Alteration in serum osteocalcin levels in patients with diabetic nephropathy

*Salem.E.S,*Phebe.L.Abdel-Messeih,* and H.H.Mansour

*Health Radiation Research Department, National Centre for Radiation Research and Technology (NCRRT), Atomic Energy authority, Cairo, Egypt

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ABSTRACT

The fact that bone mass density (BMD) is not useful for assessing fracture risk in diabetic patients (DM) seems problematic, because those populations are increasing in every country. Osteocalcin (OC) is synthesized by osteoblasts and is considered to be a marker of bone formation. The present study was carried out to evaluate the usefulness of OC as noninvasive biomarker of bone formation in diabetes mellitus type 2 (uncomplicated) and diabetic nephropathy. Immunoradiometric assay(IRMA) was used for the quantitative measurement of human intact OC both N-terminal and C-terminal fragments in the serum of the control and the studied groups. OC levels in the uncomplicated diabetic group were significantly lower while in the diabetic nephropathy group was significantly higher compared to control values . There was a weak negative correlation between OC and both fasting blood glucose and glycated Hb % in the diabetic group. In diabetic nephropathy patients, a weak positive correlation was observed between OC and protein creatinine ratio. The results concluded that changes in bone remodelling marker OC are present in both DM type2 and diabetic nephropathy explaining osteopenia and osteoporosis observed in both cases. Therefore, an effective glycaemic control should be the hallmark of prevention and treatment of diabetes mellitus induced osteoporosis.

Keywords: Diabetic nephropathy, Immunoradiometric assay, Osteocalcin

Introduction

Osteoporosis, a global age-related health problem in both elderly male and female, insidiously deteriorates the micro-structure of bone culminating in fragility, fracture, pain and disability. It can be caused by acceleration of bone resorption and/or deceleration of bone formation. Risk factors are abnormal high plasma parathyroid hormone (PTH) levels, advancing age, genetic background, cigarette smoking and chronic use of some medications. Furthermore, other medical condition particularly DM is also risk factor for osteoporotic bone loss (1).

Osteocalcin (OC) is a non-collagenous, vitamin K-dependent protein produced by osteoblasts which are mononucleated cells that are derived from mesenchymal stem cells. OC protein bone glycoprotein A contains three residues of the amino acid gamma-carboxyglutamic acid (2).Recently, a novel function of the skeleton on energy metabolism, have been described.OC is an osteoblast-derived hormone regulating insulin secretion, insulin sensitivity in peripheral tissues, and energy expenditure (3). It has been established that bone is an important endocrine organ to regulate glucose/ lipid metabolism through increasing insulin secretion from the pancreas. Insulin signaling in osteoblasts regulates both bone acquisition and bone resorption(4).
DM is a group of pandemic debilitating metabolic diseases featuring chronic hyperglycemia which results from defective insulin secretion and/or insulin actions. Such chronic hyperglycemia typically elicits dysfunction and failure of various organs resulting in diabetic retinopathy, nephropathy, cardiomyopathy and angiopathy. In addition, DM has been found to be associated with metabolic bone disease, osteoporosis and low impact fracture (5). Recent study has revealed that DM is associated with an increased risk of fractures (6). Bone disease in DM is characterized by low bone formation. The mechanisms by which bone turnover is decreased are explained mainly by impaired secretion of parathyroid hormone (PTH) and osteoblast dysfunction (7). In diabetic patients, one of the conditions that first affect the kidney is the release of small quantities of protein in the urine (microalbuminuria). Albuminuria is considered as a marker of diabetic nephropathy (DN). DN is one of the more common long term complications of DM. It results from direct vascular abnormalities that accompany DM. Patients suffering from chronic kidney disease usually present with complications of bone anomalies that vary from fracture to extraskelatal calcification due to mineral ion dysregulation (8).

The aim of the present study is to evaluate the usefulness of osteocalcin as a noninvasive biochemical marker of bone formation in diabetes mellitus type 2 and diabetic nephropathy.

**Subjects and methods:**

The present study was carried on twenty patients and ten controls. The patients were attending the outpatient diabetic clinic of the International Institute of Diabetes and Endocrinology.

These patients were categorized into two groups:

**Group 1:** included ten male patients with DM type 2 (uncomplicated by nephropathy). Their mean age was 49.3±1.45.

**Group 2:** included ten male patients with diabetic nephropathy. They were diagnosed on basis of elevated protein creatinine ratio in in urine. Their mean age was 49.1±1.35.

The **control group:** consisted of ten healthy male subjects. Their mean age was 49.2±1.4. They underwent their routine visit at the preventive health service on the same day of the patients.

All chosen subjects were subjected to throughout history taking as well as complete physical examination to exclude any other health problem that may affect the results of the study such as: thyroid disturbances, growth hormone deficiency or clinical signs or symptoms of osteoporosis. They were not taking any medications known to influence bone metabolism.

As a circadian rhythm of OC in human serum has been described, blood samples were taken from the studied groups between 8.00 and 8.30 a.m., in the fasting state and before insulin injections or oral hypoglycemic medications were taken. Morning urine sample was also taken.

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Laboratory investigations:

Serum and urinary creatinine, protein/creatinine ratio in urine, blood glucose levels both FBG and PPBG, HbA1c and osteocalcin were determined.

Assessment of serum and urinary creatinine levels were determined by the colorimetric method Jaffe without deproteinization by biotecnia instruments via L18-00156roma (9). Protein creatinine ratio were determined by a photometric test for protein using pyrogallol red provided by Diasysis Diagnostic system GmbH (10), then the value of urinary protein was divided by the value of urinary creatinine.

- Assessment of fasting blood glucose (FBG) and postprandial blood glucose (PPBG) levels were done by quantitative determination of glucose by spectrum diagnostic liquizime GOD-PAP (single reagent) (11).

Assessment of glycated hemoglobin (HbA1C%) was carried out by quantitative chromatographic spectrophotometric determination of glycohemoglobin in whole blood using a kit provided by biosystem reagents and instruments, Barcelona (Spain) (12).

- Immunoradiometric assay kit (host IRMA) which is based on coated-tube separation was used for in-vitro quantitative measurement of human intact osteocalcin both N-terminal and C-terminal fragments in the serum of the control and the studied group. The kit was purchased from DIA source ImmunoAssays S.A. Belgium Catalog number KIP1381 (13).

STATISTICAL ANALYSIS

All data were expressed as mean±standard deviation of the different groups. Statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Tukey-Kramer for multiple comparison test. A p-value of 0.05 or less was taken as a criterion for a statistically significant difference. Correlation coefficient was used to evaluate the effects of the studied groups compared with the control group (14).

RESULTS

Physical characteristics and laboratory investigations of adult males with type II DM (uncomplicated DM) and diabetic nephropathy compared to control are demonstrated in Table (1)
Table (1): Physical characteristics and laboratory investigations of studied groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control N=10</th>
<th>DM N=10</th>
<th>Diabetic nephropathy N=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49.20 ± 1.40(^a)</td>
<td>49.30 ± 1.45(^a)</td>
<td>49.10 ± 2.35(^a)</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>79.70 ± 6.03(^*a)</td>
<td>211.30 ± 14.25(^b)</td>
<td>178.00 ± 18.90(^*c)</td>
</tr>
<tr>
<td>PPBG (mg/dl)</td>
<td>97.50 ± 7.62(^*a)</td>
<td>274.10 ± 29.4(^*b)</td>
<td>245.30 ± 28.40(^*c)</td>
</tr>
<tr>
<td>HbA(_1c) (%)</td>
<td>5.20 ± 0.50(^*a)</td>
<td>8.07 ± 0.50(^b)</td>
<td>7.50 ± 0.70(^b)</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.77 ± 0.10(^a)</td>
<td>0.86 ± 0.10(^a)</td>
<td>3.44 ± 0.56(^*ab)</td>
</tr>
<tr>
<td>Protein/creatinine</td>
<td>0.103 ± 0.02(^a)</td>
<td>0.091 ± 0.05(^a)</td>
<td>2.60 ± 0.50(^*ab)</td>
</tr>
<tr>
<td>Osteocalcin (ng/ml)</td>
<td>4.95 ± 0.43(^a)</td>
<td>3.08 ± 0.46(^*b)</td>
<td>9.21 ± 1.44(^*c)</td>
</tr>
</tbody>
</table>

Values with different superscripts in the same row are significantly different at a P value ≤0.5 *highly significant (P<0.001).

There were no significant changes regarding age in the studied groups. In case of diabetes, there was a significant increase in both fasting and postprandial glucose levels in both the uncomplicated group and the diabetic nephropathy group (P<0.01). Glycated haemoglobin % showed a significant increase in both groups when compared to the control group. Yet, the increase in the diabetic nephropathy patients was insignificantly lower than the uncomplicated diabetic group (P>0.05) (table (1) and figures 1,2& 3).

Figure (1): Fasting blood glucose levels (mg/dl) in the studied groups
Values with different superscripts are significantly different at a P value ≤0.5
Figure (2): Postprandial blood glucose levels (mg/100ml blood) in the studied groups
Values with different superscripts are significantly different at a P value ≤0.5

As regard to kidney function tests, in the uncomplicated diabetic group, serum creatinine levels were insignificantly higher (p>0.05) while protein creatinine ratio was insignificantly lower (P>0.05) than controls. However, the diabetic nephropathy patients showed significantly high (P<0.001) creatinine levels and protein creatinine ratio compared to controls and the uncomplicated diabetic group as shown in figures (4 &5) and Table (1)
As shown in figure (6), osteocalcin levels in the uncomplicated diabetic group were significantly lower than the controls (P<0.001). On the other hand, osteocalcin levels in the diabetic nephropathy group was statistically higher than both controls and the uncomplicated diabetic group.
As shown in figures (7, 8), in the uncomplicated DM, there is weak negative correlations between osteocalcin and both fasting blood glucose ($r^2=0.54$) and glycated Hb % ($r^2=0.59$). In the diabetic nephropathy group, a weak positive correlation ($r^2=0.45$) between osteocalcin and protein/creatinine ratio was found as illustrated in figure (9).

Figure (7): Correlation between osteocalcin (ng/ml) and fasting blood glucose (mg/100ml) in the uncomplicated DM group

$y = -22.417x + 280.39$

$R^2 = 0.5415$
DISCUSSION

The past five years have witnessed the emergence and discovery of unexpected functions played by the skeleton in whole organism physiology. Among these described tasks is the role of bone in the control of energy metabolism, which is achieved through the secretion of osteocalcin (OC), an osteoblasts-derived hormone, regulating insulin secretion and energy expenditure (15). Although
several investigators have long addressed the question of how DM induces osteopenia and osteoporosis, the exact underlying mechanism is still elusive. However, it is widely accepted that hyperglycemia is a silent factor that has direct and indirect deleterious effects on osteoblast function and formation resulting in decreased bone formation and bone quality with increased bone resorption (1).

The possible mechanisms underlying impaired mechanical properties of the bone in DM had been suggested to be related to the increase in advanced glycation end products (AGE) or non-enzymatic cross-links within collagen fibers which in turn lead to deterioration in structural and mechanical properties of bone and eventually decrease in bone strength (16). In accordance with Pieschmann et al. (17), Pedrazzoni et al. (18) and Bouillon et al. (19), our data demonstrated that serum OC levels are decreased in patients with type 2 DM indicating that bone formation is decreased in these patients. The pathomechanism leading to decreased bone formation in DM has not been well clarified till now. As insulin stimulates the uptake of amino acid and collagen synthesis in bone, insulin deficiency might be one possible etiologic factor of diabetic osteopathy.

Ishida et al. (20) speculated that insulin exerted an indirect beneficial influence through metabolic amelioration of the decreased bone turnover and circulating OC in diabetic or that insulin had a direct stimulating effect on the osteoblast via the insulin receptor. Accumulating evidence indicates that the bone metabolism and energy homeostasis are associated with each other. Insulin signaling plays a pivotal role in bone metabolism. It has been reported that insulin stimulated the differentiation of osteoblastic cells and that activation of insulin receptor substrate, the downstream of insulin receptor, also leads to osteoblastogenesis (3). Serum OC levels have been found to represent de novo synthesis of osteocalcin by the osteoblasts (19).

Markers of bone remodelling including osteocalcin seem to be influenced by the deleterious effects of diabetic complications. Progressive decline in renal functions has been well described in patients with type 2 DM (20). The results of the present study support those found by Inukai et al. (21) who indicated that changes in bone remodeling markers such as OC levels are present even in early stages of diabetic nephropathy. The authors suggested that circulating intact PTH is important in restoring the reduced OC levels in diabetic patients probably as a reflection of bone remodelling. The present study showed increased OC levels in patients with diabetic nephropathy. Similar results were obtained by Foresta et al. (19) who studied bone markers in chronic kidney disease and declared progressive increase in serum OC levels with intact PTH and alkaline phosphatase levels reflecting severity of bone disease. Bonakdaran et al. (22) related increased OC levels in patients with diabetic nephropathy to impaired renal functions with consequent lower excretion of materials from the kidneys.

Consistent with our results Wongdee and Charoenphandhu (1) found a negative correlation between OC level and fasting blood glucose. Such correlation was independent of age, duration of DM, body stature and glucose or fat metabolism (1). Also Hwang et al. (25) declared that plasma OC level is inversely associated with the development of DM.

Saito and Marumo (16) came into conclusion that the increase in advanced glycation end products leads to deterioration in structural and mechanical properties of bone. In our study, a negative weak correlation between OC and glycated Hb % levels was found in the uncomplicated diabetic group. In the diabetic nephropathy group of patients the present study documented weak positive correlation \( r^2 = 0.45 \) between OC and protein creatinine ratio. The results of the present study concluded that changes in bone remodelling marker OC are present in both DM and diabetic nephropathy, explaining osteopenia and osteoporosis observed in both cases. Therefore, an effective glycaemic control should be the hallmark of prevention and treatment of DM induced osteoporosis.
REFERENCES