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Research Article

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The association between serum Osteocalcin levels and atherosclerosis in Syrian patients with/without Diabetes Mellitus type 2

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ABSTRACT

Patients with diabetes type 2 have a higher risk of atherosclerosis disease. In addition to insulin resistance which results in growth and differentiation failure of osteoblasts, leading to failure in Osteocalcin secretion. This study is carried out to investigate the association of serum Osteocalcin with atherosclerosis in patients with type 2diabetes and in patients with atherosclerosis without diabetes. Serum Osteocalcin was measured by ELIZA method, Fasting blood glucose, LDL, total cholesterol, TG were measured by enzymatic colorimetric method in 65 subjects.(20 atherosclerotic but non diabetic patients;24 diabetic type 2 atherosclrrotic patients and 21 apparently healthy subjects(control group). The mean serum osteocalcin concentration for diabetic type 2 atherosclrrotic patients and atherosclerotic patients without diabetes was 13.483±3.325 ng/ml,20.516±3.481ng/ml, respectively. Oste ocalcin levels were associated inversely with Fasting serum glucose, LDL, TG, Tcho ($P<0.05$). As a Conclusions, Low Serum Osteocalcin levels are significantly associated with atherosclerosis.

Keywords: Osteocalcin, atherosclerosis, diabetes type 2, LDL(low density lipoprotein), TG(triglycerides).

INTRODUCTION

Atherothrombotic cardiovascular disease is the leading cause of death worldwide, despite significant progress in the management of critical risk factors. A major reason for this trend is the ongoing epidemic of type 2 diabetes(1).

The increased risk of cardiovascular disease in patients with diabetes type 2 reflected the emergence of atherosclerotic disease, which is considered an important indicator of coronary heart disease and stroke in older subject(2).

Insulin activates substrate receptor insulin in osteoblasts, then the activation of cellular pathways which are essential for the growth of osteoblasts - differentiation and survival. It was found that when there was resistance to insulin, failure occurs in the growth and differentiation of osteoblasts ,and thus a failure in the secretion of the bone protein one of which is osteocalcin(3).

Osteocalcin, also known (bone gla protein), one of the osteoblast-specific proteins, has several hormonal features and is secreted in the general circulation from osteoblastic cells. This protein is a specific biochemical marker of bone turnover and bone formation, involved in bone mineralization and calcium homeostasis (4,5).

Observational studies suggest an association of circulating OCN with atherosclerosis risk(6). The recently proposed proofs suggested the role of this protein in regulatatin of glucose and lipid metabolism, linking bone metabolism with energy homeostasis(4).

According with these observations, it is plausible to consider serum osteocalcin as a promising candidate for risk assessment and a potential intervention target for atherosclerosis diseases, and can be useful in trying to alleviate the morbidity and mortality in these patients. So, this study aimed to determine serum osteocalcin levels in Syrian atherosclerotic patients with and without diabetes mellitus type 2,in attempt to evaluate the relationship between serum osteocalcin and atherosclerosis in these patients.

Experimental section

The study involved 65 individuals (37men:28 women)recruited from vascular surgery dep.t at Al Assad University Hospital, between June and December 2014.

Patients were divided into the following groups:

- Group 1: (control group):which included 21 apparently healthy individuals mean age ± SD: (57.95±6.48 years),that do not suffer diabetes or atherosclerosis and not taking any medications.
- Group 2: Patients with diabetes mellitus type 2 and atherosclerosis,(24 patients mean age ± SD: (59.1±5.01 years).
- Group3: Patients with atherosclerosis and without diabetes,(20 patients mean age ± SD : (58.75±8.02 years).

All patients underwent ultrasonographic study by color duplex of carotid, lower limbs and upper limbs arteries at the Department of vascular surgery, to evidence any atherosclerosis disease by determining the presence or absence of thickness or plaques in intima-media layers.

Serum samples were obtained between 8-10 AM, from the patient in the fasting state, Blood samples were centrifuged at (4000 rpm, RCF=1789xg)for 10 min at 4°C, to isolate serum. Serum was stored at -20°C for future analyses.

Osteocalcin(bone gla protein) was measured by using ELISA Kit provided by Sunred Company, Shanghai.

Glucose, LDL, TG, Tchol were measured with enzymatic colorimetric method by Hitachi 911 device using kits of Audit Diagnostics Corporation Irish.

We excluded Patients having any thyroid (hypothyroidism and hyperthyroidism) or parathyroid (hyperparathyroidism and hypoparathyroidism) disorders ; the same was done to Patients diagnosed with bone disorders as osteoporosis or Paget's disease and patients with fractures or metastatic bone tumor or Patients treated with glucocorticoids or bisphosphonate or vitamins K and D.

Statistical analysis:

The statistical analysis of data was performed using SSPS and Excel 2010.

Data were expressed as mean ± SD

The data was analyzed using One Way Analysis of variance (ANOVA) followed by Bonferroni testing to compare the results between groups.

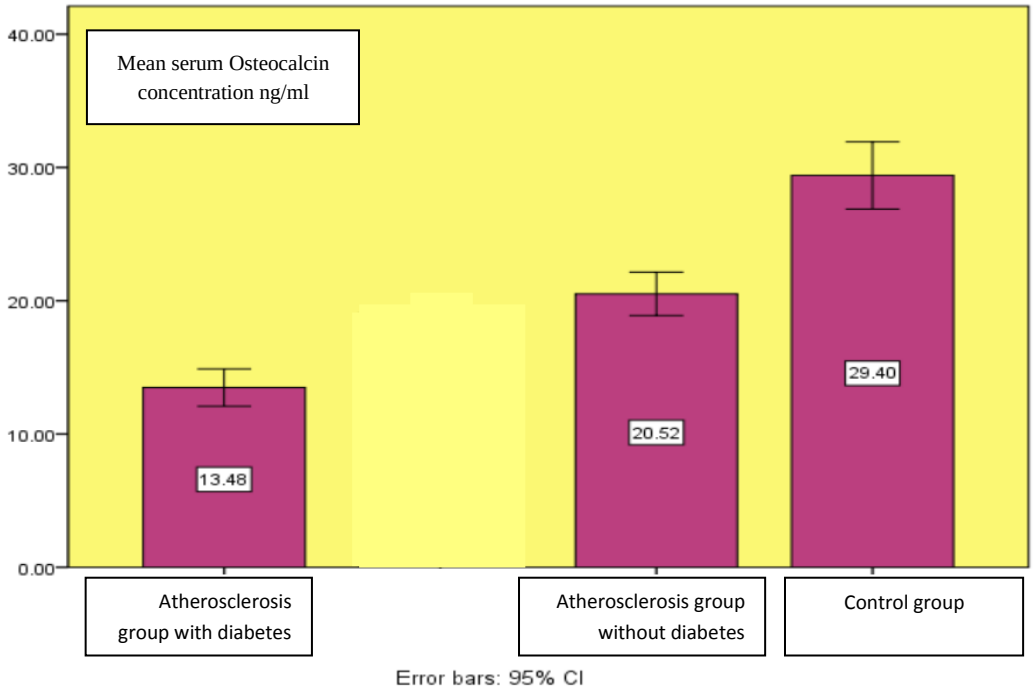
Pearson correlation is used to study the correlation between studies parameters.

$P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

The mean serum Osteocalcin values± SD in the studied groups were as follows:
29.400±5.546 ng/ml in control group (n = 21).
13.483 ± 3.325 ng/ml in atherosclerotic group with Diabetes (n = 24).
20.516±3.481ng/ml in atherosclerotic group without Diabetes(n=20)
By ANOVA test was observed statistically significant difference
($P < 0.001$) between osteocalcin levels in the studied groups.

By Benfferoni test to compare Osteocalcin levels between the studied groups, was also observed a noticeable statistically significant difference(P <0.001) in atherosclerotic group with diabetes and the atherosclerotic group without diabetes compared with the control group(p<0.001).The difference was statistically significant(P <0.001) between the atherosclerotic group with diabetes compared with atherosclerotic group without diabetes (Figure 1).



Figure(1):Mean±SD of Osteocalcin concentration(ng/ml) in the studied groups

The mean serum FBG, LDL, Tchol, TG values± SD in the studied groups are presented in Table (1).

Table(1).Illustrated the mean±SD of Fasting blood glucose, total cholesterol, TG and LDL

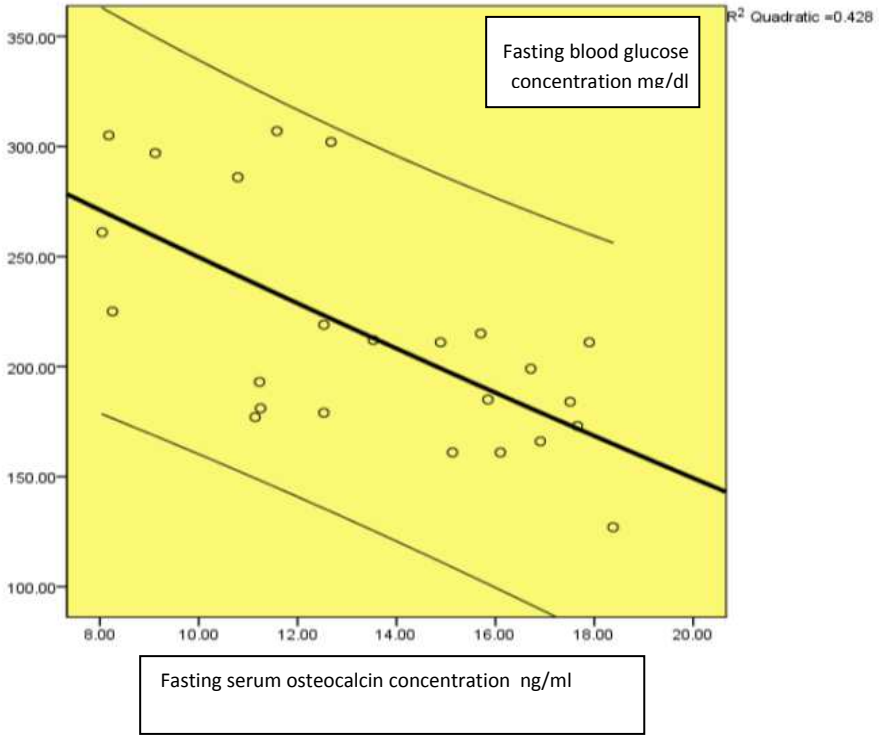
Parameters	Control groups	Atherosclerosis group with diabetes	Atherosclerosis group without diabetes
FBG	93±6.426 mg/dl	214.041±52.081 mg/dl	96.350±8.892 mg/dl
LDL	104.333±10.408 mg/dl	148.041±28.930 mg/dl	120.450±28.115 mg/dl
TG	121.285±38.574 mg/dl	197.625±32.808 mg/dl	141.600±43.795 mg/dl
Tcho	164.523±14.417 mg/dl	218.5±55.752 mg/dl	180.250±32.217mg/dl

There was a significant correlation between osteocalcin and fasting serum glucose, LDL, TG, Tcho in diabetic type 2 atherosclerotic patients (r=-0.654, p=0.001)(Fig.2),(r=-0.604, p=0.002) (Fig.3).

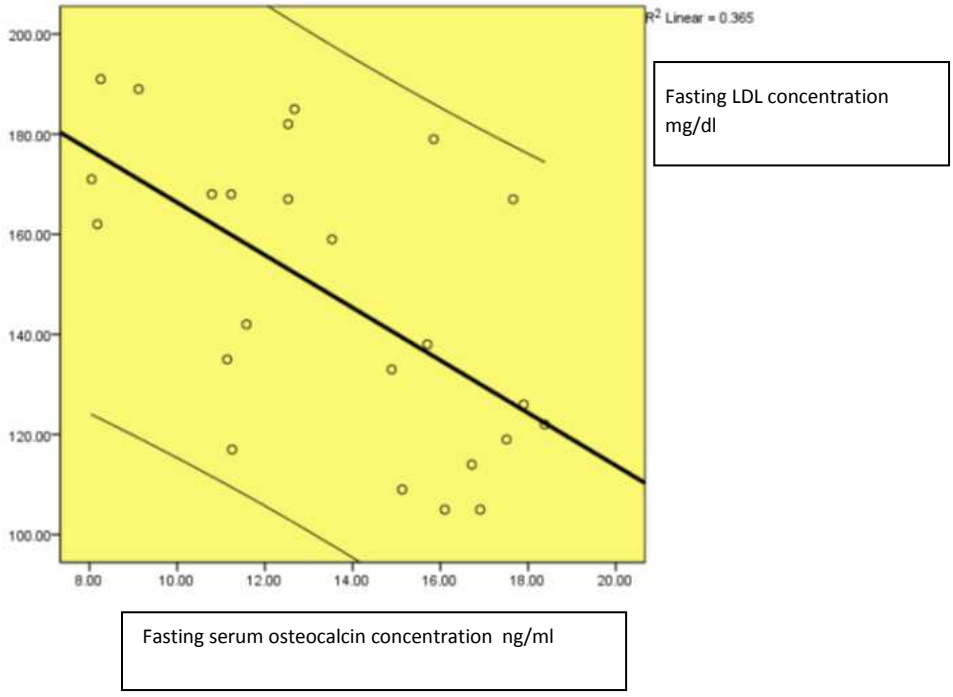
(r=-0.520, p=0.009) (Fig.4), (r=-0.649, p=0.001) (Fig.5) respectively.

There was a significant correlation between osteocalcin LDL, TG and Tcho in atherosclerotic but non diabetic patients.

(r=-0.509, p=0.022) (Fig.6), (r=-0.507, p=0.022) (Fig.7), (r=-0.577, p=0.008) (Fig.8).



Figure(2):The relation of osteocalcin levels and Fasting blood glucose concentration in diabetic type 2 atherosclerotic patients



Figure(3):The relation of osteocalcin levels and Fasting LDL concentration in diabetic type 2 atherosclerotic patients

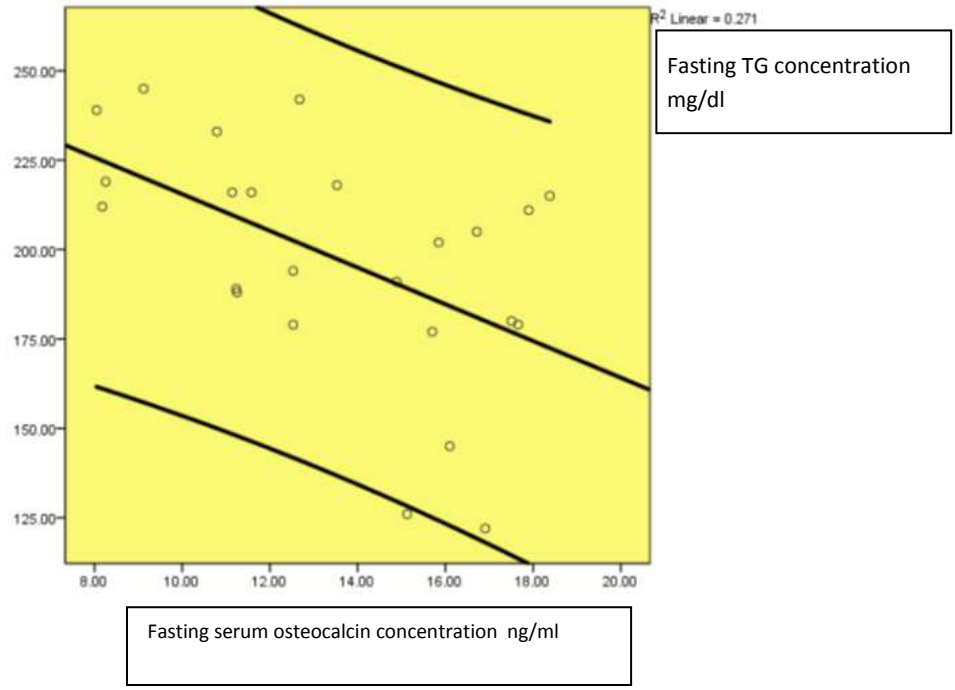


Figure (4): The relation of osteocalcin levels and fasting TG concentration in diabetic type 2 atherosclerotic patients

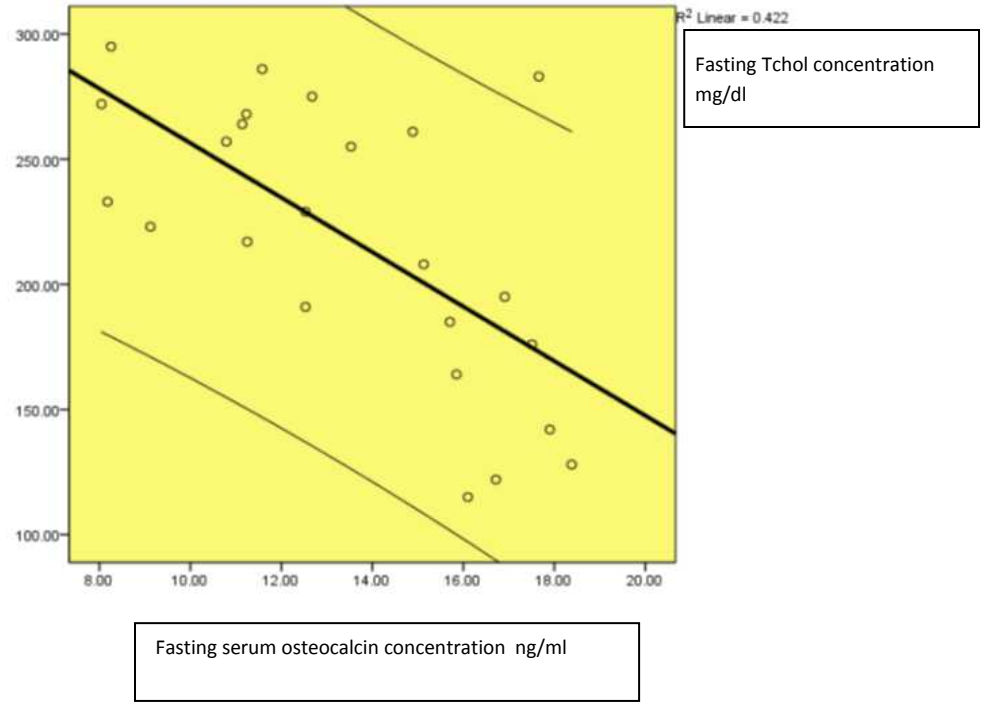
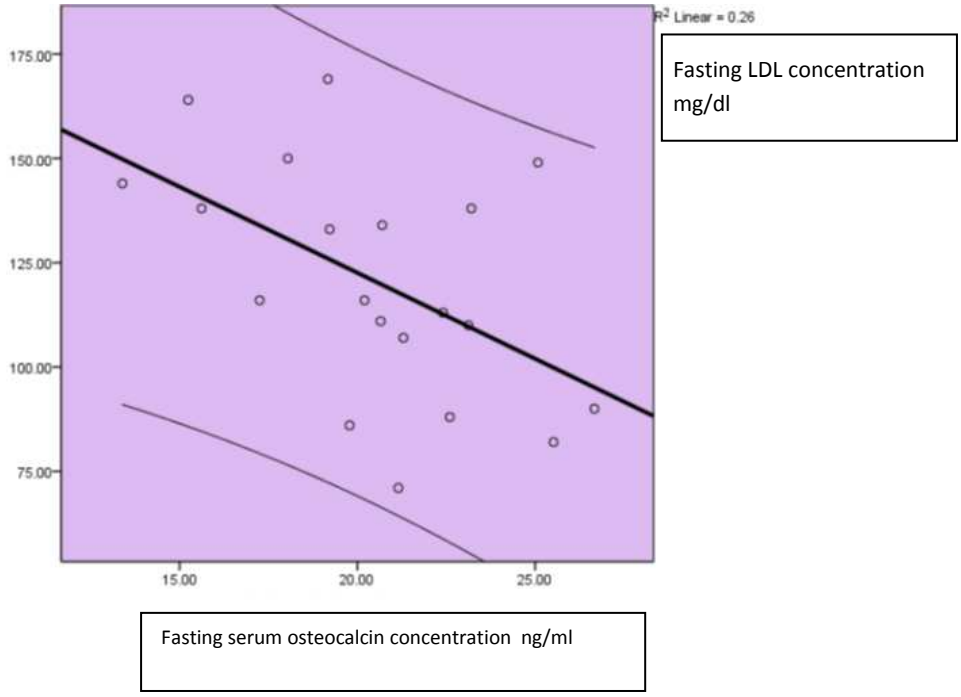


Figure (5): The relation of osteocalcin levels and fasting Tcholin diabetic type 2 atherosclerotic patients



Figure(6):The Relation of osteocalcin levels and fasting LDL concentration in atherosclerotic but non diabetic patients

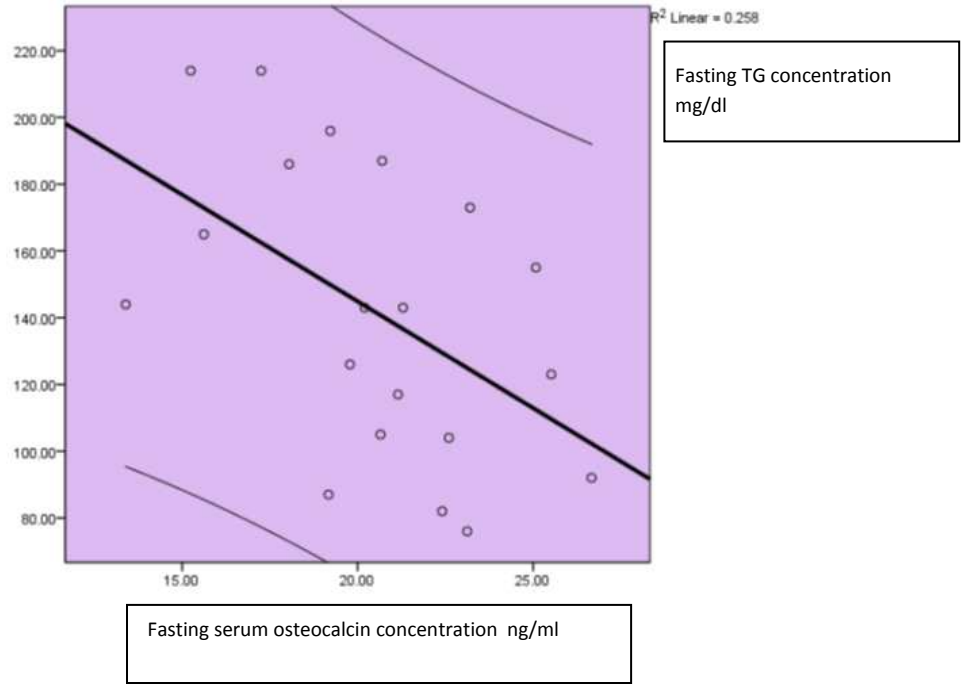


Figure (7): The Relation of osteocalcin levels and fasting TG concentration in atherosclerotic but non diabetic patients

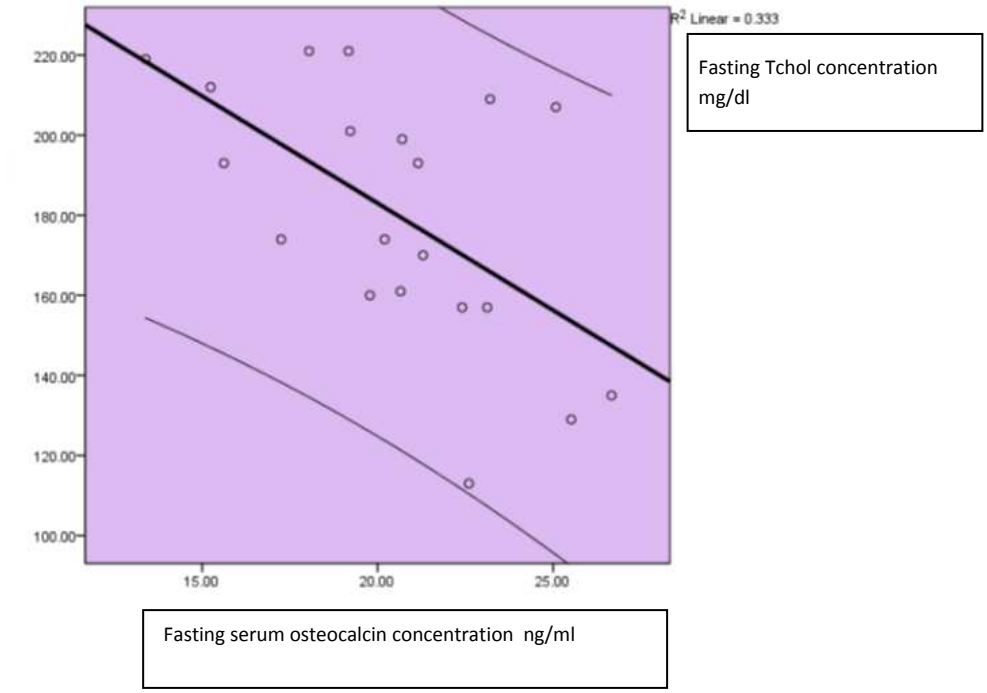


Figure (8): The Relation of osteocalcin levels and fasting Tchoin atherosclerotic but non diabetic patients

Our study showed that serum osteocalcin concentrations were significantly lower ($p<0.001$) in patients with atherosclerosis and diabetes type 2 compared to the control group.

There was a significant reduction($p<0.001$) in Osteocalcin concentration in patients with atherosclerosis without diabetes compared to control group.

Serum osteocalcin concentrations were reduced significant ($p <0.001$) in the patients of atherosclerosis with diabetes compared to patients with atherosclerosis but without diabetes.

P. Pennisi and his colleagues in 2003 study similarly showed that osteocalcin levels decrease in patients with atherosclerotic carotid and peripheral arteries(7).

Our study also revealed a reverse statistically significant between osteocalcin and fasting serum glucose, LDL, TG, Tcho in patients with diabetes and atherosclerosis disease. Patients ($r=-0.654, p=0.001$),($r=-0.604, p=0.002$), ($r=-0.520, p=0.009$,($r=-0.649, p=0.001$) respectively.

Saleem and his colleagues study in 2010 also similarly pointed that Serum OC inversely correlate with fasting glucose(8).

Sheng L and his colleagues in 2013 pointed that Serum OC inversely correlate with FBG, LDL,TG, Tcho(2).

Yeap and his colleagues in 2010 showed that Serum OC inversely correlate with TG, FBG.(9)

and the correlation between serum osteocalcin and fasting LDL, TG, Tchol in patients with atherosclerosis without diabetes was reverse and statistically significant in atherosclerotic but non diabetic patients ($r=-0.509, p=0.022$), ($r=-0.507, p=0.022$), ($r=-0.577, p=0.008$) respectively.

Osteoblastic differentiation of pre-osteoblasts from bone is inhibited by minimally oxidised LDL, whereas oxidised lipids enhance differentiation of osteoblast-like cells from the artery wall, ultimately inducing vascular mineralisation and calcification(7).

There is evidence to show the influence of bone proteins on cardiovascular disease. During atherogenesis, the bone matrix proteins including osteocalcin, may have a regulatory role in the atherosclerotic calcification process(10).

Recent evidences suggest that osteoblast-like cells are present in the vasculature and capable of calcifying vascular cells(2).These procalcific processes are counterbalanced by local and circulating inhibitors of calcification suggesting that decreased expression or activity of these mediators (Osteocalcin) may also contribute to pathological cardiovascular calcification. Vitamin K-dependent protein such as (osteocalcin), prevents calcification by inhibiting bone morphogenetic protein (BMP) signaling which enhances calcification(11).

CONCLUSION

Our study indicated that low serum osteocalcin levels were significantly associated with atherosclerosis in patients with or without type 2 diabetes.

This may reflect the role of osteocalcin as a circulating endocrine factor, which regulates glucose metabolism and there by vascular risk implicating that osteocalcin might be a new therapeutic target for the treatment of atherosclerosis disorders.

Prospective studies are needed to assess the role of serum osteocalcin as an early indicator for developing atherosclerosis.

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