Association between Bone Turnover Markers and Adipocytokines among Obese Patients with Type 2 Diabetes

Introduction

Type 2 diabetes mellitus (T2DM) now affects at least 285 million people worldwide, and this number will raise to 438 million by the year 2030 [1]. Type 1 and type 2 diabetes mellitus are both associated with bone damage [2,3]. There is clear evidence that patients with type 2 diabetes mellitus (T2DM) have increased fracture risk despite having high bone mineral density (BMD) and body mass index (BMI). Thus, poor bone quality has been implicated as a mechanism contributing to diabetic skeletal fragility [4].

Bone turnover biomarkers (BTMs) reflect bone formation and resorption, and therefore inform the status of bone remodeling, which is a mechanism underlying osteoporosis [5]. In diabetic patients, bone remodeling appears to be impaired as both markers of bone formation and markers of bone resorption are decreased when compared to healthy subjects [6-9]. Individuals with type 2 diabetes were reported to have higher BMD despite elevated risk of fractures [10,11]. Thus, BTM can potentially be a more sensitive surrogate marker than BMD in assessing fracture risk among patients with diabetes, due to a possible linkage between BTMs and glucose metabolism [12,13].

Bone turnover biomarkers (BTMs) include both bone formation and resorption markers. Bone formation markers are synthesized by osteoblasts and therefore reflect specific osteoblastic functions, and these include bone alkaline phosphatase (ALP) [14], osteocalcin (OC) [15], and procollagen type I N propeptide (PINP). These bone formation markers have been proposed as possible predictors for osteoporosis [16-19] and hip fracture risk [20,21]. Bone resorption markers are degradation products of type I collagen [22] and include C-terminal crosslinking telopeptide of type I collagen (CTX-I) and N-terminal crosslinking telopeptide of type I collagen (NTX-I) [22]. Increased CTX-I and NTX-I levels have also been reported to be inversely correlated with BMD among women [16,18] and to be significant predictors for non-spine fractures independent of BMD in several longitudinal studies [16,23,24].

Body fat and bone mineral density (BMD) are directly related [25]. Although mechanical loading may contribute to this relationship, other factors are also involved [26]. One such factor is adiponectin which is a recently discovered adipocytokine that is specifically and highly expressed in human adipose tissue [27] as adiponectin acts directly on bone to induce human osteoblast proliferation and differentiation, and to increase osteoclast

Abstract

Background: Type 2 diabetes (T2DM) is a chronic and progressive disease that is strongly associated with increased the risk of fracture due to altered bone microarchitecture and/or poor quality as key factors.

Objective: The present study aimed to detect the association between bone turnover markers and adipocytokines among obese patients T2DM.

Material and Methods: One hundred obese Saudi patients with T2DM (57 men and 43 women). Their age was 45.38 ± 5.12 year, and a control group included one hundred healthy volunteers, who was gender and age matched.

Results: Obese T2DM patients had significantly lower values of adiponectin, Bone Alkaline Phosphatase (BAP) and Bone Cross-linked N-telopeptides of Type Collagen (NTX) in addition to significantly higher values of leptin, resistin, visfatin levels in comparison to controls. Also, serum level of adiponectin showed an inverse relationship with BAP and NTX. While Serum levels leptin, resistin and visfatin showed an inverse relationship with BAP and NTX.

Conclusion: Within the limit of this study, serum level of adiponectin showed a direct relationship with bone turnover markers. While Serum levels leptin, resistin and visfatin showed an inverse relationship with bone turnover markers in obese patients with type 2 diabetes mellitus.

Keywords: Adipocytokines; Type 2 Diabetes; Obesity; Bone Turnover
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Materials and Methods

Subjects

One hundred Saudi obese T2DM patients (57 men and 43 women) with body mass index (BMI) ranging from 30 to 36 kg/m², were selected from the out-patient diabetic clinic of the King Abdulaziz Teaching Hospital. They were checked for fasting/random glucose levels. Only participants having blood sugar levels more than 5.6 mmol/l or random blood sugar level more than 7.8 mmol/l (impaired blood sugar) were included in this study and were further checked for type 2 diabetes mellitus as per recent American Diabetes Association criteria i.e. fasting blood sugar ≥7.0 mmol/l or post-prandial blood sugar ≥11.1 mmol/l [2-h plasma glucose 11.1 mmol/l during an oral glucose tolerance test] and glycosylated hemoglobin (HbA1c%) > 6.5% [37]. Exclusion criteria included smokers, congestive heart failure, pregnant female patients, hypertension, chronic liver disease, coronary artery disease, angiopathy, myocardial infarction, cerebral infarction and infectious disease. Subjects were also excluded from the study if they had conditions affecting bone metabolism, including diseases of the kidney, liver, parathyroid, or thyroid, diabetes mellitus, hyperprolactinemia, oophorectomy, rheumatoid arthritis, ankylosing spondylitis, malabsorption syndromes, malignant tumors, hematologic diseases, previous pathological fractures. If the subjects had received treatment with glucocorticoids, estrogens, thyroid hormone, parathyroid hormone, fluoride, bisphosphonate, calcitonin, thiazide diuretics, barbiturates, anti-seizure medication, they were also excluded.

A detailed clinical history and physical examinations were conducted which included the age, sex, symptoms suggestive of diabetes and family history of diabetes. Physical examinations included anthropometric measurements such as height, weight, body mass index (BMI) and waist circumference. Also, one hundred apparently healthy, medically free, and treatment naive individuals were recruited to serve as non-diabetic control. Informed written consent was obtained from each included subjects.

Laboratory analysis

For the biochemical estimations, venous blood samples were collected between 7:00 A.M. and 9:00 A.M. after fasting overnight and the samples were allowed to clot. Thereafter, the samples were centrifuged and divided into aliquots and stored at -70 °C until assayed.

Serum adiponectin, leptin, resistin, visfatin measurement: The levels of adiponectin in human serum were determined using adiponectin ELISA (Phoenix Pharmaceuticals, Inc., Belmont, CA). Serum leptin, resistin, and visfatin levels were detected using ELISA (Phoenix Pharmaceuticals). The intra- and inter-assay CVs were 5.0 and 6.0% for adiponectin, 6.0 and 7.1% for leptin, 6.1 and 7.3% for resistin, and 5.6 and 7.0% for visfatin.

Bone turnover biochemical markers measurement: The serum concentration of bone alkaline phosphatase (BAP) as the markers of bone formation were measured with ELISA kits (BAP from Metra™ BAP ELISA kit, Quidel Corp., San Diego, CA). As a marker of bone resorption, serum cross-linked N-telopeptides of type I collagen (NTX) were measured using an ELISA kit (Osteomark, Ostex, Inc., Seattle, WA). The intra- and inter-assay CVs were 5.1 and 7.0% for BAP, 5.3 and 7.9% for NTX, respectively [38].

Statistical analysis

Independent t-test was used to compare mean differences between both groups. Statistical analysis of data was performed using SPSS (Chicago, IL, USA) version 17. The degree of correlation between bone turnover markers and adipocytokines among obese T2DM patients was detected by Pearson’s product moment correlation coefficients (r).

Results

One hundred obese Saudi T2DM patients were enrolled including 57 men and 43 women, had a mean age of 45.3 ± 5.12 year and one hundred healthy subjects, had a mean age of 43.7 ± 6.31 years, there was no significant differences in body mass index between both groups. However, T2DM patients were more insulin resistant as indicated by higher values of insulin and HOMA-IR, and lower values of the quantitative insulin-sensitivity check index JQUICKI (Also, T2DM patients showed significantly higher glucose, insulin, Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index (Table 1).

Table 2 summarizes the comparison between T2DM patients and matched controls. T2DM patients were more leptin, resistin, visfatin, BAP and NTX in addition to significantly lower values of adiponectin levels in comparison to controls (Table 2). Table 3 summarizes the relationship between parameters of bone turnover markers and adipocytokines among obese T2DM patients. Serum levels of level of adiponectin showed a direct relationship with BAP and NTX. While Serum levels leptin, resistin and visfatin showed an inverse relationship with BAP and NTX (Table 3).
**Table 1:** Baseline characteristics of type 2 diabetic patients and control subjects.

<table>
<thead>
<tr>
<th></th>
<th>Diabetic Group</th>
<th>Control Group</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>Age, year</td>
<td>45.38 ± 5.12</td>
<td>43.72 ± 6.31</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>57/43</td>
<td>55/44</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>33.62 ± 3.14</td>
<td>34.85 ± 3.29</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>FBS, mg/dl</td>
<td>184.58 ± 10.17</td>
<td>90.26 ± 5.35</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>PPS, mg/dl</td>
<td>241.24 ± 15.32</td>
<td>119.76 ± 9.66</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Insulin, mU/l</td>
<td>15.41 ± 3.11</td>
<td>8.57 ± 2.48</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.123 ± 0.016</td>
<td>0.169 ± 0.021</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>5.75 ± 1.58</td>
<td>2.63 ± 1.14</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>HBA1c, %</td>
<td>9.13 ± 2.74</td>
<td>6.25 ± 1.15</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

BMI: Body Mass Index; FBS: Fasting Blood Sugar; PPS: Postprandial Blood Sugar; HOMA-IR: Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index; QUICKI: The quantitative insulin-sensitivity check index; HBA1c: Glycosylated Hemoglobin; (*) indicates a significant difference between the two groups, P < 0.05.

**Table 2:** Mean value and significance of biochemical parameters of type 2 diabetic patients and control subjects.

<table>
<thead>
<tr>
<th></th>
<th>Diabetic Group</th>
<th>Control Group</th>
<th>T-Value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin, μg/ml</td>
<td>6.73 ± 2.34</td>
<td>13.46 ± 4.91</td>
<td>7.64</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>24.25 ± 8.71</td>
<td>12.18 ± 4.26</td>
<td>6.83</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Resistin, ng/ml</td>
<td>7.88 ± 1.94</td>
<td>5.22 ± 1.32</td>
<td>6.14</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Visfatin, ng/mL</td>
<td>37.21 ± 10.16</td>
<td>22.63 ± 9.68</td>
<td>7.55</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>BAP, U/l</td>
<td>18.13 ± 6.81</td>
<td>28.74 ± 11.43</td>
<td>7.18</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>NTX, nmol BCE</td>
<td>12.95 ± 5.23</td>
<td>16.33 ± 7.15</td>
<td>6.22</td>
<td>P &lt; 0.05</td>
</tr>
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</table>

BAP: Bone Alkaline Phosphatase; NTX: Bone Cross-linked N-telopeptides of Type I Collagen; (*) indicates a significant difference between the two groups, P < 0.05.

**Table 3:** Pearson’s correlation coefficients test value of the studied variables in the type 2 diabetic group.

<table>
<thead>
<tr>
<th></th>
<th>BAP (U/l)</th>
<th>NTX (nmol BCE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin, μg/ml</td>
<td>0.587*</td>
<td>0.571*</td>
</tr>
<tr>
<td>Leptin, ng/ml</td>
<td>0.612*</td>
<td>0.628*</td>
</tr>
<tr>
<td>Resistin, ng/ml</td>
<td>0.576*</td>
<td>0.613*</td>
</tr>
<tr>
<td>Visfatin, ng/mL</td>
<td>0.625*</td>
<td>0.615*</td>
</tr>
</tbody>
</table>

BAP: Bone Alkaline Phosphatase; NTX: N-telopeptides of Type I Collagen; Spearman’s correlation was used *: P < 0.05.

**Discussion**

Recently, there is a growing interest in the association between diabetes and alterations in bone metabolism. On the other hand, the effects of type 2 diabetes on bone are less clear-cut, whereas bone mineral density has been reported to be either unchanged or modestly increased, incidence of low-stress fractures is almost doubled, pointing to a decrease in the bone quality of these patients [39]. Thus, we investigated the relationships between serum levels of adipocytokines, including adiponectin, leptin, resistin & visfatin and bone turnover biochemical markers (Serum BAP as a bone formation marker, and serum NTX as a bone resorption marker were assayed) among obese type 2 diabetic patients.

Our main result finding of the present study was that adiponectin significant was positively correlated with BAP and NTX. This finding supports the hypothesis that adiponectin may play a significant role in regulating bone mass among obese type 2 diabetic patients. Adiponectin was negatively correlated with fat mass, while the leptin showed a positive correlation. The correlation between leptin and fat mass remained significant. Furthermore, the finding that adiponectin was positively correlated with NTX and BAP. Thus, adiponectin may exert a negative effect on bone mass by promoting excessive bone resorption associated with bone loss among obese type 2 diabetic patients. Several experiments have shown that adiponectin could stimulate bone formation [40,41] and regulate bone turnover [42]. Several studies also documented a significant positive relationship between serum adiponectin and bone markers.
However, Peng et al. [45] investigated the relationship between serum adipocytokine levels and both bone marrow density and bone turnover biochemical markers in 232 Chinese men their age ranged between 20-80 years, they concluded that there were significant positive correlations between adiponectin and NTX, BAP [45]. Also, Wu et al. [46] investigated the relationships between serum adiponectin concentration and bone mineral density, and bone biochemical markers in 265 premenopausal and 336 postmenopausal Chinese women and proved that there were significant positive correlations between adiponectin and NTX, BAP in postmenopausal women. However, in premenopausal women, adiponectin was not related to NTX and BAP [46]. Thus, adiponectin appears to mediate bone formation and bone remodeling, and serum hypoadiponectinemia may cause low turnover and lead to bone fragility in patients with type 2 diabetes [47].

The exact mechanism by which adiponectin is strongly associated with bone formation and bone remodeling can be explained by several characteristics of adiponectin provide plausible avenues for modulation of bone metabolism. Adiponectin bears marked structural similarity to TNF-α family members, examples including RANK-L and osteoprotegerin, two proteins involved in regulation of osteoclastogenesis. Adiponectin activates [48] as well as inhibits [49] NF-kB, a ubiquitous transcription factor which is critical for osteoclastogenesis, providing a mechanism by which adiponectin may affect bone. Recombinant adiponectin prevents adipogenesis in bone marrow derived pre-adipocytes, suggesting that it can influence the marrow environment [50]. Adiponectin is structurally similar to CORS26 (collagenous repeat-containing sequence of 26-kDa protein), a novel protein discovered in a mouse embryonic fibroblast cell line, which is likely to be involved in skeletogenesis [51]. Another similar protein is Type X collagen, and mutations in the COL10A1 gene are known to cause metaphyseal chondrodysplasia (type Schmid) [52,53]. Thus, while the precise effects of adiponectin on the skeleton are not known, there are several potential mechanisms.

In this study, we observed that baseline serum leptin, resistin and visfatin levels inversely correlated with changes in NTX and BAP. However, previous studies stated that the relationship between serum leptin level and bone turnover markers was still controversial [54-57]. Tamura et al. [54] investigated the associations of serum level of leptin or adiponectin with bone mineral density, serum osteocalcin, and urinary N-terminal telopeptide of type I collagen (NTX) in 40 Japanese patients with type 2 diabetes mellitus and revealed that there were significant positive correlations between serum leptin or adiponectin level and z score at the distal radius and a significant negative correlation between serum leptin and urinary NTX, a marker of bone resorption [54]. Wu et al. [55] proved that there were no significant correlations between leptin concentrations and NTX and BAP in premenopausal and postmenopausal women [55]. Peng et al. [56] concluded that there were significant positive correlations between adiponectin and NTX and BAP. There were no significant correlations between leptin, resistin, visfatin levels and NTX and BAP among Chinese men [56]. Moreover, Fisher and coworkers determined the relationship of serum resistin concentrations to biochemical parameters of bone turnover and mineral metabolism among 256 elderly patients with hip fracture and founded a positive association between serum resistin, parathyroid and calcium levels in patients with cervical hip fracture [57]. Moreover, the major limitations of the present study is only Saudi obese T2DM patients enrolled in the study, so the value of this study only related only to Saudi subjects, also small sample size in the two groups may limit the possibility of generalization of the findings in the present study. Finally, within the limit of this study, serum level of adiponectin showed a direct relationship with bone turnover markers. While serum levels leptin, resistin and visfatin showed an inverse relationship with bone turnover markers in obese patients with type 2 diabetes mellitus.

References


