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Original Research Article

Serum Osteocalcin Level in Patients with Type II Diabetes

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Abstract

Osteocalcin, the second most abundant protein in bone tissue is secreted by osteoblasts, was thought to participate in mineralization and calcium ion homeostasis. In addition to its use as biomarker in osteoporosis, recent studies have identified osteocalcin as an endocrine regulator of glucose metabolism, stimulating beta-cell insulin secretion and reducing peripheral insulin resistance. Insulin signalling in osteoblasts improves glucose handling directly by increasing secretion of active osteocalcin and indirectly by enhancing bone resorption which releases osteocalcin into the bloodstream. Thus in type 2 diabetes patient with insulin resistance serum osteocalcin level is decreased, which in turn affecting bone mineralisation. ***Aim:*** To look for an association of serum osteocalcin in type 2 diabetes mellitus. ***Materials and Method:*** The study population consisted of 46 type 2 diabetic patients as cases and 44 healthy subjects as contols. Fasting venous blood was collected from each subject and estimations of Serum Osteocalcin, Fasting Insulin, glycated haemoglobin, Serum ionized calcium ,Homeostatic model assessment (HOMA) is a calculated method used to quantify insulin resistance were done. ***Result:*** The serum osteocalcin was decreased in diabetic patients and was found to be statistically significant (P value 0.03). Serum osteocalcin negatively correlated with fasting blood sugar (r=-0.233), HbA1c (r=-0.160) and was statistically significant. Serum osteocalcin did not correlate with insulin resistance assessed by HOMA-IR, and fasting insulin. ***Conclusion:*** Serum osteocalcin was decreased in Type 2 Diabetic patients and negatively correlated with glycemic control. Thus a good glycemic control is essential part of bone health in diabetics individual.

Keywords: Osteocalcin, Type 2 Diabetes mellitus, HbA1c Glycated hemoglobin, HOMA-IR Homeostatic model Assessment- Insulin Resistance.

INTRODUCTION

Diabetes forms a part of a larger global epidemic among non-communicable diseases. [1]. Indian subcontinent facing a mammoth diabetes epidemic, with about 40.9 million diabetics, contributes to nearly about 15% of the world's burden. Studies show that this burden will increase to about 70 million by 2025 [2].

Type 2 Diabetes Mellitus consists of an multiple array of dysfunctions, resulting from the combination of inadequate insulin secretion and resistance to insulin action, characterized by hyperglycemia [3]. In addition to the micro-vascular and macro-vascular complications [4], the metabolic effects of diabetes mellitus can affect bone structure, metabolism, and mineral density and the extent to which type 2 diabetes predisposes to increased risk of fracture is still debated [5].

Recently a novel pathway has emerged, linking the bone to glucose metabolism [6]. Osteocalcin (OC), a biomarker of bone formation has emerged as an element which has insulin-sensitizing properties [7]. A recent study has demonstrated the interactions between the bone and energy metabolism, which has revealed that insulin receptor is indicated in osteoblast [8]. Under Insulin signalling, osteoclastic bone resorption releases osteocalcin [9]. Osteocalcin is then expelled into the circulation and has its effects on target tissues like adipose tissue and the beta cells of pancreas by increasing insulin production and beta cell proliferation [10].

Experiments wherein the gene for Osteocalcin was deleted resulted in phenotypes with insulin resistance, glucose intolerance and visceral [11]. When recombinant Osteocalcin was admistered in vivo it showed improvement in the secretion of insulin and there was better tolerance of glucose [12]. Compiling these data it is clear that Osteocalcin has a key role in

regulating the skeletal-glucose metabolism and to maintain homeostasis. Decreasing or increasing signaling of insulin in the Osteoblast stimulate or suppresses glucose metabolism which is dependent on the bone resorption. Hence in feed forward loop insulin signaling in the Osteoblast activates Osteocalcin which stimulates glucose metabolism [13].

In humans various studies show, diminished Osteocalcin concentration in the serum of T_2DM patients and is related to Fasting Plasma Glucose (FPG), Glycated Hemoglobin (HbA1c) level, Homeostasis-Model Assessment Insulin Resistance (HOMA-IR) index and fasting insulin level. However to our knowledge, there are only few studies which evaluate the relation between total Osteocalcin with changes in glucose metabolism Therefore we conducted a study to determine whether Osteocalcin is associated with insulin resistance and Glycated Hemoglobin (HbA1c) level, in Type 2 diabetic patients.

MATERIALS METHODS

This study was was done in 2 groups, namely, apparently healthy controls and subjects with Type 2 Diabetes Mellitus. The control group comprised of 44 apparently healthy subjects with no significant medical illness. The case group comprised of patients with type 2 diabetes mellitus and were selected from patients attending the OPD of the Department of Diabetology, at our Medical College, Chennai .The control group and case were matched for age, gender, and body mass index (BMI). Diabetes was diagnosed according to the 1999 World Health Organization criteria [7]. Complete physical examinations and routine biochemical analyses of blood were performed. The study was approved by the ethics committee of our hospital. Written informed consent was obtained from all participants. Participants with the following conditions were excluded: hepatic or renal dysfunction, history of malignancy, and fracture within 1 year or taking medications known to influence bone and calcium metabolism, such as vitamin D, bisphosphonate, calcitonin, estrogen, tamoxifen, or corticosteroids. Patients on insulin treatment, and chronic bed ridden patients were excluded from study.

Biochemical Analyses

6 ml of peripheral venous blood was withdrawn from all the study subjects under sterile conditions from antecubital vein after overnight fasting of 8- 10 hours. A physical examination, including the measurement of height, weight, waist circumference, blood pressure (BP), was performed for each subject. BMI was calculated as weight divided by squared height. Two ml of blood was transferred into the test tube containing EDTA for HbA1C estimation. The remaining 4 ml of blood was transferred to a plain tube. Blood samples were centrifuged at –4° centigrade. Serum samples for analysis of osteocalcin, fasting insulin was stored at –80° centigrade until analysis. Serum insulin concentration was measured by ELISA (Calbiotech kit). HbA1c was determined by HPLC D10 (Bio-Rad Inc). Fasting plasma glucose, Serum urea, serum creatinine, Serum triglyceride (TG), total cholesterol (TC), were measured by enzymatic procedures using an automatic analyser by commercially available kit. Serum osteocalcin was measured by ELISA (epitope diagnostics). The assay quantitatively determination of both human osteocalcin (1-49) (N-terminal) and osteocalcin (1-43) (mid-regional osteocalcin) levels. Insulin resistance was estimated using the homeostasis model assessment (HOMA-IR), with the formula: Fasting Insulin (mU/l) x Fasting Glucose (mg/dl)/405. Ionized calcium was measured in Roche 9180 ion selective electrode (ISE). Statistical analysis was performed using SPSS package. Pearson's correlation was used for univariate analysis. The groups were compared using Student's T test. p-value of <0.05 is considered significant.

Aim

To look for an association of Serum Osteocalcin level in type 2 Diabetes Mellitus.

Objectives

- To Measure Serum Osteocalcin, Fasting blood sugar, Insulin, Glycated haemoglobin in study population.
- To correlate Serum Osteocalcin concentration with Fasting Glucose and Glycated Haemoglobin.
- To find association between Serum Osteocalcin, Insulin Resistance and Insulin. Using data from a Biochemical evaluation in patients with Type 2 Diabetes and in Control.

RESULTS

This study done to evaluate serum Osteocalcin in type 2 Diabetes Mellitus was done in total of 90 subjects, of which 46 with known Diabetes mellitus were taken as cases and 44 Patients without DM were taken as controls. The baseline anthropometric and biochemical characteristics of control and type 2 diabetic patients group are presented in Table-1.

Table-1: Anthropometric and Metabolic Parameters in Control Group and In Patients with Type 2 Diabetes Mellitus (DM2)

Parameters	Control group (n=44)	Type 2 Diabetes mellitus group (n=46)	P value
Age	49.02	50.61	
Ht	157.50±7.309	154.50±8.49	0.117
Wt	67.16±9.160	66.24±11.89	0.683
BMI	27.07±3.24	27.80±5.45	0.446
Serum biochemical parameters			
Fasting glucose (mg/dl)	90.09±11.76	170.8±17.1	0.000*
Hba1c	5.60±0.33	8.25±1.78	0.000*
- Fasting insulin (µIU/ml)	6.19±3.3	8.25±5.77	0.08*
HOMA-IR	1.25±0.744	3.45±3.2	0.000*
Ionized calcium	1.16±0.14	1.23±0.14	0.024*
Osteocalcin (ng/ml)	19.87±11.8	6.73± 2.40	0.03*

*Shows significance p value

The diabetic group had significantly higher BMI, FPG, fasting insulin, HOMA-IR, HbA1C than the control group, whereas osteocalcin level was significantly lower in the diabetic group (Table-2).

Table-2: Correlation of FBS, HbA1C, Insulin, Insulin Resistance (IR) Vs Serum Osteocalcin in Type 2 Diabetes Mellitus

		Correlations	P value
F BS vs osteocalcin	Pearson Correlation	-.172	0.008*
Insulin vs osteocalcin	Pearson Correlation	.164	0.275
HbA1C vs osteocalcin	Pearson Correlation	-.148	0 .002*
IR vs osteocalcin	Pearson Correlation	.027	0.860

*Shows significance p value. After adjustment for age, gender, BMI, serum osteocalcin level was significantly inversely associated with FPG, HbA1C.

DISCUSSION

In this study, we attempted to explore the role of Serum Osteocalcin in Type 2 Diabetes Mellitus. The Diabetics and Controls were matched with respect to the confounding variables like Age, Gender and BMI. The biochemical parameters were assessed between the groups. Among the parameters, Fasting Blood Glucose, Ionized Calcium, HbA1c, Insulin Resistance, Insulin, and Serum Osteocalcin were statistically significant between the Cases and Control.

The Serum Osteocalcin level was found to be decreased in Diabetic patients and was statistically significant. Serum Osteocalcin negatively correlated with Fasting Blood Sugar and HbA1c and was statistically significant. These results were in agreement with previous studies by Daniela Grădinaru *et al*., [14] P. D. Sarkar *et al*., [15], Wang *et al.*, [16] and suggest that Osteocalcin may be involved in the glycemic homeostasis. In our study, Serum Osteocalcin did not correlate with Insulin Resistance assessed by HOMA-IR and Fasting Insulin.

It is well known from Clinical and Experimental observations by Merlotti *et al*., [17], Swhwetz *et al*., [18], that Seum Osteocalcin levels are lower in Diabetic patients than in normal individuals. Studies have demonstrated that in hyperglycemic states, the osteoblast mass and function are decreased, which suppresses Osteocalcin synthesis and secretion.

Different cross-sectional and prospective studies done in healthy subjects by Kanazawa *et al.*, [19], Oestreweroff *et al.*, [20] have demonstrated a positive association between total Osteocalcin levels and improved Fasting Blood Glucose and a reduced concentration of Glycated Haemoglobin levels. Yu-Qian Ba 113 *et al*., showed that serum osteocalcin concentrations increased with improved glucose control. High osteocalcin levels were associated with subsequent improvements in glucose variability during glucose-lowering treatment [21].

In addition, a recent prospective analysis by Ngarmukos *et al*., [22] carried out in middle-aged men showed that low Osteocalcin levels were related to a high risk of developing Type 2 diabetes at 10 years. Lee *et al*., showed that diabetes mice without Osteocalcin gene expression have a decreased beta-cell mass while mice engineered with an increase of active Osteocalcin have increased beta-cell proliferation and increased insulin levels. These studies provided a proof-of-principle that Osteocalcin could be an important regulator of beta-cell mass and glucose homeostasis [14].

Osteocalcin has long been accepted as an osteoblast-specific product. The majority of Osteocalcin, secreted by the osteoblast is deposited in extracellular bone matrix and serum Osteocalcin represents the fraction of total Osteocalcin that has not been adsorbed to hydroxyapatite in bone matrix. The unadsorbed fraction of Osteocalcin, during resorption is converted to uncarboxylated form and released into circulation where it influences energy homeostasis. This unadsorbed fraction of Osteocalcin is shown to increase beta cell mass enhances glucose tolerance and improves insulin sensitivity and acts as an insulin secretagogue increasing blood insulin levels [24]. Gene expression analysis of islets cell lines demonstrated that Osteocalcin directly enhances the expression of the insulin genes as well as those encoding cyclin - dependent kinase 4 (Cdk4), cyclin D1 (Ccnd1) and D2 (Ccnd2). These findings explain the positive effect that Osteocalcin has on both insulin production and beta cell proliferation. Likewise, in vitro and vivo analyses demonstrated that Osteocalcin signals directly to adipocytes, where it promotes the expression of the gene encoding adiponectin (Adipoq) increasing insulin sensitivity [25].

In our study, Serum Osteocalcin does not correlate with Insulin Resistance by HOMA-IR, and Fasting Insulin which is in concordant with Wang *et al.*, [16] Gower *et al*., [26] also suggested that total Osteocalcin is not associated with hepatic insulin sensitivity. Pitas *et al.*, [27] suggested that the association between Osteocalcin and Glycemia may be causal. Although we selected subjects with apparent Type 2 diabetes, this may confound the interaction between Osteocalcin levels and insulin resistance. Also, drug interventions may make it more difficult to interpret the correlation between Osteocalcin levels and Insulin Resistance. The observed inverse association between Osteocalcin and Insulin Resistance in humans, as measured by HOMA-IR, appears to be partially mediated by secretion of adiponectin. Measurement of adiponectin is therefore needed to test this hypothesis in study group.

However, Several Chinese studies showed that total Osteocalcin was not related to HOMA-IR [16]. Total Osteocalcin was an independent associated factor to HbA1c. These results suggested that decreased serum Total Osteocalcin was related with long-term hyperglycemia but had little impact on Insulin Resistance. The results of this study are similar to those of the other two Chinese studies [28, 29], but different from those of the other foreign studies. Wang *et al*., suggested that this difference in study populations is caused by races, genes, sunlight exposure and diet habits [16].

Although Calcium value in both diabetic and control population was in a normal range, calcium was significantly higher in the diabetic compared to control group. No correlation was found between plasma glucose or HbA1c value and calcium level. The findings from this study is in accordance with levy *et al*., [30] and showed that the difference in ionized calcium between diabetic and control were small. This elevation of calcium level is attributed as a part of calcium homeostasis in diabetics.

The elevation of calcium level can cause increased push of calcium into the cells via mass effect, thereby altering cellular calcium homeostasis. This might result in increased insulin secretion since Insulin secretion is a calcium-dependent biological process [30]. Indirect evidence is shown from observing diabetic animal models. Stephen *et al*., suggested that hyperglcemia elevates cytosolic free calcium [31]. Barbagallo *et al*., suggested that glucose related excess calcium was seen in diabetics [32]. The findings in our study proved that serum Osteocalcin level is decreased in type 2 diabetes mellitus. Here, serum Osteocalcin level is inversely related to fasting blood sugar and glycemic status of the individual.

CONCLUSION

From the discussion held on the results obtained in the study on serum Osteocalcin in type 2 DM patients the following conclusion is arrived at regarding the biochemical parameters: Serum Osteocalcin levels are significantly decreased in Type 2 Diabetic patients. Serum Osteocalcin levels inversely associated with HbA1c and Fasting Blood Sugar. Serum Osteocalcin levels does not correlate with insulin and insulin resistance by HOMA-IR

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