

Research article

Osteocalcin – A hidden factor in diabetes mellitus: A cross sectional study

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Abstract

Background: Osteocalcin, a bone gamma carboxy glutamic acid protein (BGP) is synthesised from osteoblasts. Experimental animal studies reported the role of osteocalcin in glucose homeostasis. Research is going on still to find out the role of osteocalcin in humans. Hence we also designed this study. **Aim:** To find out the association of serum osteocalcin and glycemic status in type 2 Diabetic subjects and healthy controls. **Materials and methods:** This is a cross sectional study done at Chettinad Hospital and Research Institute, Chennai with 84 male participants in the age group of 30 -60 years. Out of 84, 42 were healthy subjects and 42 were previously diagnosed Type 2 Diabetes with 5 years duration. Participants on exogenous insulin or with any other chronic illnesses such as hypertension, kidney diseases, cardiovascular diseases, bone disorders, drug intake such as vitamin D, calcium were excluded. After obtaining informed consent, demographic details and personal history were recorded in the form of Questionnaire. 5ml of venous blood was collected for estimating serum osteocalcin, fasting plasma glucose, serum insulin and processed on the same day. Fasting plasma glucose was estimated using HEXOKINASE method in Siemens DADE XPAND auto analyser. Serum osteocalcin, Serum insulin was estimated using SANDWICH IMMUNOLUMINOMETRIC ASSAY method in MAGLUMI1000 chemiluminescence fully auto analyser. Insulin resistance was determined using HOMA-IR model. Statistical analysis was done using SPSS software version 21. **Results:** Serum osteocalcin level was significantly low in diabetic subjects compared to healthy subjects (p value 0.000) and it shows a negative association with fasting plasma glucose, serum Insulin, HOMA-IR, BMI, Waist circumference. **Conclusion:** Our study suggested that serum osteocalcin have a significant role in glucose homeostasis. This puts up a new foothold in the field of diabetes especially in the line of treatment.

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Introduction

Diabetes is considered as one of the oldest disease of the humans, discovered by the ancient Egyptians before 3000 years. At the same time, Hindu physicians also discovered this condition and termed it as “Madhumeha” which means honey urine. Araetus of Cappodocia in 81-133 AD, coined the name “Diabetes” [1-2]. Now, Diabetes has emerged as a global burden.

Diabetes mellitus is a group of metabolic disorder characterised by hyperglycemia resulting from defect in the insulin secretion, insulin action or both. Diabetes mellitus is classified in to four types. They are Type 1 DM, Type 2 DM, Other Specific types and gestational diabetes mellitus [3]. Among all these types, Type 2 diabetes mellitus is the most commonest type. Globally, people living with diabetes were estimated to be around 366 million in the year 2011. Data from various studies shown that it will be rocket up to 552 million by the year 2030 [4].

Men are more prone to develop diabetes compared to women. The prevalence of diabetes was increasing in the developing countries [5]. India ranks second in the

diabetic population. Studies reported that India will shortly become the capital of diabetes. As stated by International Diabetes Federation, 40.9 million Indian people were living with diabetes. This number would be expected to increase around 69.9 million by the year 2025 [6].

Also, data from World Health Organisation (WHO) showed the prevalence of diabetes in India is around 52 million and this number is expected to increase around 87 million in 2030 [7]. Studies also shown that genetically Indians are at more risk of developing diabetes mellitus [8].

The National Urban Diabetes Study (NUDS) showed a high prevalence of diabetes among Southern India when compared to Northern India [9]. The increased prevalence of diabetes in Southern India was also observed in a large community based on study done by Indian Council of Medical Research (ICMR). This study reported that people living with diabetes in Tamilnadu was estimated to be around 4.8 million [10].

The prevalence of diabetes is increasing due to life style changes, altered food habits, decreased physical activity,

smoking, alcohol and to lesser extent, genetic factors [11-12].

According to Chennai Urban Population Study, the diabetic people are more at risk of developing microvascular and macrovascular complications such as diabetic retinopathy, diabetic nephropathy, diabetic neuropathy, coronary heart disease, stroke. This life threatening complication leads to increased mortality and morbidity rate among diabetic people [13].

Since mortality and morbidity is increased due to diabetes, so many researches are going on globally to help the vast population of diabetic patients. Recent animal studies found out the interaction of osteocalcin, a osteoblast specific protein with Insulin.

Osteocalcin is a 49 amino acid glutamic acid rich, non collagenous protein with a molecular weight of 5800 kilo Daltons. It is synthesised from osteoblastic cells of the bone matrix. It is also known as bone gamma carboxy glutamic acid protein or bone gla protein. The gene responsible for synthesis of osteocalcin is BGLAP gene located in the long arm of chromosome 1q25-q31. This gene is expressed mainly in osteoblastic cells and to a minor extent in odontoblasts. Transcription of this gene is stimulated by vitamin D. Vitamin D enters the osteoblasts and binds with its cognate receptor (VDR) present inside the cell. After binding to its receptor (VDR), this receptor- hormone complex (VDR-Vitamin D complex), traverses into the nucleus and binds with the vitamin D responsive element region present in the DNA. This binding stimulates the transcription of BGLAP gene, which is ultimately translated in to a peptide, preproosteocalcin.

Post translationally, proteolytic cleavage of preproosteocalcin takes place to release proosteocalcin, a 75 amino acid peptide and a peptide containing 23 amino acid. This 23 amino acid peptide is removed after cleavage. The pro-osteocalcin undergoes vitamin K dependent carboxylation of glutamic acid residues at position 17, 21, 24 by the enzyme, gamma glutamyl carboxylase resulting in the formation of Gla residues. It is known as pro-Gla osteocalcin. Some portion of pro-osteocalcin remains as such without undergoing carboxylation and is known as pro-Glu osteocalcin. These two forms of osteocalcin, pro-Gla osteocalcin and pro-Glu osteocalcin again undergoes proteolytic cleavage to produce carboxylated osteocalcin and undercarboxylated osteocalcin. By a calcium mediated process, both the forms of osteocalcin are released from the osteoblasts.

Carboxylated osteocalcin binds to free calcium and hydroxyapatite, permitting the deposition of osteocalcin on mineralized bone matrix. Undercarboxylated osteocalcin have decreased affinity towards hydroxyapatite and it is released into the systemic circulation and it is biologically active [17].

Osteocalcin constitutes about 2% of total body protein. Although it is present in meagre levels, it exerts most

important biological effects in our body. Osteocalcin plays a pivotal role in calcium homeostasis and bone mineralisation. Formerly it is used as a marker for osteogenesis. Recent experimental animal studies have thrown light upon its endocrine actions. Among its endocrine actions, increasing insulin secretion and sensitivity is of utmost importance [14-15]. Still research is going on to find out the endocrine actions of osteocalcin in humans. Hence, we also designed this study in human beings to find out the association of serum osteocalcin and glycemic status in type 2 diabetes subjects and healthy controls.

Experimental

Materials and methods

This Cross sectional study done at Chettinad Hospital and Research Institute, Kelambakkam, Chennai from the period of July 2015 to August 2015, was approved by Ethical Committee Board of Chettinad Hospital and Research Institute (Ethical approval no IHEC/02). Eighty four male participants in the age group of 31 to 60 years were recruited. Of which, forty two were previously diagnosed as type 2 diabetes mellitus with five years of duration (cases) and forty two were healthy males (controls). Participants on exogenous insulin, any other illnesses such as hypertension, chronic kidney disease, coronary heart disease, bone disorders, drug intake such as vitamin D, calcium were excluded from the study. After obtaining informed consent from the participants, demographic details and personal history were obtained in the form of questionnaire.

Height was measured in centimetres. Weight was measured in electronic balance. Body mass index or Quetelet index was calculated using the formula, weight in kilograms divided by height in meter². Waist circumference and hip circumference were measured according to World Health Organisation (WHO) criteria 2008. According to WHO criteria 2008, the waist circumference should be measured at the midpoint between the last palpable rib and the top of the iliac crest. Hip circumference should be measured around the widest portion of the buttock [22]. Waist to hip ratio was calculated by the formula, waist circumference divided by hip circumference. 5ml of fasting venous blood samples were collected in grey top and red top vacutainers. After centrifuging, plasma and serum were separated and processed on the same day for estimating parameters such as fasting plasma glucose (FPG), serum osteocalcin and serum insulin. fasting plasma glucose (FPG) was estimated using HEXOKINASE method using SIEMENS DADE XPAND auto analyzer. Serum osteocalcin and serum insulin was estimated using SANDWICH IMMUNOLUMINOMETRIC ASSAY method using MAGLUMI 1000 fully auto chemiluminescence immunoassay analyzer. Insulin resistance was determined

using HOMA-IR model (Homeostatic Model of Assessment- Insulin Resistance). HOMA-IR was calculated using the formula, Fasting Insulin (mIU/L) X Fasting Glucose (mg/dL)/405. Statistical analysis was done using SPSS software version 21.

Results and discussion

Results

Table 1 shows the mean, standard deviation (SD), Independent t test between various parameters among cases and controls. Weight was significantly higher in case group (mean- 75.52) when compared to control group (mean-62.10) (p value 0.000), body mass index (BMI) was significantly higher in case group (mean-26.83) when compared to control group (mean- 21.15) (p value 0.000), waist circumference (WC) was significantly higher in case group (mean-102.69) when compared to control group (mean- 89.71) (p value 0.000), Hip Circumference did not show any significant difference between both case (mean-105.45) and control group (mean- 106.81) (p value 0.175), waist to hip ratio (W/H ratio) was significantly higher in case group (mean- 0.98) when compared to control group (mean- 0.84) (p value 0.000). Fasting plasma glucose level (FPG) was significantly higher in case group (mean-158.33) when compared to control group (mean-90.86) (p value 0.000).

Serum osteocalcin level was significantly lower in case group (mean-4.53) compared to control group (mean-10.76) (p value 0.000). Serum insulin level was significantly higher in case group (mean-18.67) compared to control group (mean-11.20) (p value 0.000). Homeostatic Model of Assessment- Insulin Resistance (HOMA-IR) was significantly higher in case group (mean-8.217) when compared to control group (mean-2.584) (p value 0.000).

Table 2 shows the Pearson's correlation of serum osteocalcin with various parameters among cases and controls. Serum osteocalcin was negatively correlated with body mass index (BMI) (r value = - 0.825, - 0.933), waist circumference (WC) (r value = - 0.784, - 0.887), waist to hip ratio (W/H ratio) (r value = -0.739, -0.868) in both cases and controls. Also the correlation was statistically significant among both the cases and controls. Table 3 shows the Pearson's correlation of serum osteocalcin with fasting plasma glucose, serum insulin and HOMA-IR among cases and controls. Serum osteocalcin was negatively correlated with fasting plasma glucose (FPG) (r value= -0.781,-0.892) as shown in Graph 1, Serum Insulin levels (r value=0.878,- 0.851) as shown in Graph 2, HOMA-IR model (r value= -0.782,-0.846) as shown in Graph 3 in both cases and controls. Also the correlation was statistically significant among both the cases and controls.

Table 1. Mean, SD, t-test levels of various parameters among cases and controls.

Parameters	Groups		Controls		Independent Samples t-test Sig.
	Cases	SD	Mean	SD	
Age	49.62	7.46	48.00	10.78	0.426(NS)
Height (cms)	167.83	3.41	171.29	4.60	0.000(HS)
Weight (kgs)	75.52	5.09	62.10	4.25	0.000(HS)
BMI	26.83	1.94	21.15	0.97	0.000(HS)
Waist Circumference (cms)	102.69	3.76	89.71	5.09	0.000(HS)
Hip Circumference (cms)	105.45	5.75	106.81	2.82	0.175(NS)
Waist-Hip Ratio	0.98	0.08	0.84	0.03	0.000 (HS)
FPG (mg/dl)	158.33	49.70	90.86	6.76	0.000(HS)
S. Osteocalcin (ng/mL)	4.53	2.94	10.76	6.37	0.000(HS)
S. Insulin (µIU/ml)	18.67	8.49	11.20	4.76	0.000(HS)
HOMA IR	8.217	6.452	2.584	1.264	0.000(HS)

HS – HIGHLY SIGNIFICANT

NS – NOT SIGNIFICANT

Table 2. Pearson's Correlation of Serum Osteocalcin with various parameters among Cases and Controls.

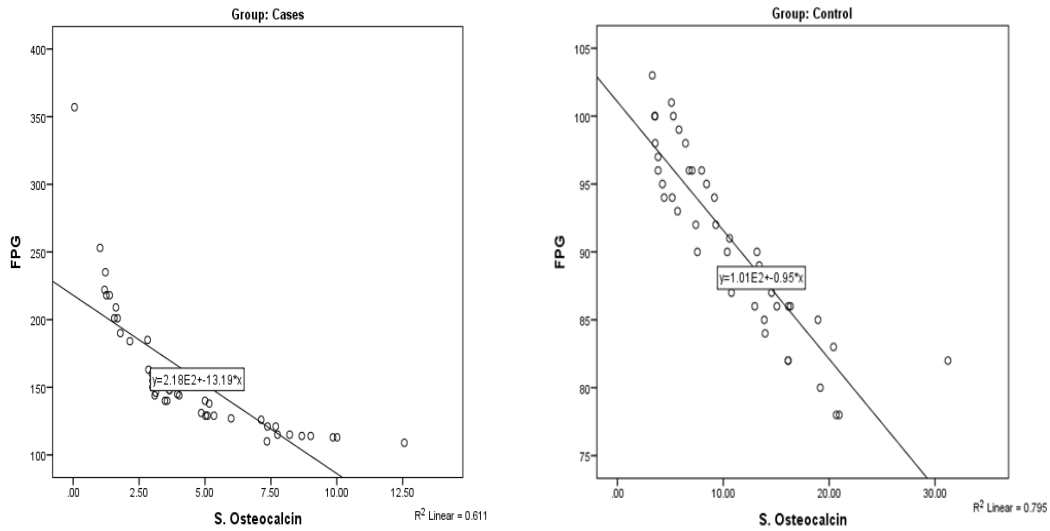
Parameter	Groups	Body mass Index (cms)	Waist Circumference (cms)	Waist-Hip Ratio
Serum Osteocalcin (ng/mL)	Cases	-0.825**	-0.784**	-0.739**
	Controls	-0.933**	-0.887**	-0.868**

** . Correlation is significant at the 0.01 level (2-tailed).

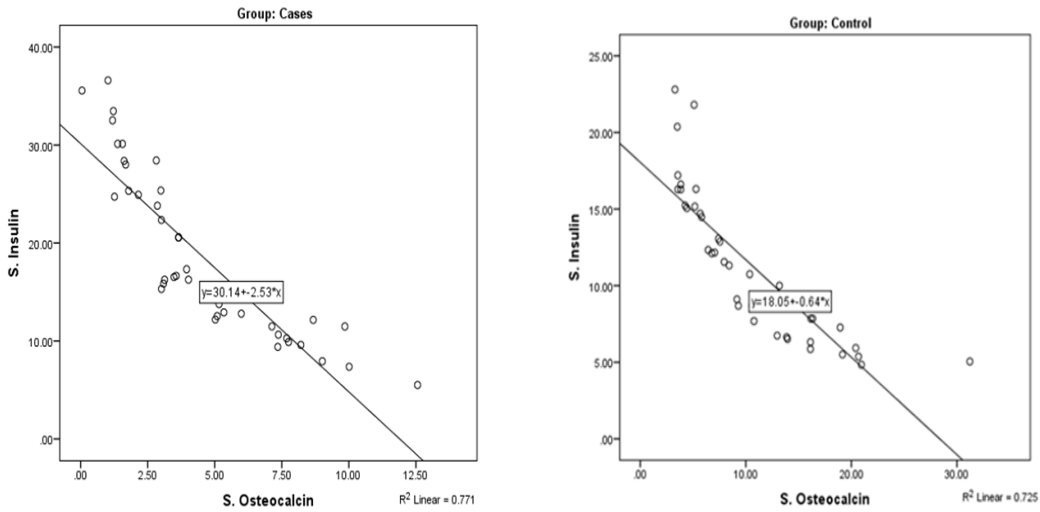
Table 3. Pearson's Correlation of Serum Osteocalcin with Fasting Plasma Glucose, Serum Insulin and HOMA-IR among Cases and Controls.

Parameters	Groups	Fasting Plasma Glucose (mg/dl)	Serum Insulin (µIU/ml)	HOMA IR
Serum Osteocalcin (ng/mL)	Cases	-0.781**	-0.878**	-0.782**
	Controls	-0.892**	-0.851**	-0.846**

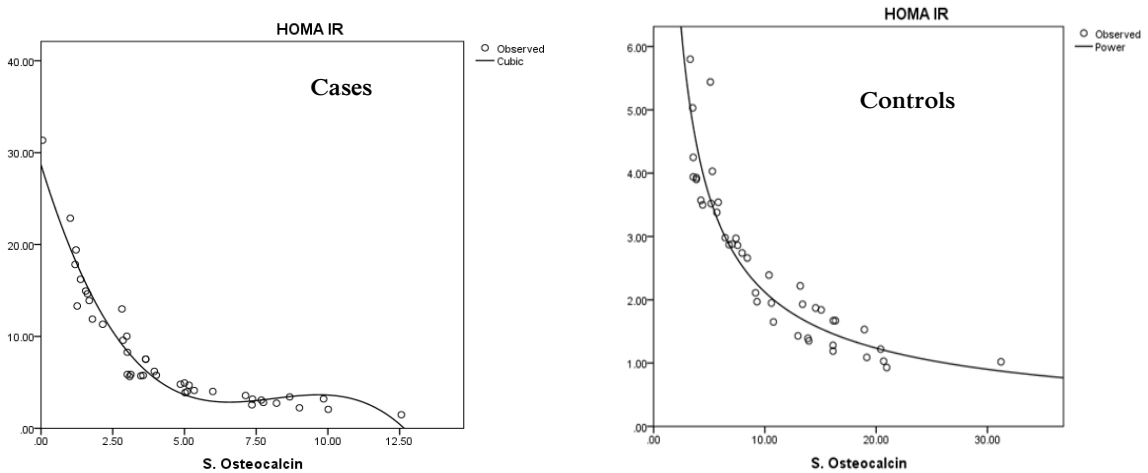
** . Correlation is significant at the 0.01 level (2-tailed).



Graph 1. Shows negative correlation between serum osteocalcin level and fasting plasma glucose among cases and controls.



Graph 2. Shows negative correlation between serum osteocalcin level and serum insulin level among cases and controls.



Graph 3. Shows negative correlation between serum osteocalcin level and homeostatic model of assessment- insulin resistance (HOMA-IR) among cases and controls.

Discussion

Osteocalcin, a protein with 49 amino acid is produced, modified and released from the osteoblasts and acted so far as a classical biomarker for bone turn over but recent animal studies reveal its role in energy metabolism and increasing insulin secretion and sensitivity. The study on osteocalcin started by Lee *et al* in 2007, where the focus was initially on Esp gene which codes for a protein, extracellular osteoarticular protein (Osteoarticular Protein Tyrosine Phosphatase OST-PTP) which expressed in the osteoblasts, sertoli cells and embryonic stem cells. This gene is upregulated during the differentiation of osteoblasts and down regulated during mineralization of Osteoblasts. This Esp gene is responsible for the carboxylation of osteocalcin [14, 17, 23]. Lee *et al* showed that mice lacking extracellular osteoarticular protein tyrosine phosphatase (OST-PTP) protein encoded by Esp gene become hypoglycemic, protected from obesity and Glucose intolerance by down regulating the carboxylated osteocalcin [14].

Our study is about the association of serum osteocalcin levels in type 2 diabetes. Our study is based on the findings from the animal studies. To our knowledge, this is the first time, a study on osteocalcin is being conducted in south India. There was one study published from Jabalpur, Madhya Pradesh but osteocalcin was measured by enzyme linked immunosorbent assay system (ELISA) technique [16]. But in our study, osteocalcin and insulin are measured by highly sensitive chemiluminescence immunoassay (CLIA) technique. Our study established a firm association between serum osteocalcin levels, fasting plasma glucose levels and insulin resistance in south Indian male population.

Animal studies proved that undercarboxylated osteocalcin was modifying glucose metabolism and thereby involved in the regulation of body energy metabolism. It further shows that undercarboxylated osteocalcin (UOC) exerted direct action on the beta cells of the pancreas increasing their mass and increasing the insulin secretion. More than that, undercarboxylated osteocalcin had a cross talk with white adipocytes. It was increasing the concentration of adiponectin and thereby improving the insulin sensitivity [17].

Though we did not determine the estimation of adiponectin and beta cell mass in humans, our study showed an excellent negative correlation between serum osteocalcin and fasting plasma glucose and insulin as stated in other articles [18-19].

Our current study includes 84 South Indian males (42 diabetic people and 42 healthy people). In our study, serum osteocalcin is well correlating with fasting plasma glucose and serum insulin levels in both healthy people and diabetic people that is low osteocalcin is associated with hyperglycemia, increased insulin levels and vice versa in all age groups (31-60 years). We also observed a

significant inverse relationship between serum osteocalcin, waist circumference and body mass index (BMI).

Homeostatic Model of Assessment- Insulin Resistance (HOMA-IR) also shows a strong negative association with serum osteocalcin and insulin resistance both in diabetic subjects and in healthy subjects. Mechanism of action is as stated below, insulin secreted from the pancreatic beta cells act through the insulin receptors (IR) in the osteoblastic cells and mediates the production of osteocalcin. Deletion of this insulin receptors (IR) in these mice model showed decrease in the osteoblast number and reduced osteoclast activity which leads to decreased release of total and undercarboxylated osteocalcin from bone, poor glycemic control, increased fat mass and decrease in bone mineral density [17].

RANKL is a major cytokine responsible for differentiation and activation of osteoclasts. osteoprotegerin, a protein secreted from the osteoblasts acts as a decoy receptor for RANKL. Binding of osteoprotegerin to RANKL inhibits the activation of osteoclasts, thereby it prevents bone resorption. But Insulin signalling in the osteoblastic cells decreases the secretion of osteoprotegerin which causes increase in the activity of osteoclasts. This enhanced osteoclastic activity leads to the extracellular bone matrix acidification and bone resorption. Bone resorption promotes the decarboxylation of osteocalcin which ultimately results in the increased release of undercarboxylated osteocalcin from the bone. All these studies clearly demonstrated the existence of a feedback loop between osteocalcin, insulin, bone resorption and glucose metabolism [17, 24]. This has proved by a study done by Kaji *et al* in 2009 and Yamauchi *et al*, which shows increased fasting plasma glucose levels in osteoporotic women under treatment with drugs that inhibit bone resorption. Inhibiting bone resorption also decreases serum undercarboxylated osteocalcin levels resulting in increased fasting plasma glucose levels [20-21].

Animal studies have even proved that exogenous intravenous administration of recombinant undercarboxylated osteocalcin improved glucose utilization. Hence this study can be conducted in large human population, both as prospective and retrospective cohort. Exogenous undercarboxylated osteocalcin administration and its effects on normalising the glycemic status needs further research using the parameters like undercarboxylated osteocalcin, Fasting plasma glucose levels and insulin levels. This is expected to bring euglycemic status and bring down the complications of diabetes mellitus.

Hence the beneficial effects of circulating undercarboxylated osteocalcin should be identified in future large scale studies.

Conclusion

The present study revealed valuable information about undercarboxylated osteocalcin and glycemic status. We found out that serum undercarboxylated osteocalcin concentration was inversely associated with fasting plasma glucose level, body mass index (BMI), waist circumference (WC), waist to hip ratio (W/H ratio), fasting serum insulin levels and Homeostatic Model of Assessment- Insulin Resistance (HOMA-IR) model in both type 2 diabetic male subjects and healthy male subjects. This further puts up a new foothold in the field of diabetes. This pivotal role of osteocalcin, if proved in future large scale human studies, will present as a boon to the vast population of diabetic patients as a therapeutic modality.

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