SERUM OSTEOCALCIN LEVEL IN TYPE II DIABETES MELLITUS AS AMARKER FOR EARLY DETECTION OF OSTEOPOROSIS

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ABSTRACT

Background: Diabetic osteoporosis is caused by reduced bone mineral content due to the abnormal levels of sugar, protein, fat, and macroelements, such as calcium and phosphorus. Beside metabolic bone diseases, these changes at times lead to pathological fractures. Still, the effect of type 2 diabetes (T2DM) on bone mineral density remains controversial. There are potential mechanisms behind the increased fracture risk that occurs in patients with diabetes, even those with increased bone mineral density. One potential link between diabetes and bone is the osteoblast-produced factor, osteocalcin. Objective: The aim of this study was to determine the level of osteocalcin in type 2 diabetic patients as a marker for early detection of osteoporosis. This may help early prediction and treatment of osteoporosis before bone mass density (BMD) affected.

Subjects and Methods: Sixty diabetic patients (30 males and 30 females) aged 33–61 years were recruited and classified by DEXA into three equal groups: patients with type 2 diabetes mellitus with osteoporosis, patients with osteopenia, and patients with normal bone, as well as twenty apparently healthy controls. The inclusion and exclusion criteria were applied for both patients and controls. All subjects included in this study were subjected to the following: Full history taking and clinical examination, laboratory investigations including complete blood picture (CBC), fasting and post prandial serum glucose levels, serum creatinine, lipid profile (LDL, HDL, total cholesterol and triglycerides) and complete urine analysis. Serological test for osteocalcin by ELISA technique was used. Results: The results of the present study showed that serum osteocalcin level significantly decreased in diabetic patients with osteoporosis, osteopenia, and normal bone as compared to healthy control subjects. Also, there was significant decrease in serum osteocalcin level in type 2 diabetic patients with osteoporosis as compared to those with osteopenia and normal bone density groups. Significant negative correlation was found between osteocalcin and HbA1c, LDL, duration of diabetes and TG in all diabetic patients groups, and positive correlation between osteocalcin, HDL, and BMD in all diabetic groups. Conclusion: Osteocalcin may have a role on prediction of osteoporosis in diabetic patients even before bone mineral density affected.

Key words: Osteocalcin, diabetes mellitus, osteoporosis.

INTRODUCTION

Diabetes mellitus is a pandemic and a chronic metabolic disorder with substantial morbidity and mortality that is characterized by the presence of high blood glucose (Chin et al., 2014). About 374 million people in the world are under the threat of this deleterious health problem (Sealand et al., 2013).
Osteoporosis (OP) is often described as a silent disease because it is typically asymptomatic until a fracture occurs. The disease negatively and significantly impacts morbidity and mortality as it can lead to severe pain, deformity, disability, and death. The signs of OP are deterioration of the microstructure of bone specifically at trabecular sites including vertebrae, ribs and hips, culmination in fragility fractures, pain and disability (Wilden-Kirk et al., 2011). The occurrence of OP is prevalent among the aging women than the aging men although corticosteroid treatment, intake of excessive alcohol, cigarette smoking, low calcium intake and hypogonadism may be the secondary cause (Chin et al., 2014).

The worldwide prevalence of osteoporosis is estimated to be greater than 200 million people, with the majority being women (Osteoporosis – General Statistics, 2012).

Bone mineral density (BMD) has been shown to be higher in people with T2DM (Ma et al., 2012). It leads to increased skeletal fragility and microarchitectural deterioration of bone tissue, causing a decrease in bone mineral density (BMD), bone quality, and strength (Reyes and Moreno, 2005).

It is difficult to ascertain whether there is a difference in the distribution of osteoporotic fractures in patients with T2DM because age, gender, and obesity may have separate effects on fracture incidence (Viegas et al., 2011).

Osteocalcin (OC) is a product of differentiated osteoblasts, formed by 46 to 50 amino acids residue protein and is released into the general circulation (Villaf?n-Bernal et al., 2011). Osteocalcin is also known as bone gamma-carboxyglutamic acid (Gla) protein, it is the most abundant noncollagenous protein of bone matrix (Razzaque, 2011). Osteocalcin has been reported to exert a profound effect on glucose homeostasis, insulin sensitivity and fat metabolism (Ferron et al., 2008).

Different experimental observations indicated the existence of a feed forward loop linking insulin, bone resorption, and osteocalcin activity as a potential mechanism for the association between bone and glucose metabolism. In support of these findings, the daily injection of osteocalcin in normal mice (in doses between 3 and 30 ng/g/day) led to an improved glucose homeostasis by increasing beta-cell function and insulin sensitivity (Ferron et al., 2012).

**SUBJECTS AND METHODS**

The subjects in current study were divided into four equal groups (20 subjects each). Group I was healthy subject as control with mean age 43.1 ± 9.1; Group II was diabetic patients with osteoporosis and mean age 52.2 ± 8.5, Group III was diabetic patients with osteopenia and mean age 50.8 ±6.2, and Group IV was diabetic patients and normal bone with mean age 48.9 ± 8.4. Patients with type II diabetes mellitus (20 males and 20 females) attended the clinic of Endocrinology Department in Sayed Galal University Hospital. They had no apparent complication of diabetes, and they were classified by DEXA. Type 2 diabetic patients were diagnosed according to criteria of the American Diabetes Association (2006). For subjects of all groups written consent and history
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was obtained. Full clinical examination was done including neurological and fundus examination, complete blood picture, fasting and post prandial serum glucose levels, serum creatinine, lipid profile (LDL, HDL, total cholesterol and triglycerides) and complete urine analysis.

Exclusion criteria: Cases complaining from other diseases

Specific investigations: Estimation of serum osteocalcin by ELISA method manufactured by Epitope diagnostic inc,San Diego, CA92121, USA (Nagasue et al.,2003). Bone mineral density was measured by DEXA (Kanis and Gluer, 2000).

Sampling: Ten ml venous blood was withdrawn aseptically from each subject. Of them, 2.5 ml were added to EDTA-containing tube for estimation of CBC and HbA1c. The remaining 8 ml were added to plain tube for serum separation. All biochemical parameters were freshly measured. Part of serum was stored at -80°C for measurement of osteocalcin. Another two ml blood were withdrawn 2 hours after average meal, and used for measurement of 2 hours postprandial serum glucose level.

Analytical Methods: Osteocalcin was measured by ELISA according to the method of Nagasue et al. (2003). Serum glucose was measured according to the method of Young (2001) using spin react kit. Cholesterol was measured according to the method of Burtis and Ashwood (1999) by spin react kit. Estimation of serum low density lipoprotein was detected according to the Friedewald Formula (LDL = total cholesterol – [HDL+ triglycerides/5])

Statistical analysis: All statistical analyses were performed using descriptive statistics. Mean ± SD for the outcome variables of interest were computed. One-way analysis of variance with repeated measures was used for comparison of dependent variables. P<0.05 was considered to be significant. Statistical data were analyzed to evaluate the differences between the groups using the student’s t-test. Statistical analysis was done using the Statistic Package for Social Science Version 17 (SPSS 17.0).

RESULTS

As regards the clinidemographic and laboratory assessment, there was a significant increase of FBS, PP blood glucose, glycated hemoglobin, urea and creatinine in diabetic groups compared to control group (Table 1).

Osteocalcin levels in all diabetic groups were significantly lower than controls. However, no significant difference was found between diabetic groups and controls with respect to cholesterol, TG and LDL. Significant decrease in HDL, osteocalcin and BMD (femur T-score and lumbar T-score) were found in all diabetic groups compared to control groups (Table2).

Correlation analysis between serum osteocalcin and other variables of all diabetic groups and control revealed the presence of significant negative correlation between osteocalcin and HbA1c (r= -0.368 –fig.1).
Table (1): Demographic and biochemical characteristics of all studied groups (Mean ± SD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-diabetic (Controls) n= 20</th>
<th>Diabetic group with osteoporosis n = 20</th>
<th>Diabetic group with osteopenia n =20</th>
<th>Diabetic group with normal bone n = 20</th>
<th>ANOVA (F) test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>43.1 ± 9.1</td>
<td>52.2 ± 8.5</td>
<td>50.8 ±6.2</td>
<td>48.9 ± 8.4</td>
<td>F = 5.1</td>
<td>0.003*</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>84.2 ± 11.9</td>
<td>121.7 ± 31.96</td>
<td>126.4±26.3</td>
<td>135.6 ±30.4</td>
<td>F = 13.3</td>
<td>0.000*</td>
</tr>
<tr>
<td>P.Pblood glucose (mg/dl)</td>
<td>113.8 ± 13.8</td>
<td>194.9 ± 60.0</td>
<td>199.4 ±29.0</td>
<td>183.5 ±26.2</td>
<td>F = 0.000</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>189.4 ± 30.3</td>
<td>191.35 ±29.6</td>
<td>200.0 ±38.2</td>
<td>169.1 ±36.1</td>
<td>F = 2.3</td>
<td>0.08</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>112.0 ± 36.4</td>
<td>143.3 ±36.4</td>
<td>129.8 ±26.8</td>
<td>109.4 ±60.0</td>
<td>F = 2.6</td>
<td>0.5</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>116.5 ± 21.9</td>
<td>122.8 ±12.8</td>
<td>105.0 ±13.8</td>
<td>102.7 ±17.6</td>
<td>F = 5.3</td>
<td>0.002*</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>56.1 ± 7.6</td>
<td>35.7 ±6.3</td>
<td>37.5 ±11.6</td>
<td>47.9 ±9.3</td>
<td>F = 22.8</td>
<td>0.000*</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>30.8 ± 4.6</td>
<td>37.5 ±10.6</td>
<td>35.1 ±6.1</td>
<td>32.1 ±7.2</td>
<td>F = 2.9</td>
<td>0.04*</td>
</tr>
<tr>
<td>Creatinin (mg/dl)</td>
<td>0.58 ± 0.2</td>
<td>1.0 ± 0.31</td>
<td>1.0± 0.3</td>
<td>1.0± 0.29</td>
<td>F = 11.6</td>
<td>0.000*</td>
</tr>
<tr>
<td>Osteocalcin (ng/ml)</td>
<td>13.9 – 19</td>
<td>2.4 – 13.4</td>
<td>7.0 ±2.8</td>
<td>0.000*</td>
<td>F=165.0</td>
<td>0.000*</td>
</tr>
<tr>
<td>Range</td>
<td>16.7 ± 1.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

*Significant.

Table (2): Comparison between diabetic patients and non-diabetic subjects (controls) regarding serum osteocalcin level and bone density scores.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-diabetic (Control) n= 20</th>
<th>Diabetic group n = 60</th>
<th>Sig.test</th>
<th>P-value</th>
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<tr>
<td>Osteocalcin (ng/ml)</td>
<td></td>
<td></td>
<td>F = 165.0</td>
<td>0.000*</td>
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<tr>
<td>Range</td>
<td>13.9 – 19</td>
<td>2.4 – 13.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>16.7 ± 1.6</td>
<td>7.1 ± 2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femur T-score</td>
<td></td>
<td></td>
<td>F =395.6</td>
<td>0.000*</td>
</tr>
<tr>
<td>Range</td>
<td>1.2 -2.3</td>
<td>-3.80 - 1.90</td>
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<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.6950±.27237</td>
<td>-1.3 ±1.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumbar T-score</td>
<td></td>
<td></td>
<td>F =773.8</td>
<td>0.000*</td>
</tr>
<tr>
<td>Range</td>
<td>1.10 - 2.50</td>
<td>-4.60 - 1.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.69 ± .34928</td>
<td>-1.69 ± 2.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant.
DISCUSSION

Diabetes mellitus is a pandemic metabolic disease with morbidity and mortality, bone and mineral abnormalities in patients with diabetes mellitus may be caused by direct effect of insulin deficiency or resistance, advanced glycation of bone matrix protein and abnormal cytokines and adipokines productions (Kanazawa et al., 2009).

Skeleton was considered as a dynamic connective tissue which was essential for mobility, calcium homeostasis, and hematopoietic niche. However, more and more evidences indicate that skeleton works not only as a structural scaffold, but also as an endocrine organ which regulates several metabolic processes (Shao et al., 2014). The relationship between type 2 diabetes and osteoporosis is complicated. Multiple studies have demonstrated an association between type 2 diabetes and fracture (Bonds et al., 2006).

OP is a painless weakening of the bones with a harmful impact on morbidity and mortality. It leads to increased skeletal fragility and microarchitectural deterioration of bone tissue, causing a
decrease in bone mineral density (BMD), bone quality, and strength (Raisz, 2005).

Osteoporosis is the most important metabolic bone disease in patients with diabetes mellitus. The relationship between T2DM and osteoporosis is complicated. Multiple studies have demonstrated an association between T2DM and fracture (Sealand et al., 2013).

Osteocalcin is a small protein secreted by osteoblasts that can undergo γ-carboxylation. The γ-carboxylated form binds hydroxyapatite and is abundant in bone extracellular matrix. In contrast, the under carboxylated circulating form has several hormonal features that regulates glucose metabolism and fat mass (Lee et al., 2007). It appears that osteocalcin increased insulin secretion, lower blood glucose, increased insulin sensitivity, and decreased visceral fat in both genders (Aurora et al., 2013).

In the present study, serum osteocalcin significantly decreased in diabetic patients with osteoporosis as compared to diabetic patients with osteopenia or healthy control. This was in agreement with the results obtained by Reyes-Garcia et al. (2013) who reported that osteocalcin and TRAP levels were significantly lower among diabetes patients than non-diabetic subjects, and they suggested that type 2DM was in a state of low bone turnover. Also, the present study was in agreement with the results obtained by Kindblom et al. (2009) who demonstrated an association of bone turnover biomarkers, especially of osteocalcin with levels of blood glucose and HbA1c.

Booth et al. (2013) reported that impaired bone turnover in type 2 diabetes appears to result from decreased bone formation. They also suggested that poor glycemic control in type 2 diabetes may contribute to osteopenia. Cutrin et al. (2007) assessed the effect of chronic hyperglycemia on bone mineral density (BMD) and bone remodeling in patients with type 2 diabetes mellitus. Their results demonstrate that hyperglycemia is not associated with increased bone resorption in type 2 diabetes mellitus and that BMD is not altered in type 2 diabetes mellitus.

Imet al. (2012) found an association between OC and vertebral fractures in type 2 diabetic patients. Bhatto et al. (2013) stated that it is uncertain whether bone markers may be of use in the prediction of fractures in diabetic patients.

In the current study, lipid profile showed that the mean serum levels of total cholesterol, and LDL-C in diabetic group with osteoporosis was significantly higher than those of control group and then those of the diabetic group with osteopenia. The serum level of HDL-C was significantly lower in the diabetic group with osteoporosis than in the control group, and the diabetic group with osteopenia.TG showed non-significant change in diabetic patients with osteoporosis group as compared to those with osteopenia and control groups. These results came in agreement with Ruiz-Gaspa et al.(2007). who stated that osteocalcin plays a role in the lipid lowering effects of statins. In a cross-sectional study in type 2 diabetes, HDL-C level was independently inversely associated with osteocalcin in men, where in premenopausal women triglycerides was positive independent factor influencing osteocalcin (Zhou et al., 2010).
On the other hand, some studies found no significant association between osteocalcin and lipid profile variables (Lee et al., 2012). Few other reports also showed a negative association between osteocalcin levels and triglycerides in blacks and non-Hispanic whites (Saleem et al., 2010).

The significant negative correlation between osteocalcin and HbA1c in diabetic patients and control may indicate a role of glycemic control in osteocalcin level.

Abdelsalam (2013) stated that there was a highly significant negative correlation between serum osteocalcin level and fasting blood glucose, 2 h postprandial blood glucose, HbA1c, fasting serum insulin, HOMA-IR, total cholesterol, serum triglycerides, LDL-cholesterol and highly significant positive correlation with HDL-cholesterol. Serum osteocalcin level may have a role in glucose homeostasis in gestational diabetes mellitus.

In conclusion: Skeleton has an endocrine function via osteocalcin and plays an important role in energy metabolism, especially in glucose metabolism. Osteocalcin promotes proliferation of β cells, insulin secretion, and insulin sensitivity. Measurement of osteocalcin seems more a helpful marker for early detection of osteoporosis in diabetic patients even with normal BMD. This may help early prediction and treatment of osteoporosis before BMD affected.

Further studies with larger sample sizes are needed to elucidate the physiological relevance of osteocalcin function in normal individual and patients with metabolic and other diseases in humans.

REFERENCES


خليفة البحث: تحدث هشاشة العظام في مرض السكري من النوع الثاني نتيجة انخفاض كثافة المعادن مثل الكالسيوم والفسفور في العظام مما يؤدي إلى كسور العظام، وهذا ناتج عن احتلال مستويات السكر والبروتينات والدهون. وتظل علاقة مرض السكري بحالة العظام موضوعا للجدل.

وتغير العلاقة بين السكر من النوع الثاني وحالة العظام علاقة معقدة إلى حد كبير. ونلاحظ أن زيادة شحمية خطر حدوث كسور العظام في مرضى السكري من النوع الثاني حتى عند زيادة كثافة المعادن في العظام.

والأنسلاك الأسثيكوالسين الذي تفرزه بانيات العظام يسبب ذلك، ويعمل الأسثيكوالسين كهورمون في الجسم، حيث يحفز خلايا بيتا في البنكرياس لإطلاق سراح الأنسلاك.

الهدف من البحث: يهدف هذا البحث إلى تحديد نسبة الأسثيكوالسين في مصل مرضى السكري مما يساعد في التشخيص والعلاج المبكر لحالة هشاشة العظام المصاحبة لمريض السكري من النوع الثاني وذلك قبل أن يحدث أي تغيير في كثافة العظام.

المرضى وطرق البحث: شملت هذه الدراسة ستميني مصابين بداء السكري من النوع الثاني، وقد تم تقسيم المرضى طبقاً لقياس كثافة العظام باستخدام جهاز الديكاسا إلى ثلاث مجموعات: المجموعة الأولى: مرضى السكر من النوع الثاني ويعانون من هشاشة عظام، والمجموعة الثانية: مرضى السكري من النوع الثاني ويعانون من تقصي بحالة العظام (الأسيتونيا)، والمجموعة الثالثة: مرضى السكري من النوع الثاني ولا يعانون من أي نقص في كثافة العظام. بالإضافة إلى ذلك، فقد تم الاستعانة ببعض فرد أصحاء كمجموعة ضابطة.

وقد تم فحص المرضى كلينيكياً، وتمانج الرؤية والرجوع إلى مصل الدم في كل المرضى من أجل مراقبة حالة هشاشة العظام والكثافة الكبدية البترولية. وتستخدم الأسثيكالسين في مصل الدم بطريقة القياس الإرادي من الأنسلاك (البيزة). النتائج: أظهرت الدراسة انخفاضاً ذا دالة إحصائية في مستوى الأسثيكالسين في مصل الدم في مجموعات المرضى السكري من النوع الثاني مقارنة مع مراقبة حالة الأنسلاك، وجدت علاقة ذات دالة إحصائية في مستوى الأسثيكالسين في مصل الدم حيث تناضح الأسثيكالسين تناضح مع الدهون منخفضة الكثافة ونسبة الدهون السكري ومستوى الدهون، وتحديد مع كثافة العظام والدهون عالية الكثافة في كل مجموعات مرضى السكري من النوع الثاني، وليس له علاقة بالعمر ومستوى السكر الصائم في الدم والكوليستيرول في كل مجموعات مرضى السكري.

الاستنتاج: ومنه سبب يمكن إستنتاج أن الأسثيكوالسين قد يكون له دوراً في التنبؤ المبكر بمرض هشاشة العظام المصاحب لمرض السكري من النوع الثاني.