Serum Osteocalcin level in Patients with Type 2 Diabetes Mellitus

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Abstract

Background: Osteocalcin (osteoblast-derived protein) acts as a hormone regulating glucose metabolism and fat mass. Lower serum concentrations of the osteocalcin have been associated with poorer glycemic control, insulin resistance and with the development of type 2 diabetes (T2DM). The current study aimed to measure the level of serum osteocalcin in type 2 diabetic patients with it’s variabilities like BMI, HbA1c, smoking. Methods: A case-control study was conducted from March 2019 to October 2019. A convenient general population samples of 90 persons (40 type 2 diabetic patients and 50 normal control) with mean age (51.87 ±9.11), were collected randomly from Al Hussein teaching hospital in Nasiriyah city in south of Iraq. Independent-sample t-test was used to tell us how significant differences between the groups. Results: Osteocalcin was significantly lower in diabetic patients in comparison with control group (p. value <
There was a significant statistical difference in the mean of osteocalcin between the two groups of glycemic control (HBA1C<7 and HBA1C>7) in diabetic patients (P. value< 0.05). In addition, there was statistically significant difference in the mean of osteocalcin in the two groups of assessment of body mass index (BMI) (P.value< 0.05).

**Conclusion:** There was a significant difference in osteocalcin level between diabetic and control group. There was a significant inverse relationship between HBA1C and serum osteocalcin level in type 2 diabetic patients. Increase in BMI was associated with a significant reduction in serum osteocalcin level in type 2 diabetic patient.

**Keywords:** serum osteocalcin, type 2 diabetes mellitus, bone formation.

**Introduction**

Diabetes mellitus is a general term for a group of metabolic disorders with the main feature of chronic hyperglycaemia[^1]. It results from either impaired insulin secretion or impaired insulin efficacy or, most often, both[^2]. The classical symptoms of diabetes are polyuria, polydipsia, polyphagia and weight loss[^3]. Symptoms may progress rapidly during weeks or months in type 1 diabetes while they mainly progress more slowly and may be absent in type 2 DM[^3]. Type 2 diabetes is typically a chronic disease associated with a ten-year shorter life expectancy. The development of type 2 diabetes is caused by a combination of lifestyle and genetic factors[^4]. There are several risk factors for developing type 2 DM. These include a suboptimal intrauterine environment, low birth weight, sedentary life style, obesity, gestational diabetes and aging[^5].
There are many complications for DM which are either early or late complications involving cardiovascular disease, hypoglycemia, eye complications, neuropathy, limb loss and nephropathy\(^6\). These complications mainly occur as a result of poorly controlled blood sugar level specially hyperglycemia over a prolonged period of time \(^6\).

Osteocalcin (OC) is a type of noncollagenous protein which is synthesized and secreted by osteoblast. Physiological functions of OC include maintaining normal bone mineralization, suppressing abnormal hydroxyapatite formation, and slowing down growth cartilage mineralization \(^7\). Osteocalcin (OC) plays important role in the communication between the skeleton and glucose homeostasis.

Diabetes is well known to affect bone integrity, because mature osteoblastic cells become weakened by abnormal glucose metabolism \(^8\). Type 2 diabetes mellitus (T2DM) is associated with an increased risk of fracture, although bone mineral density (BMD) is unaffected or even higher in diabetic patients \(^9\). The reasons include likely a combination of features, including the duration of disease, inadequate glycemic control, greater risk of falling as a consequence of hypoglycemia, osteopenia, impairment of bone quality, and side effects of medication, which may lead to a higher risk of bone fragility and fractures \(^9\).

In addition, diabetes complications and osteoporotic fractures are two of the most important causes of morbidity and mortality in older patients and share many features including genetic susceptibility, molecular mechanisms, and environmental factors \(^10\). Interestingly, OC seems also to have a role in energy metabolism. In its undercarboxylated form, OC stimulates insulin secretion and enhances insulin sensitivity in both adipose and muscle tissue.
An inverse association between OC and metabolic syndrome has been observed, suggesting that reduced levels of osteocalcin may impact in the pathophysiology of T2DM [11].

Materials and Method

The current study of case–control design included 90 persons (40 type 2 diabetic patients and 50 normal control) with age group (35-70) of mean age for all sample, mean ±SD (51.87 ±9.11). The study was conducted during the time between March 2019 to October 2019. The participants in the current study were collected randomly from Al Hussein teaching hospital in Nasiriya city in south of Iraq. The patients group had 40 patients (14 males and 26 female) with type 2 diabetes.

Inclusion criteria:

1. Type 2 diabetic patients who were diagnosed by physicians.
2. Age of patients was >35 years old.

Exclusion criteria: Chronic kidney disease, Chronic liver disease, Osteoporosis or bone disorder such as fracture or tumor, Atherosclerosis and ischemic heart disease, Pregnancy, Malignancy, History of prolong steroid use, History of bisphosphonate use, History of Vitamin D, Calcium use, Use of the following agents: Thiazolidinedione, statin, warfarin, vitamin A and other hormones, Insulin treatment, Anemia, Thyroid disease, Parathyroid disease, Trauma, major surgery and other stress.

Control group: The criteria included: Controls matched to patients with regard to geographical distribution, sex and age.

Ethical Considerations: Arrangements were done to get approvals from the executive office of the Iraqi Board of Health specialization and verbal consent
were obtained from all participants. The purpose of study was explained to all participants.

**Method**

**History:** A uniform case sheet was determined for every participant in the study which include age, sex, history of smoking, medical history, family history, drug history and DM (duration and treatment).

**Clinical examination and physical measurement:** Full examination was done for all patients. Body weight and height were measured by digital weight and height scale. Body mass index was calculated according to the following equation: $$\text{BMI} = \frac{\text{weight (Kg)}}{\text{height (m}^2)}$$ [12, 13]. Blood pressure measurement, ECG, serum calcium and DEXA scan were also done for all study groups.

**Biochemical analysis:** Sample of blood was taken from all the study group for laboratory investigation of HbA1C which was done by Rapid Quantitative Test for Finecare FIA System.

**Assay of OC:** Osteocalcin was detected by Human OC/BGP (Osteocalcin) with batch number E-EL-H1343 enzyme-linked immunosorbent assay. The kit was provided by Elabscience Biotechnology Co., Ltd in United States. Blood sample was collected and centrifuged for all participants, and the serum was kept in the fridge at the temperature of $-20^\circ$C for tOC.

**Statistical analysis**

Data were expressed as mean ± SD. Independent-sample t-test, Pearson correlation analysis and stepwise multiple regression analysis had been used to compare the difference between two groups of the study. Statistical analysis was performed by using computer programs statistical package of social sciences (SPSS) version 23 and Microsoft excel in data performing.
(Statistical significance was obtained at \( p<0.05 \) value).

**RESULTS**

**Demographic and clinical-biochemical characteristics of study population**

The current study included 90 subjects (40 as diabetic patients and 50 as a control). The highest proportion of participants were females (56%) and age group more than 50 (59%). The demographic and clinical -biochemical characteristics of recruited individuals were presented in table 1.

**Table 1: Demographic and clinico-biochemical characteristics of study population**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>Diabetic</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35-50</td>
<td>19 (38%)</td>
<td>18 (45%)</td>
<td>37 (41%)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>31 (62%)</td>
<td>22 (55%)</td>
<td>53 (59%)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>20 (40%)</td>
<td>14 (35%)</td>
<td>34 (37%)</td>
</tr>
<tr>
<td>Female</td>
<td>30 (60%)</td>
<td>26 (65%)</td>
<td>56 (53%)</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30</td>
<td>29 (58%)</td>
<td>15 (37.5%)</td>
<td>44 (49%)</td>
</tr>
<tr>
<td>&gt;30</td>
<td>21 (42%)</td>
<td>25 (62.5%)</td>
<td>46 (51%)</td>
</tr>
<tr>
<td><strong>HBA1C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;7</td>
<td>50(100%)</td>
<td>11 (27.5%)</td>
<td>61 (68%)</td>
</tr>
<tr>
<td>&gt;7</td>
<td>0 (0.0%)</td>
<td>29 (72.5%)</td>
<td>29 (32%)</td>
</tr>
<tr>
<td><strong>Duration of disease</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5</td>
<td>0 (0.0%)</td>
<td>18 (45%)</td>
<td>18 (20%)</td>
</tr>
<tr>
<td>&gt;5</td>
<td>0 (0.0%)</td>
<td>22 (55%)</td>
<td>22 (24%)</td>
</tr>
<tr>
<td><strong>Smoking status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non smoker</td>
<td>36 (72%)</td>
<td>27 (67.5%)</td>
<td>63 (70%)</td>
</tr>
<tr>
<td>Smoker</td>
<td>14 (28%)</td>
<td>13 (32.5%)</td>
<td>27 (30%)</td>
</tr>
</tbody>
</table>
2. Comparison of serum osteocalcin of diabetic patients versus control group

There was statistically significant difference in osteocalcin level between diabetic and control group. Osteocalcin was significantly lower in diabetic patients in comparison with control group (p. value < 0.0001) as shown in table below (Table 2).

**Table 2: Comparison of serum osteocalcin of diabetic patients versus control group**

<table>
<thead>
<tr>
<th>parameter</th>
<th>Diabetic No%=40(44%) Mean ± SD</th>
<th>Control No%=50(56%) Mean ± SD</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteocalcin</td>
<td>34.7140±33.055</td>
<td>66.2669±37.372</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Odds ratio=10.54

**Figure1:** shows distribution of serum osteocalcin of diabetic patients versus control group

3. Effect of gender on osteocalcin in diabetic patient:

There was no statistically significant difference in the mean of osteocalcin between male, female diabetic patients (p. value> 0.05) as shown in table below (Table 3).

**Table 3: Effect of gender on osteocalcin in diabetic patient**

<table>
<thead>
<tr>
<th>parameter</th>
<th>Sex</th>
<th>No. %</th>
<th>Mean ± SD</th>
<th>p. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteocalcin</td>
<td>M</td>
<td>14(35%)</td>
<td>35.7785 ± 35.49478</td>
<td>0.871</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>26(65%)</td>
<td>34.1408 ± 32.37989</td>
<td></td>
</tr>
</tbody>
</table>

4. Effect of age on osteocalcin in diabetic patient

There was no statistically significant difference in the mean of osteocalcin between age groups (30-50) and > 50 in diabetic patients (P.value> 0.05) as shown in table below( Table 4).
Table 4: Effect of age on osteocalcin in diabetic patient

<table>
<thead>
<tr>
<th>parameter</th>
<th>Age</th>
<th>No. %</th>
<th>Mean ± SD</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteocalcin</td>
<td>(35-50)</td>
<td>18(45%)</td>
<td>37.8008± 37.52043</td>
<td>0.529</td>
</tr>
<tr>
<td></td>
<td>(more than 50)</td>
<td>22(55%)</td>
<td>32.1884± 29.57648</td>
<td></td>
</tr>
</tbody>
</table>

5. Effect of glycemic control on osteocalcin in diabetic patient

Regarding the effect of glycemic control on osteocalcin in diabetic group, there was significant statistical difference in the mean of osteocalcin between the two groups of glycemic control (HBA1C<7 and HBA1C>7) in diabetic patients (P. value< 0.05)(Table 5).

Table 5: Effect of glycemic control on osteocalcin in diabetic patient

<table>
<thead>
<tr>
<th>parameter</th>
<th>HBA1C</th>
<th>No. %</th>
<th>Mean ± SD</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteocalcin</td>
<td>&lt;7</td>
<td>11(27.5%)</td>
<td>57.7743±40.11483</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>≥7</td>
<td>29(72.5%)</td>
<td>25.9670±25.67233</td>
<td></td>
</tr>
</tbody>
</table>

3.5: Shows negative correlation between osteocalcin and HBA1C

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6. Effect of body mass index on osteocalcin in diabetic patients

There was statistically significant difference in the mean of osteocalcin in the two groups of assessment of body mass index (BMI) (P.value<0.05) (Table 6)

<table>
<thead>
<tr>
<th>parameter</th>
<th>BMI</th>
<th>No.</th>
<th>Mean ± SD</th>
<th>p.value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;30Kg/m²</td>
<td>15 (37.5%)</td>
<td>47.5453±38.73688</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>≥30Kg/m²</td>
<td>25 (62.5%)</td>
<td>27.0152±27.12257</td>
<td></td>
</tr>
</tbody>
</table>

7. Effect of duration of diabetes on osteocalcin in diabetic patients

There was no statistical significant differences in the mean of osteocalcin in groups of duration of diabetic patients (less than 5 years and more than 5 years) (P. value > 0.05) as shown below (Table 7).

<table>
<thead>
<tr>
<th>parameter</th>
<th>Duration of diabetes</th>
<th>No.</th>
<th>Mean ± SD</th>
<th>p. value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>less than 5</td>
<td>18 (45 %)</td>
<td>37.8634±35.26816</td>
<td>0.283</td>
</tr>
<tr>
<td></td>
<td>more than 5</td>
<td>22 (55 %)</td>
<td>32.1371±31.73125</td>
<td></td>
</tr>
</tbody>
</table>

Discussion

Comparison of serum osteocalcin of diabetic patients versus control group

The current study, which involved 40 type 2 diabetic patients, revealed a significant lowering of osteocalcin level in type 2 diabetic patients (P.value<0.05), compared to control group. The observation of decrease
osteocalcin level in type 2 diabetic patients is of a particular interest, because the results suggested that decreased serum tOC was related with long-term hyperglycemia and may had impact on insulin resistance [14].

Several studies indicated that hyperglycemia induces a low turnover of bone with osteoblast dysfunction and suppresses serum osteocalcin levels [15]. Gerdhem et al. [16] have shown that serum osteocalcin level, was lower in women with diabetes after correction for covariance of body weight and serum creatinine. Okazaki et al. [15] have also shown that serum osteocalcin level was low before treatment and elevated after treatment of diabetes.

These findings agreed with Ghiraldini et al who designed a clinical trial in 32 T2DM patients and 19 patients without diabetes. Baseline data indicated that OC levels were higher in systematically healthy patients than those with poorly controlled T2DM patients [17].

Also these results are similar to Ogail Yousif Dawod et al results, 2017 who revealed that serum TOC level was significantly low in diabetic patients compared to healthy control individuals [18]. Additionally, Zhou, et al., showed that serum osteocalcin was inversely correlated with blood sugar and positively correlated with insulin secretion in the Chinese population [19].

**Effect of age and gender on osteocalcin in diabetic patient.**

This study showed no statistical significant change in mean of osteocalcin (P. value>0.05) between two age groups in diabetic patients and two groups of gender. This may be explained by effect of diabetes that overcomes the physiological effect of age and gender on osteocalcin.

This result agreed with Nagwa Amr Lacine et al, 2017 who noted the correlation between serum osteocalcin and the various parameters among the
studied patients showed that there were no statistically significant correlations with age \[20\].

These findings disagreed with Nahid J. et al., 2018 who found that lower bone turnover (indicated by lower serum OC) occurred in Mexican American men with T2D who had poorer glycemic control \[21\].

**Effect of glycemic control on osteocalcin in diabetic patient**

Analysis of effect of glycemic control on osteocalcin level in type2 diabetic, revealed significant differences in the mean of osteocalcin in type 2 diabetic patients (p.value <0.05). There was inverse relationship between serum osteocalcin level and HBA1C. This result agreed with I. Kanazawa et.al. 2011 who reported that total osteocalcin was inversely correlated with HbA1c, and undercarboxylated osteocalcin was inversely correlated with fasting blood glucose \[22\].

Our results was in accordance with many Chinese studies as for example Qingping W et al., who studied the correlations between serum osteocalcin and glucose metabolism in patients with T2DM, they demonstrated that total osteocalcin was inversely correlated with HbA1c , and suggested that decreased serum osteocalcin was related with long-term hyperglycemia but had little impact on insulin resistance \[22\].

**Effect of body mass index on osteocalcin in diabetic patient.**

This study showed a significant statistical change in the mean of osteocalcin in type2 diabetic patients between the two groups of assessment of body mass index (BMI).

This finding is consistent with L. Darwish et al, May 2019 who concluded that higher body mass was associated with higher uOCN.
(rho = 0.423, \( p = 0.009 \)) in participants without T2DM, but with lower concentrations of both ucOC \( (p = 0.006) \) and cOC \( (p = 0.003) \) in participants with T2DM \cite{23}. In addition, this result disagreed with Nagwa Amr Lacine et al, 2017 who could not prove significant correlation between osteocalcin and BMI which are related to insulin resistance \cite{20}.

Effect of duration of diabetes on osteocalcin in diabetic patients

There was no significant statistical difference in the mean of osteocalcin in the two groups of duration of diabetes. These results agreed with results of Nahid J. et al., 2018 which showed that duration of disease was not associated with serum OC \cite{21}. A possible explanation is that due to the effect of other confounding factors like glycemic control (HbA1C) and BMI that may affect on osteocalcin level regardless the duration of diabetes. Conclusions

1. There was a significant change in osteocalcin level in type2 diabetic patients in comparison with control subjects. These results indicated that OC reduction may be associated with the aggravation of glucose metabolism disorders.

2. HBA1C is inversely correlated with osteocalcin level in type2 diabetic patients. So that serum osteocalcin level may be considered as an indicator to the severity of glycemic control.

3. BMI is associated with a significant change in osteocalcin level in type2 diabetic patients. These findings support the recent notion that osteocalcin is important for both bone metabolism, glucose metabolism and fat mass.

Recommendations and limitations

1. Large sample size to get a wide range to study the effect of osteocalcin on glucose metabolism in type2 diabetic patients.
2. Long-term prospective studies should be performed in order to evaluate the clinical course of abnormal glucose metabolism in the natural history of diabetic disease.

3. Longitudinal studies are needed to further confirm whether the change of osteocalcin level, as an initiating factor, has some impact on insulin secretion and sensitivity.

4. Different kits are needed to determine the serum cOC levels. Some human studies showed that cOC had a greater impact than uncarboxylated OC on insulin sensitivity.

References:

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