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Serum osteocalcin levels in metabolic syndrome

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Abstract

Introduction: Osteocalcin has recently been proposed to play an important role in modulating glucose, lipid and energy metabolism. This study was undertaken to determine serum osteocalcin levels in metabolic syndrome and to study its association with measures of insulin resistance and cardiovascular risk factors.

Methods: The study included 45 cases diagnosed as having metabolic syndrome defined by NCEP ATPIII criteria and 45 normal healthy subjects as controls. A fasting serum sample was collected from each subject and assayed for Fasting Blood Sugar, Lipid Profile, Insulin and Osteocalcin. Body Mass index (BMI), Waist hip ratio (WHR) and Homeostatic model assessment (HOMA) were calculated.

Results: The mean serum Osteocalcin (ng/mL) levels were 4.56±1.75 and 10.302±1.96 in cases and controls respectively ($p<0.001$). In cases, Hyperglycemia and Hypertriglyceridemia were the most prevalent risk factors. The cases had a statistically significant negative correlation between osteocalcin and BMIand Triglyceride and a statistically significant positive correlation between osteocalcin and High Density Lipoprotein (HDL)($r = +0.964$, $p< 0.001$). Metabolic syndrome subjects had lower serum osteocalcin levels. Greater the number of risk factors present, the lower was the osteocalcin level. The strongest positive association was found between osteocalcin and HDL. Osteocalcin was negatively associated with HOMA, FBS, BMI and triglyceride.

Conclusions: These findings suggest that osteocalcin may have an essential role in modulating glucose and energy metabolism and in the pathophysiology of metabolic syndrome. Further studies may determine the utility of osteocalcin for cardiovascular risk prediction and strategies to increase osteocalcin levels, thus efficiently optimizing glucose, lipid and energy utilization.

Keywords: Osteocalcin; Metabolic syndrome; Body Mass Index; HOMA; Insulin resistance

Introduction

Osteocalcin, a 49 amino acid non-collagenous protein produced by osteoblasts of the bone and stored in the hydroxyapatite matrix, is found in low concentrations in the circulation. Osteocalcin promotes osteoblastic differentiation, osteocyte maturation resulting in increased bone mineral density and bone formation rate[1].

A large number of studies have highlighted the role of osteocalcin as a hormone, on energy metabolism and cardiovascular system[2-6]. Animal and human studies have shown that serum osteocalcin levels are inversely associated with blood glucose level and body fat mass, directly correlated with insulin secretion, improved insulin sensitivity and adiponectin levels[2-6]. Low serum osteocalcin level has also been linked atherosclerosis and adverse cardiovascular changes like increased carotid intima-media thickness, coronary and carotid atherosclerosis[4].

Metabolic Syndrome is the clustering of interrelated modifiable cardiovascular risk factors that includes central adiposity, hypertension, hyperglycemia, hypertriglyceridemia with low high density lipoprotein cholesterol levels, hypercoagulability, insulin resistance and inflammation. Metabolic syndrome is strongly predicts the long term risk of diabetes and atherosclerotic cardiovascular disease[7]. Increasing prevalence of metabolic syndrome can be attributed to changes in nutrition, lifestyle and socioeconomic status, rural-to-urban migration, increasing obesity and sedentary lifestyles[7]. Prevalence of metabolic syndrome in an Indian population was 33.5% (24.9% in males, 43.2% in females), according to a study conducted in urban Orissa on 1178 subjects and 22.3% (25% in males and 22% in females) in an adult population in rural Karnataka[8,9].

According to the updated present AHA/NHLBI statement (Modified NCEP ATPIII criteria), presence of any three of the following five modifiable cardiovascular risk factors would make a clinical diagnosis of metabolic syndrome 1) Elevated waist circumference (WC) (with ethnicity specific values for South Asians) ≥90 cm in men and ≥80 cm in women, 2) Elevated serum triglycerides (TG) ≥150 mg/dL, 3) Reduced serum high density lipoprotein cholesterol(HDL)<40 mg/dL in men and <50 mg/dL in women, 4) Elevated blood pressure ≥130 mm Hg systolic blood pressure (SBP) and/or ≥85 mm Hg diastolic blood pressure (DBP) or on antihypertensive drug treatment in a patient with a history of hypertension, 5) Elevated fasting glucose (FBS) ≥100 mg/dL or on drug treatment for elevated glucose[10-12].

Studies exploring the association of serum osteocalcin levels and metabolic factors in normal

healthy persons and in subjects of metabolic syndrome have had contrasting conclusions[4-6,13-15].

This study was undertaken to determine serum osteocalcin levels in subjects of metabolic syndrome and to study the association between serum osteocalcin levels with measures of insulin resistance and metabolic factors in subjects of metabolic syndrome and in normal healthy persons.

Materials and Methods

A cross sectional case control study was undertaken after obtaining approval from the institutional ethics committee. Males and pre-menopausal females in the age group 20-50 years who satisfied the inclusion criteria were taken as study subjects. Group 1 or cases had 45subjects attending the medicine out-patient department (OPD) at M S Ramaiah Medical College and Hospitals, diagnosed as having metabolic syndrome and Group 2 or Controls had 45 normal healthy individuals attending the same OPD for a routine health check-up. Subjects were diagnosed as having metabolic syndrome, according to the updated present AHA/NHLBI statement, by the presence of any three of the five criteria detailed above. Healthy individuals, who did not have even a single criterion of the metabolic syndrome, were included as controls for the study. The exclusion criteria were as follows- Pregnant females, lactating mothers, postmenopausal women, subjects with an abnormal Prothrombin time, history of thyroid dysfunctions, heart diseases, fractures (<6 months ago), hepatic disease, renal disease, acute illnesses, infections, patients admitted for surgery, patients on bisphosphonates, calcium supplements, steroids, warfarin, insulin, thiazides, diazoxides, pentamidine, phenytoin, α interferons, history of intestinal malabsorption disorder, vitamin D insufficiency, Vitamin K insufficiency, osteoporosis or malignancy. After a written informed consent was taken, a detailed history, physical examination and anthropometric measurements were done for each subject as per standard protocols[16]. A blood sample was collected in a plain tube from each study subject, after an overnight fast of 10-12 hours, with due aseptic precautions in the phlebotomy section of the diagnostic laboratory. The blood samples were allowed to clot and were centrifuged at 4000 rpm for 8-10 minutes. After separation, the following lab investigations were done on the samples on Roche Cobas 6000 c501, fully automated analyzer at the diagnostic laboratory-Fasting blood sugar (FBS) by Hexokinase method (Roche Diagnostics, Mannheim), Serum total cholesterol (TC)by enzymatic colorimetric method using cholesterol oxidase (Roche Diagnostics, Mannheim), Serum triglyceride (TG) by enzymatic colorimetric method using glycerol phosphate oxidase (Roche Diagnostics, Mannheim), Serum high density lipoprotein cholesterol (HDL) enzymatic colorimetric method using cholesterol oxidase and esterase (Roche Diagnostics, Mannheim), Low density lipoprotein cholesterol (LDL) estimated by direct Assay based on precipitation method (Roche Diagnostics, Mannheim). All of the assays were routinely monitored by participation in external quality-control programs and using assayed chemistry and assayed immunoassay plus controls (Bio-Rad Lab, Hercules, CA, USA). After the above investigations, the serum samples were aliquoted, labeled and stored at -80°C in the diagnostic laboratory and were analyzed for Serum Insulin and Serum Osteocalcin using ELISA kits manufactured by BioVendor Laboratories Limited, USA and Quidel Corporation, USA respectively. The inter and intra assay coefficients of variation all analytes remained within 5% during the test period. Homeostatic model assessment (HOMA) was used to quantify insulin resistance HOMA-IR = (glucose X insulin)/22.5(Insulin concentration in μU/L and glucose in mmol/L)[17]. Fasting insulin levels ≥12mU/l and HOMA ≥2.6 were considered as indicative of the presence insulin resistance in a study subject[18]. Anthropometric measurements were used to calculate Body mass index (BMI) using the formula: weight (kg)/height (m^2). The cut offs for Body Mass Index (BMI) in Indian subjects has been given by Misra. A et al and is as follows- BMI of 18 – 22.9 falls into normal category, BMI of 21–24.9 as pre obese, BMI of 25–29.9 as obesity (grade 1) and BMI of ≥30 as obesity(grade 2)[12]. The study subjects were also grouped as normal/non-obese and centrally obese on the basis of waist to hip ratio (WHR), according to Misra.A et al, wherein in females the waist to hip ratio <0.80 is normal, and in males <0.88 is normal[12].

Statistical Analysis: Data was entered in Microsoft excel and analysed using SPSS (Statistical Package for Social Science, Ver.10.0.5) package. Normality of data was tested using Shapiro-Wilk test. Proportions were compared using Chi-square (χ^2) test of significance. Proportion of Cases belonging to specific group of parameter or having a particular problem was expressed in absolute number and percentage**.** The student 't' test was used to determine whether there was a statistical difference between groups in the parameters measured if the data is normal. In all the above test "p" value of less than 0.05 was accepted as indicating statistical significance.

Results

The present study was done with 90 study subjects. The mean ± SD of age in years in cases was 43.36±5.77 and 42.22±7.65 in controls. Amongst controls, 48.9% were males and 51.1% were females, in cases, 53.3% were males and 46.7% were females without a statistically significant gender difference. The anthropometric, clinical and biochemical characteristics of the study groups are given in Table 1. There was a statistically significant difference between the two

study groups in BMI, WHR, WC, SBP, DBP, FBS, Serum TG, serum HDL, Fasting Serum Insulin, serum Osteocalcin and HOMA IR. The mean serum Osteocalcin (ng/mL) levels were 4.56±1.75 and 10.302±1.96 in cases and controls respectively.

Based on BMR cut-offs mentioned earlier, amongst the cases, 1(2.2%) was pre obese, 30 subjects (66.7%) had grade 1obesity and 14(31.1%) had grade 2 obesity. Amongst the controls, 16(35.6%) subjects had normal BMI and 29(64.4%) subjects were pre-obese or at risk ($p<0.001$). Based on waist hip ratio cut-offs, in male cases 6 (25%) were normal (waist hip ratio <0.88) and 18(75%) were centrally obese (waist hip ratio >0.88). All female (n=21) subjects amongst the cases were centrally obese (waist hip ratio >0.80). All controls had a normal waist to hip ratio. The difference in waist hip ratio was statistically significant between cases and controls ($p<0.001$).

The study subjects were grouped as shown in table 2, based on the fasting serum insulin values into normal, non-insulin resistant group (fasting serum insulin <12 µIU/L) and insulin resistant group(fasting serum insulin ≥12 µIU/L). 36(80%) cases had serum insulin ≥12 µIU/L showing a statistically significant increase in serum insulin levels in cases as compared to controls. 2 subjects (4.44%) amongst the controls had fasting serum insulin values ≥12 µIU/L. HOMA-IR ≥2.6 is considered as insulin resistance and <2.6 is normal was used, amongst cases 97.8% had HOMA-IR ≥2.6 and all controls had HOMA-IR <2.6. Amongst male cases 1 (4.2%) was normal (HOMA-IR<2.6) and 23(95.8%) were insulin resistant (HOMA-IR>2.6) ($p<0.001$). All female subjects [21(100%)] amongst cases, were insulin resistant (HOMA-IR>2.6) ($p<0.001$).

Table 3 shows the prevalence of the components of metabolic syndrome amongst the cases. The most prevalent component of metabolic syndrome present in the cases were Hyperglycemia (FBS ≥100 mg/dL) and Hypertriglyceridemia (TG ≥150 mg/dL). These components were present together in 93.3% of the cases followed by the other components. Amongst male subjects of MS, the most prevalent components were Hyperglycemia (Fasting Blood Sugar ≥100 mg/dL) and Hypertension (≥130 mm Hg systolic blood pressure and/or ≥85 mm Hg diastolic blood pressure), present in 95.8% of all male cases. All the female subjects of metabolic syndrome had Hypertriglyceridemia (TG ≥150 mg/dL), Decreased HDL (<50 mg/dL in women) and Central Obesity (WC ≥80 cm in women).

It was found that 51.1% (n=23) of the cases had all the 5 components of the metabolic syndrome, 40% (n=18) had 4 criteria and 8.8% (n=4) had 3 components present. A comparison of mean ± SD values of anthropometric and Biochemical parameters in subjects with 3 or 4 criteria and subjects with 5 criteria is shown in table 4. A moderately significant statistical difference was seen between the two groups in terms of BMI, WC, WHR, FBS, TG, fasting serum insulin and HOMA-IR ($p<0.05$). A strongly significant statistical difference was seen between the two groups in terms of HDL and serum osteocalcin ($p<0.001$).

Table 5 shows the Pearson correlation between osteocalcin and other study variables. There was a statistically significant negative correlation between osteocalcin (ng/ml) and BMI($r = -0.824$, $p <0.001$) (Fig. 1) and Triglyceride($r = -0.684$, $p <0.001$) in only cases and not in controls. There was a statistically significant positive correlation between HDL and osteocalcin($r = +0.964$, $p< 0.001$) only in the cases. Both in cases and controls, when osteocalcin was compared with FBS, WC, WHR, serum insulin and HOMA-IR, a statistically significant negative correlation was found ($p<0.001$).

Discussion

The present study was undertaken to determine the levels of osteocalcin in subjects of metabolic syndrome and to study its association with the components of metabolic syndrome.

Recent studies suggest an endocrine role for osteocalcin beyond its erstwhile known functions of increasing bone mineral density and bone formation rate[1-6]. These studies focus on the role of osteocalcin on energy metabolism and cardiovascular system[1-6].

The present study was done with a total of 90 subjects in the age group of 20-50 years with 45 healthy controls and 45 subjects with ≥ 3 criteria of MS defined as per NCEP-ATP III[10-12]. Prevalence of metabolic syndrome was found to be maximum in the age group 41-50 years (73.3%), followed by the age group 31-40 years(24.4%). There was no statistically significant difference in the prevalence of metabolic syndrome between males and females in the present study (53.3% of cases were males and 46.7% cases were females), this finding being similar to that of another study done in a rural population of Karnataka[9]. Other studies done in India, have shown increased prevalence of MS was in males[19], or females[8].

On comparing anthropometric, clinical and biochemical characteristics of the study groups, there was a statistically significant difference between the two study groups in terms of BMI, WHR, WC, SBP, DBP, FBS, serum TG, serum HDL, Fasting Serum Insulin, serum Osteocalcin and HOMA IR. This is consistent with the findings of other studies[8,9].

In the present study, the mean serum Osteocalcin (ng/mL) levels were 4.56±1.75 and 10.302±1.96 in cases and controls respectively. This is similar to osteocalcin levels detected in Indian subjects in a study done in newly diagnosed diabetics (4.06±1.97ng/mL) and healthy controls (9.62±3.2 ng/mL) and in other studies[4,6,20].

Eight percent of the subjects with metabolic syndrome were found to be insulin resistant, with Fasting serum insulin ≥12 µIU/L and HOMA-IR ≥2.6. The most prevalent component of metabolic syndrome

present in the cases was Hyperglycemia (FBS≥100 mg/dL) and Hypertriglyceridemia (TG ≥150 mg/dL). All the 5 components of the metabolic syndrome were present together in 51.1% of the cases while 48.8% had 3or 4 components present. Osteocalcin levels were higher in the group with 3 or 4 metabolic components than in those with 5 components, indicating lower osteocalcin levels in the presence of more metabolic components. This is in line with the findings of other studies done in Asians, Blacks and non-Hispanic whites[21,22]. There was a significant negative association between osteocalcin and FBS, WC, WHR, serum insulin and HOMA-IR in all the study subjects. In subjects with metabolic syndrome, there was a statistically significant negative correlation on comparing osteocalcin with BMI and Triglyceride and statistically significant positive correlation on comparing osteocalcin with HDL. This indicates that serum osteocalcin levels are significantly associated with insulin resistance, BMI and other individual components of Metabolic Syndrome. The strongest negative and positive relationship was found on comparing osteocalcin with BMI and osteocalcin with HDL respectively in metabolic syndrome subjects. Similar results were seen in other studies[3,21,22]. In a study done by Fernández-Real JM et al., osteocalcin was inversely related to metabolic markers like BMI, WC, FBS, insulin and triglycerides, and directly related to adiponectin. In the same study, the more the severity of the metabolic syndrome the lower were the levels of osteocalcin found, independent of the glycemic changes[3]. Chin KY et al., demonstrated that serum osteocalcin level was significantly associated with BMI, Body Fat Mass, WC and serum HDL cholesterol level[23]. Though the definitive mechanism is not known, Chin KY et al., suggested that the positive association between osteocalcin and HDL is mediated by adiponectin. Osteocalcin and adiponectin levels have a direct association. Adiponectin is positively related to HDL cholesterol level as it decreases the catabolic rate of ApoA-I (major apolipoprotein particle of HDL)[23].

Osteocalcin has since long been used as a serum marker for bone formation and osteoblast number. Studies on osteocalcin knockout mice, have revealed that osteocalcin regulates body energy metabolism by modulating lipid and glucose storage and expenditure. Such mice were hyperglycemic, had reduced insulin secretion and sensitivity, decreased islet size, number and increased adipocyte mass and number[2]. A number of invivo and invitro experiments have found that osteocalcin is not only secreted by osteoblasts but also from adipocytes and megakaryocytes and it directly and/or indirectly promotes insulin and adiponectin secretion and improved muscle and adipocyte sensitivity to insulin. Osteocalcin also induces the expression of genes regulating energy expenditure and adiponectin secretion. Increased production of reactive oxygen species and subdued osteoblast functioning is caused by insulin resistance and hyperglycemia of type 2 diabetes leading to decreased production and secretion of osteocalcin. Hyperglycemia can also be toxic to the osteoblasts directly. Studies have proposed the existence of a feedback loop wherein osteocalcin enhances pancreatic beta cell proliferation, insulin secretion and sensitivity[24-27].

The study has a few drawbacks- subjects comprised people who visited a tertiary care hospital for a routine health check-up or for follow up of a metabolic complication and thus might not be representative of the general population. This cross sectional study could not determine a causal relationship between osteocalcin and Metabolic Syndrome. Moreover carboxylated and undercarboxylated forms of osteocalcin were not measured. Nevertheless, only a few studies in India have studied the association of osteocalcin and metabolic risk factors.

The present study detected lower serum osteocalcin levels in subjects of metabolic syndrome compared to normal healthy controls. Lower serum osteocalcin was associated with increased FBS, serum fasting insulin, insulin resistance and decreased serum HDL levels. These findings suggest that osteocalcin may have an extensive role in modulating glucose and energy metabolism and in the pathophysiology of metabolic syndrome. Further studies might be valuable in determining strategies that pursue the use of osteocalcin for cardiovascular risk prediction and those that could increase osteocalcin levels, leading to a decrease in the prevalence of metabolic syndrome and non-communicable diseases in India.

Table 1: Anthropometric, Clinical and biochemical parameters in the study subjects

	Cases n=45 Mean± SD	**Controls n=45 Mean± SD**	**p value**
Body Mass Index(BMI)	29.02±3.099	23.03±1.464	<0.001**
Waist to Hip Ratio (WHR)	0.895±0.061	0.78±0.035	<0.001**
Waist Circumference (WC)(cms)	90.73±4.82	77.33±3.275	<0.001**
Systolic Blood Pressure (SBP)(mm/Hg)	134.4±9.07	120.13±6.33	<0.001**
Diastolic Blood Pressure (DBP) (mm/Hg)	87.82±5.14	78.98±4.48	<0.001**

Fasting Blood Sugar (FBS) (mg/dl)	161.8 ±38.65	87.53±8.10	<0.001**
Total Cholesterol (mg/dl)	195.93±51.91	169.93±28.7	0.004
Triglyceride (TG) (mg/dl)	177.93±41.99	92.18±28.39	<0.001**
High Density Lipoprotein (HDL) (mg/dl)	31.56±10.4	50.49±8.39	<0.001**
S. Creatinine (mg/dl)	0.8862 ±0.20	0.7944±0.11	0.011
S. Alanine Transaminase (U/L)	32.36±9.038	21.11±6.17	<0.001**
Fasting Serum Insulin (µ IU/L)	13.22 ±1.66	4.81±2.33	<0.001**
Osteocalcin (ng/mL)	4.56±1.75	10.302±1.96	<0.001**
HOMA IR	5.26±2.0	1.05±0.54	<0.001**

[Suggestive significance (p value: 0.05<p<0.10), *Moderately significant (p value: 0.01 to ≤ 0.05), **Strongly significant (p value: ≤0.01) Student t test]

Table 2: Fasting serum insulin levels and HOMA-IR in study subjects

	Cases n=45 n(%)	**Controls n=45 n(%)**	**$\chi 2$ value**	**'p' value**
Fasting serum insulin (<12 µIU/L)	9(20%)	43(95.55%)	68.824	<0.001
Fasting serum insulin ≥12 µIU/L (Insulin Resistance)	36(80%)	2(4.4%)		
HOMA-IR <2.6 (normal)	9(20%)	45(100%)	86.087	<0.001
HOMA-IR ≥2.6 (insulin resistance)	36(80%)	0(0%)		

Table 3: Distribution of cases under the different criteria of Metabolic Syndrome

Components	**Cases n=45 [%of all cases]**	**Males [n=24] [% of male subjects]**	**Females [n=21] [% of female subjects]**
Hyperglycemia (Fasting Blood Sugar ≥100 mg/dL)	42 (93.3)	23(95.8)	19(90.44)
Hypertriglyceridemia (Serum Triglyceride ≥150 mg/dL)	42 (93.3)	21(87.5)	21 (100)
Decreased HDL (<40 mg/dL in men and <50 mg/dL in women)	38 (84.4)	17 (70.8)	21(100)
Hypertension (≥130 mm Hg systolic blood pressure and/or ≥85 mm Hg diastolic blood pressure)	38(84.4)	23(95.8)	15 (71.4)
Central Obesity (Waist circumference ≥90 cm in men and ≥80 cm in women)	37 (82.2)	16 (66.5)	21(100)

Table 4: Comparison of Anthropometric and Biochemical parameters amongst cases

	Cases with 3-4 criteria of MS n=22 Mean± SD	**Cases with 5 criteria of MS n=23 Mean± SD**	**p value**
Body Mass Index	28.09±2.9	29.9±2.8	<0.05*
Waist Circumference(cms)	89.22±3.6	92.17±5.3	<0.05*
Waist to Hip Ratio	0.87±0.05	0.91±0.06	0.05*
Systolic Blood Pressure (mm Hg)	136.36±10.2	132.52±7.3	0.16
Diastolic Blood Pressure (mm Hg)	88.72±5.3	86.95±4.7	0.25
Fasting Blood Sugar (mg/dl)	147.04±42.07	175.91±28.6	<0.05*
S.Triglyceride (mg/dl)	164±46.5	191±31.8	<0.05*
S. High Density Lipoprotein (mg/dl)	36±9.6	27.3±9.2	<0.001**
Fasting Serum Insulin (µ IU/L)	12.62±1.7	13.79±1.3	<0.05*
HOMA IR	4.49±1.2	5.98±1.4	<0.05*
Osteocalcin (ng/mL)	5.30±1.7	3.8±1.4	<0.001**

[Suggestive significance (p value: 0.05<P<0.10), *Moderately significant (p value: 0.01<P ≤ 0.05), **Strongly significant (p value: P≤0.001) Student t test]

Table 5: Correlations between Osteocalcin (ng/mL) vs parameter in study subjects

	Cases		**Controls**	
	R	**'p' value**	**r**	**'p' value**
Age (years)	0.170	0.264	.0102	0.505
Body Mass Index	-0.824	<0.001**	-0.179	0.239
Waist Circumference in cms	-0.571	<0.001**	-0.386	0.009*
Waist to Hip Ratio	-0.438	0.003*	-0.508	<0.001**
Systolic Blood Pressure in mm/Hg	0.249	0.099	0.008	0.960
Diastolic Blood Pressure in mm/Hg	0.005	0.973	-0.017	0.910
Fasting Blood Sugar in mg/dl	-0.900	<0.001**	-0.859	<0.001**
Total Cholesterol in mg/dl	-0.309	0.039	-0.242	0.109
Triglyceride in mg/dl	-0.684	<0.001**	0.032	0.833
High Density Lipoprotein in mg/dl	0.964	<0.001**	-0.099	0.517
Serum Insulin in µ IU/L	-0.838	<0.001**	-0.706	<0.001**
HOMA IR	-0.903	<0.001**	-0.735	<0.001**

[r=Correlation Coefficient, Suggestive significance (p value: 0.05<P<0.10), *Moderately significant (p value: 0.01<P ≤ 0.05), **Strongly significant (p value: P≤0.001)]

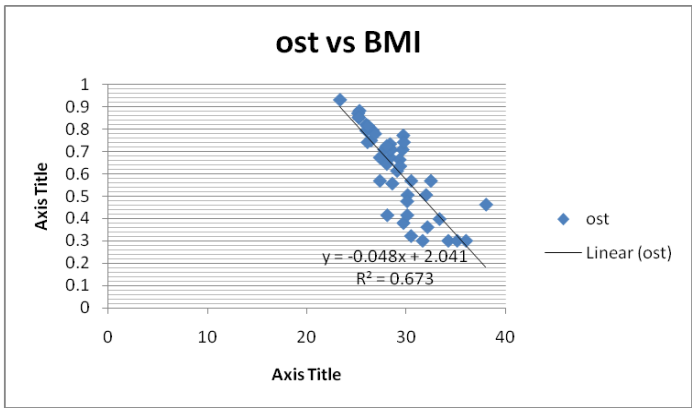

Fig. 1: Pearson correlation between osteocalcin and BMI

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