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Relationships between serum osteocalcin levels versus blood glucose, insulin resistance and markers of systemic inflammation in central Indian type 2 diabetic patients

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Abstract. – BACKGROUND: Recent studies have demonstrated an endocrine role of osteoblast derived protein osteocalcin in the regulation of blood glucose homeostasis. In addition emerging evidence suggests that subclinical inflammation is associated with altered bone metabolism. However, the relationship between osteocalcin and inflammatory markers is still unclear.

AIM: This study was aimed to investigate the association of serum osteocalcin with fasting plasma glucose, insulin resistance and markers of systemic inflammation – interleukin-6 (IL-6) and high sensitivity C-reactive protein (hs-CRP) in central Indian type 2 diabetic patients.

PATIENTS AND METHODS: This study included 108 individuals with newly diagnosed type 2 diabetes mellitus (DM) and 50 age and body mass index (BMI) matched healthy subjects as controls. Blood samples were analyzed for fasting plasma glucose, fasting insulin, interleukin-6, hs-CRP and serum osteocalcin. Insulin resistance was calculated by homeostasis model assessment (HOMA-IR).

RESULTS: In the present study we observed significantly lower level of osteocalcin in type 2 diabetic group compared to non diabetic control ($p < 0.0001$). In linear regression analysis adjusted for age, gender, BMI, waist circumference and waist to hip ratio, serum level of osteocalcin was inversely associated with fasting plasma glucose (beta = –0.015; $p = 0.0004$), fasting insulin (beta = –0.059; $p = 0.0242$), HOMA-IR (beta = –0.149; $p = 0.0011$), interleukin-6 (beta = –0.071; $p = 0.0036$) and hs-CRP levels (beta = –0.506; $p = 0.0085$) in the diabetic subjects.

CONCLUSIONS: Our findings suggest that osteoblast derived protein osteocalcin may have a wide ranging role in the pathophysiology of type 2 diabetes by being associated with both blood glucose homeostasis and systemic inflammation.

Key Words:
Type 2 diabetes mellitus, Osteocalcin, Blood glucose homeostasis, Insulin resistance, Inflammatory markers.

Introduction

Recent animal studies have demonstrated an endocrine link between bone metabolism and blood glucose homeostasis. Osteocalcin, an osteoblast derived non collagenous bone matrix protein with 49 amino acid, has several hormonal features. It is synthesized as a prepromolecule and secreted in the general circulation[1,2]. Mice lacking osteocalcin gene develops a series of phenotypic abnormalities such as decreased beta cell proliferation, decreased insulin secretion, insulin resistance and hyperglycemia than wild type mice[3]. Moreover, administration of recombinant osteocalcin into wild type mice enhances pancreatic beta cell proliferation, insulin secretion and protects them to a large extend against obesity and type 2 diabetes mellitus[4]. Lower serum osteocalcin levels are associated with increased fasting plasma glucose and insulin resistance in several cross sectional and prospective studies[5-7]. According to Hwang et al[8], the plasma osteocalcin levels are inversely associated with the development of type 2 diabetes mellitus.

A low grade chronic inflammation has been shown to promote insulin resistance and precede the onset of type 2 diabetes[9]. In addition, emerging evidence suggests that interleukin-6 (IL-6) and C-reactive protein (CRP), two sensitive physiological markers of subclinical systemic inflammation, have been associated with altered bone metabolism[10-13]. However, only a limited number of studies have investigated the relationship between osteocalcin and inflammatory cytokines. These studies reported a negative association of osteocalcin with high sensitivity C-reactive protein (hs-CRP)[5,14,15] and IL-6[5]. Till date, the relationship of osteocalcin with glucose metabolism and inflam-

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matory markers has not been evaluated in Asian Indian population. Therefore, in this cross sectional study we investigated the association of serum osteocalcin with fasting plasma glucose, insulin resistance, interleukin-6 and hs-CRP in central Indian type 2 diabetic patients.

Patients and Methods

This study was conducted in the Department of Biochemistry, Netaji Subhash Chandra Bose Medical College (NSCB) and Hospital, Jabalpur, Madhya Pradesh and approved by Institutional Ethical Committee. 108 newly diagnosed type 2 diabetic patients (56 male 52 female; mean ± SD age: 51.4 ± 9.26 yr) were selected from the Out Patient Department of NSCB Medical College and Hospital. 50 age and BMI matched healthy subjects (27 male 23 female; mean ± SD age: 50.6 ± 7.39 yr) who had come for routine health check-up in our Hospital, were taken as controls. Diabetes mellitus was confirmed according to the 1999 World Health Organization (WHO) criteria[16]. Brief clinical history of present and past illness and medical therapy was recorded from all participants. Written informed consent was obtained from study group and control before entry into the study. The exclusion criteria were: (1) pregnancy; (2) any systemic disease other than type 2 diabetes; (3) under hypoglycaemic drug or insulin treatment; (4) recent history of fracture; and (5) use of any bone active medications such as vitamin D, calcitonin, bisphosphonate or oestrogen therapy, or lipid lowering drug. Body mass index (BMI) of all subjects was calculated by using the formula weight in kg/m^2. Waist circumference (WC) and waist to hip ratio (WHR) were measured in the standing position using standard techniques.

Venous blood samples were collected after an overnight fasting in the morning in an aseptic condition from antecubital vein. Blood samples were centrifuged at –4° centigrade. Analyses of fasting plasma glucose were done immediately. Serum samples for analysis of osteocalcin, interleukin-6, hs-CRP and fasting insulin were stored at –80° centigrade until analysis. Fasting plasma glucose (FPG) was estimated by standard laboratory kit method using fully automated biochemistry analyzer (Biosystem). Serum osteocalcin was estimated by ELISA method (Quidel Corporation, San Diego, CA, USA). Fasting insulin was measured by commercially available ELISA kit (LDN, Nordhorn, Germany). Estimation of hs-CRP was done by immunoturbidimetric method (Biosystem, Foster City, CA, USA). Interleukin-6 was estimated by commercially available ELISA kit (Ray Biotech Inc, Norcross, GA, USA). Insulin resistance was calculated by homeostasis model assessment (HOMA) based on the formula: HOMA-IR = fasting serum insulin (FINS) (μIU/ml) × fasting plasma glucose (mg/dl) divided by 405[17].

Statistical Analysis

Data were expressed as the mean ± standard deviation. Comparison of baseline anthropometric and biochemical parameters between type 2 diabetic patients and non diabetic control group was done by unpaired Student's *t*-test. Linear regression analysis was done to study association between osteocalcin and biochemical parameters after multivariate adjustment for age, gender, BMI, waist circumference and waist to hip ratio. *p* value less than 0.05 was considered to be statistically significant. All data were analyzed using statistical software SPSS version 16 (SPSS Inc., Chicago, IL, USA).

Results

The baseline anthropometric and biochemical characteristics of control and type 2 diabetic patients group are presented in Table I. The diabetic group had significantly higher BMI, FPG, fasting insulin, HOMA-IR, IL-6 (17.60 ± 8.73 pg/ml vs. 9.48 ± 3.26 pg/ml) and hs-CRP (2.85 ± 1.26 mg/l vs. 1.27 ± 0.75 mg/l) ($p < 0.05$-0.0001) than the control group, whereas osteocalcin level was significantly lower in the diabetic group (4.06 ± 1.97 ng/ml vs. 9.62 ± 3.29 ng/ml; $p < 0.0001$) (Table I). Furthermore, we looked for associations of osteocalcin with glucose metabolism related parameters and markers of systemic inflammation in type 2 diabetic group using multivariate linear regression analysis. After adjustment for age, gender, BMI, waist circumference and WHR, serum osteocalcin level was significantly inversely associated with FPG ($\beta = –0.015$; $p = 0.0004$), fasting insulin ($\beta = –0.059$; $p = 0.0242$), HOMA-IR. ($\beta = –0.149$; $p = 0.0011$), IL-6 ($\beta = –0.071$; $p = 0.0036$) and hs-CRP ($\beta = –0.506$; $p = 0.0085$) (Table II).

Discussion

In line with the observation from animal experiment[3,4], in the present study we observed sig-

Table I. Baseline anthropometric and biochemical characteristics of control and type 2 diabetic patients group.

Parameters	Control group	Diabetic group	p
BMI (kg/m^2)	24.6 ± 4.42	25.96 ± 4.56	0.0408
Waist circumference (cm)	87.84 ± 9.89	89.5 ± 8.77	0.1444
Waist to hip ratio (WH)	0.935 ± 0.074	0.919 ± 0.072	0.1010
Fasting plasma glucose (mg/dl)	84.76 ± 14.81	171.2 ± 43.2	< 0.0001
Fasting insulin (µIU/ml)	7.47 ± 3.69	14.6 ± 8.54	< 0.0001
HOMA-IR	1.58 ± 0.88	6.50 ± 4.64	< 0.0001
Osteocalcin (ng/ml)	9.62 ± 3.29	4.06 ± 1.97	< 0.0001
Interleukin-6 (pg/ml)	9.48 ± 3.26	17.60 ± 8.73	< 0.0001
Hs-CRP (mg/l)	1.27 ± 0.75	2.85 ± 1.26	< 0.0001

Data are presented as mean ± SD.

nificantly lower level of osteocalcin in type 2 diabetic group compared to non diabetic control. Serum osteocalcin level was significantly and inversely associated with FPG, fasting insulin and HOMA-IR among type 2 diabetic patients. Similar observations were also made by previous researchers in human studies[5-8]. *In-vitro* and *in-vivo* reports demonstrate that hyperglycemia suppresses osteoblast functioning[18,19] and, thereby, decreased production and secretion of osteocalcin in type 2 diabetes. Hyperglycemia also induces increase production of reactive oxygen species (ROS) responsible for bone marrow ageing. However, increased glucose concentration may directly be toxic to osteoblast[20]. In a feedback loop osteocalcin enhances pancreatic beta cell proliferation, insulin secretion and sensitivity established in animal study[3]. The precise molecular mechanisms by which osteocalcin may affect blood glucose and insulin sensitivity in human are yet to be discovered.

In our study type 2 diabetic patients were found to have significantly increased level of both IL-6 and hs-CRP. Moreover, serum osteocalcin level was significantly and negatively associated with IL-6 and hs-CRP. Our findings are supported by some previous studies[5,14,15], that indicated a possible anti-inflammatory effect of osteocalcin. Although data regarding relationship between osteocalcin and inflammatory markers are limited, there is increasing evidence showing a connection between bone metabolism and subclinical inflammation in recent years. So far the literature concerning the role of IL-6 in bone metabolism in human is conflicting. Some previous works reported that serum IL-6 was negatively associated with bone mineral density (BMD) in post menopausal women[11,12], whereas others observed no significant associations in women[21,22]. Moreover, several authors demonstrated low bone mineral density with increasing concentration of hs-CRP[10,13], therefore, proposed that serum hs-CRP is closely associated with bone metabolism.

These findings suggest that low grade subclinical systemic inflammation may be associated with bone metabolism, but the exact mechanisms are not clearly understood. In bone IL-6 is synthesized by osteoblast[23], and is a key regulator for receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin, an important mediator for osteoclast differentiation and activation[24]. Moreover, IL-6 over expressing transgenic mice exhibit osteopenia with decreased osteoblast and increased osteoclast number and activity[25]. Suppressed osteoblastogenesis

Table II. Linear regression analysis for association of serum osteocalcin with biochemical parameters adjusted for age, gender, BMI, waist circumference and waist to hip ratio.

Independent variable	β	SE	Standardized β	p
Fasting plasma glucose (mg/dl)	-0.015	0.004	-0.319	0.0004
Fasting insulin (µIU/ml)	-0.059	0.026	-0.257	0.0242
HOMA-IR	-0.149	0.045	-0.352	0.0011
Interleukin-6 (pg/ml)	-0.071	0.024	-0.316	0.0036
Hs-CRP (mg/l)	-0.506	0.188	-0.324	0.0085

SE: Standard error.

has been shown as a cause and mechanism for low bone mass and impaired bone regeneration in a rat model of type 2 diabetes mellitus. Impaired osteoblast differentiation resulted in reduced expression of osteoblast specific protein osteocalcin[26]. However, the mechanism linking high-sensitivity CRP and bone metabolism has not been well understood, but involvement of IL-6 which influences hepatic C-reactive protein synthesis, has been suggested[11]. Thus, it appears that bone metabolism and inflammation are interlinked though a common mechanism involving osteoblast derived protein osteocalcin. Further studies to confirm and extend these observations as well as to elucidate the underlying mechanisms are clearly needed.

Conclusions

In the present investigation we observed significantly lower level of osteocalcin in central Indian type 2 diabetic patients compared to non diabetic control group. Lower serum osteocalcin was associated with increased fasting plasma glucose, fasting insulin and insulin resistance. Moreover, we found an inverse association of serum osteocalcin with IL-6 and hs-CRP in type 2 diabetes patients. Our findings suggest that osteoblast derived protein osteocalcin may have a wide ranging role in the pathophysiology of type 2 diabetes by being associated with both blood glucose homeostasis and systemic inflammation. Lifestyle and pharmacological approaches that increase osteocalcin levels might be valuable in the prevention and treatment of type 2 diabetes and complications.

Conflict of Interest

None to declare.

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