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STUDY OF SERUM OSTEOCALCIN LEVELS IN METABOLIC SYNDROME

Biochemistry

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ABSTRACT

Background: 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.

KEYWORDS

Osteocalcin; Metabolic syndrome; 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), .

statement 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.

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

Studsy site- Darbhanga medical college and hospital darbhanga Laheriasarai bihar, 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 DMCH Darbhanga, 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.

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. 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 (grade1) 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].

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 twostudy 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).

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

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(%)	X^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]

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