, insulin and IGF-1 deficiency suppress osteoblast proliferation [12].

A total of 37 patients diagnosed with diabetes were included in the study. It was aimed to investigate the effects of metformin use, which is used in the treatment of diabetes, which concerns very large masses by increasing in frequency and causes complications in bone formation and resorption parameters. We planned to conduct this study as there were not enough studies evaluating the bone formation and resorption parameters before and at least 3 months after the start of metformin treatment with new Type 2 DM.

Various studies have shown that the metformin used in the treatment of type 2 DM shows effects by suppressing glucose production in the liver and increasing glucose uptake and insulin impact in peripheral tissues (especially in skeletal muscle) [13]. It is known that it delays intestinal glucose absorption and prevents postprandial hyperglycemia. It is an anti-hyperglycemic agent frequently used in type 2 diabetes treatment. It has been observed that metformin monotherapy has preventive effects against diabetes complications [14].

Even though Bak et al. have shown that metformin has no effect on osteoclast formation, metformin has also been shown to inhibit osteoclast differentiation in vivo or in vitro by osteoprotegerin stimulation or inhibition of RANKL expression. It has been demonstrated that high glucose levels reverse the detrimental effect of high glucose levels on osteoblast function [15,23]. While most of the studies reporting the osteogenic effects of metformin in vitro show that metformin has no osteogenic effects or inhibits osteoblast differentiation [16].

It has been noted that part of the antihyperglycemic effect of metformin is due to a decrease in free fatty acid (FFA) release or lipid oxidation in adipose tissue. It has been shown that it reduces the average preprandial blood glucose by 60-70 mg/dl and the average A1C level by 1.5-2%. IGF-1: Although it is anabolic for bone, it may be decreased in DM [9,17]. An increase in inflammation and related cytokines accelerates bone turnover and leads to bone loss [10,11].

Metformin treatment was commenced in 37 patients included in our study. The blood glucose lowering effect of both FPG and PPG in patients using metformin was found to be statistically significant ($p < 0.05$). While metformin did not show a significant change in the mean A1C level before and after treatment, other similar studies have shown that metformin can decrease the mean A1C level by an average of 1-1.5% [20].

In similar studies, such as in a study where those who received only metformin treatment and those who received metformin combination therapy, it was determined that the decrease in A1C, FPG and PPG was more significant in those who received combination therapy than in those who received metformin. No significant difference was found in serum Ca, PTH levels in those receiving both metformin and combination therapy.

It has been shown in the study of Cortizo et.al that metformin stimulates ALP activity, collagen production, calcium deposition, and nitric oxide (NO) expression in osteoblast-like cells (MC3T3-E1) in a normal glucose environment [14,21]. In addition, articles claim that metformin stimulates osteoblastic differentiation and stimulates bone formation.

Similarly, metformin was shown to increase cell proliferation in a dose-dependent manner in a study conducted by Petty and Pearson in vitro conditions [22]. Studies are suggesting that this anti-diabetic drug not only stimulates osteoblast growth and differentiation but also has the ability to increase extracellular matrix mineralization [23].

However, different results have been found in the conducted studies. When bone formation parameters were evaluated in a randomized controlled study using metformin, glyburide, and rosiglitazone, a significant decrease was observed in bone formation parameters in both men and women. A very small change was observed in the group using glyburide [24]. A significant decrease in serum osteocalcin levels in our study, consistent with these studies, after 12 weeks of metformin treatment compared to baseline was found to be statistically significant ($p < 0.05$). It is noteworthy that osteocalcin levels decrease more in women than in men after metformin treatment. While a significant decrease in osteocalcin levels in women was statistically significant, no significant change was observed in men, although osteocalcin levels tended to decrease after metformin treatment ($p > 0.05$).

CTX, also known as the bone resorption parameter, is an important marker of bone resorption. In a randomized controlled study, an increase of 6.1% CTX in females was observed in the rosiglitazone group, while an increase of 0.2% in females and a decrease of 2.5% in males was observed in the metformin and glyburide groups. The change observed in all three groups was found to be statistically significant [25]. On the other hand, the decrease in CTX in the rosiglitazone group in men was not found to be significant (26). The decrease in CTX was found to be significant in groups using metformin and glyburide. A decrease was observed in CTX measurements in both female and male subjects at the 3rd month compared to baseline in the study, but the difference was not statistically significant ($p > 0.05$).

In our study, there was a negative correlation between FPG, P, PTH, and CTX before and after treatment. Although there was a positive correlation between PPG and CTX after treatment, there was no statistically significant ($p > 0.05$). There is a negative correlation between PPG and CTX after treatment.

While there is a positive correlation between Ca and CTX before treatment, there is a negative correlation after treatment. Although there was a negative correlation between osteocalcin and CTX before treatment, it was determined to be statistically significant ($p < 0.05$).

Although there was a negative correlation between osteocalcin and CTX before treatment, it was found to be statistically significant ($p < 0.05$). A negative correlation was observed between osteocalcin and CTX after treatment.

Conclusion

As a result, it has been put forward that the use of an antihyperglycemic agent, such as metformin, that breaks insulin resistance in patients recently diagnosed with Type 2 diabetes prevents diabetes-related complications. However, its effects on bone metabolism are controversial. In our study, short-term (3 months) metformin treatment reduces both bone formation and

resorption. The fact that the reducing effect on production was more evident in our study indicates that it may have negative effects on bone. However, further studies need to be conducted to reveal this issue.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

All authors declare no financial support.

Ethical approval

Our study was carried out with the approval number 15.05.2012 - 2012/51 from İnönü university. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or the national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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