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Original Article

Serum osteocalcin in subjects with metabolic syndrome and central obesity

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ABSTRACT

Aims: The aim of this study was to determine if osteocalcin is related to adiposity and hyperglycaemia in metabolic syndrome irrespective of the presence of diabetes mellitus.
Materials and Methods: This was a cross sectional study of 90 patients (59 men and 31 women) with metabolic syndrome as defined by the International Diabetes Federation criteria. Based on medical history 50 out of 90 patients had a diabetes. Anthropometric data were collected and blood taken for measurement of osteocalcin, fasting lipids, fasting glucose and insulin resistance (using homeostatic model assessment index, HOMA-IR).
Results: Osteocalcin correlated negatively with fasting glucose ($r = -0.366$, $p < 0.001$) and HOMA-IR ($r = -0.305$, $p < 0.05$) but not with waist circumference ($r = 0.079$), body mass index ($r = 0.028$), total cholesterol ($r = 0.061$) or triglycerides ($r = 0.009$). Diabetics had higher HOMA-IR ($p < 0.01$) and lower osteocalcin levels ($p < 0.01$) than non-diabetics. Among diabetics, osteocalcin correlated with glucose only ($r = -0.341$, $p = 0.015$). In non-diabetics, osteocalcin correlated with HOMA-IR ($r = -0.359$, $p = 0.023$) via insulin ($r = -0.402$, $p = 0.010$). Patients with impaired fasting glucose levels (5.6–6.9 mmol/L) had the same HOMA-IR as diabetics ($p = 0.076$) but not low osteocalcin ($p = 0.025$).
Conclusions: In this cross-sectional study of subjects with metabolic syndrome and central obesity, low osteocalcin was associated with diabetes but not adiposity.

1. Background

Osteocalcin is a non-collagenous protein produced by osteoblast cells and previously thought only to function in bone and mineral metabolism. However, animal studies showed that osteocalcin knock-out mice had low insulin secretion, insulin resistance, glucose intolerance and increased fat mass [1,2]. Interestingly, these pathophysiological features are similar to those of diabetes mellitus and metabolic syndrome.

Several studies have reported low osteocalcin levels in patients with diabetes and metabolic syndrome. Whilst the correlations between osteocalcin and parameters of glucose metabolism have largely been consistent with animal models, the relationship between osteocalcin and fat metabolism has been less conclusive. Dyslipidaemia in diabetes and metabolic syndrome is characterised by elevated triglycerides and reduced high density lipoprotein cholesterol (HDLC). In cross-sectional studies involving healthy and metabolic syndrome subjects, osteocalcin correlated negatively with triglycerides and positively with HDLC although with some gender differences [3–6]. However, similar studies of healthy and diabetic subjects showed the opposite or no correlation between osteocalcin and dyslipidaemia in both men and women [7–9].

Several factors may have contributed to this discordance including gender, age, diagnostic criteria for metabolic syndrome and the presence of diabetes. Metabolic syndrome is a cluster of pathophysiological disturbances attributed to insulin resistance. Patients may or may not have diabetes mellitus. Osteocalcin was lower in diabetic compared to non-diabetic metabolic syndrome patients although low osteocalcin was still associated with metabolic syndrome in non-diabetics [3]. In another study, the negative correlation between osteocalcin and glucose in subjects with metabolic phenotype was not apparent after exclusion of the diabetic sub-group [10]. The aim of this study was to determine if osteocalcin is related to adiposity and hyperglycaemia irrespective of the presence of diabetes.

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2. Methods

This was a cross-sectional study of non-hospitalised patients attending the phlebotomy clinic who were found to have metabolic syndrome using the International Diabetes Federation (IDF) criteria [11]. The cut-off values used for waist circumference (WC) were >90 cm for males and >80 cm for females for definition of central obesity. All male and female patients aged between 18 and 65 years old were eligible. The study was approved by the institution's ethics committee and all subjects gave their informed written consent.

Diabetic patients were defined as those previously diagnosed with diabetes mellitus and/or taking oral hypoglycaemic agents (OHA) or insulin. Patients with a fasting plasma glucose (FPG) < 6.9 mmol/L and no history of diabetes mellitus or treatment with OHA/insulin were considered as non-diabetics. Patients with the following conditions were excluded; severe anaemia, malignancy, hyperparathyroidism, thyroid disease, osteoporosis, pregnancy, fracture within the past 1 year or on treatment with drugs known to affect bone metabolism.

Demographic and clinical data were obtained from patient interviews and medical records. All anthropometric and biochemical measurements were taken in the morning after an overnight fast. The patient's standing height, weight and WC at the level of the umbilicus were measured using calibrated instruments. Blood pressure (BP) was measured in the seated position using a sphygmomanometer. Body mass index (BMI) was calculated as weight (kg) divided by squared height (meters).

Fasting serum lipids (triglycerides, total cholesterol and HDL-C) and FPG were measured by standardised enzymatic methods (Roche Diagnostics, Mannheim, Germany). LDL cholesterol (LDL-C) was calculated from the Friedewald equation [12]. Insulin and osteocalcin were measured on frozen stored (−70 °C) serum samples after adequate thawing. Insulin was measured by a chemiluminescent immunoassay (Siemens Healthcare Diagnostics, USA) and osteocalcin by an electrochemiluminescent immunoassay (Roche Diagnostics, Mannheim, Germany) on automated instruments. Insulin resistance was estimated using the formula for homeostasis model assessment for insulin resistance (HOMA-IR) [13].

2.1. Statistical analyses

All quantitative data were presented as mean (±standard deviation) for variables with normal distribution or median (range) for variables without normal distribution. Differences between diabetic and non-diabetic groups were tested by Chi-squared test for gender, Student t-test for normally distributed variables and Mann–Whitney test for non-Gaussian distributed variables. Bivariate correlations were determined by Spearman's rho correlation test. Linear regression analysis was used to identify factors independently associated with osteocalcin. Covariates included gender, age, WC, systolic and diastolic BP, BMI, total cholesterol, triglycerides, HDL-C and LDL-C. All statistical analyses were performed using the IBM Statistical Package for Social Sciences (version 19). Results with $p < 0.05$ were considered as significant.

3. Results

A total of 90 patients with metabolic syndrome (59 men and 31 women) were studied. Among them 80% had hypertension, 56% had diabetes mellitus, 52% had raised triglycerides or low HDL-C or both and 41% were obese with BMI > 30 kg/m^2. In terms of treatment, 82% were on lipid-lowering drugs (statin or fibrate or combined), 72% were on antihypertensive agents and 48% were taking insulin, OHA or both.

Table 1
Characteristics of metabolic syndrome subjects with and without diabetes mellitus.

Characteristic	All	Diabetic (n = 50)	Non-diabetic (n = 40)	p value
Age (year)	51.2 ± 10.7	53.5 (47)	52.4 ± 9.1	0.575
Waist circumference (cm)	101.3 ± 10.2	102.3 ± 11.9	99.9 ± 7.4	0.586
Systolic BP (mm Hg)	143 ± 17	142.6 ± 17.6	143.4 ± 16.3	0.736
Diastolic BP (mm Hg)	83 ± 11	82.7 ± 12.5	83.6 ± 9.5	0.742
BMI (kg/m^2)	29.9 ± 5.2	29.3 (30.1)	28.9 (11.7)	0.179
Fasting Glucose (mmol/L)	6.05 (2.8)	7.7 (14.8)	5.4 (2.6)	<0.001
Total cholesterol (mmol/L)	4.93 ± 1.2	4.57 (4.29)	4.96 (7.44)	0.277
Triglyceride (mmol/L)	1.60 (0.94)	1.68 ± 0.69	1.53 (12.1)	0.865
HDL-cholesterol (mmol/L)	1.17 ± 0.28	1.13 ± 0.27	1.21 ± 0.28	0.149
LDL-cholesterol (mmol/L)	3.02 ± 1.01	2.88 (4.40)	2.89 (5.03)	0.719
HOMA-IR	3.22 (4.35)	4.3 (121.3)	2.3 (5.9)	<0.001
Osteocalcin (ng/mL)	20.2 ± 11.3	14.1 (45.1)	19.9 (66.1)	0.002

Data are presented as mean ± SD or median (interquartile range). p values are for comparison between diabetic and non-diabetic patients using Chi-squared test for gender, Student t-test for parameters expressed as mean and Mann–Whitney test for parameters expressed as median.

Diabetic metabolic syndrome patients had higher FPG ($p < 0.001$) and HOMA-IR ($p < 0.001$) and lower osteocalcin ($p < 0.01$) than their non-diabetic counterparts (Table 1). There was no difference in WC, BMI, systolic BP, diastolic BP, total cholesterol, triglyceride, HDL-C and LDL-C.

In whole patients, osteocalcin correlated negatively with fasting glucose ($r = -0.366$) and HOMA-IR ($r = -0.305$), Table 2. There was no correlation between osteocalcin and WC, BMI, blood pressure or lipid parameters. HOMA-IR correlated strongly with glucose and insulin as expected and in addition correlated positively with triglycerides ($r = 0.369$) and negatively with HDL-C ($r = -0.217$).

Correlations were also determined in diabetic and non-diabetic groups, Table 3. In diabetics, osteocalcin correlated with FPG but not with HOMA-IR. In non-diabetics, osteocalcin correlated with HOMA-IR and insulin but not with FPG. HOMA-IR did not correlate with triglycerides or HDL-C in either group.

Linear regression analysis was performed with osteocalcin as the dependent variable and gender, age, WC, systolic BP, diastolic BP, BMI, FPG, total cholesterol, triglycerides, HDL-C, LDL-C, insulin and HOMA-IR as independent variables. Only FPG remained independently associated with osteocalcin, Table 4.

Non-diabetic subjects were divided into those with normal fasting glucose (NFG < 5.6 mmol/L, $n = 25$) and impaired fasting

Table 2
Correlations of osteocalcin and HOMA-IR in patients with metabolic syndrome.

Parameter	Correlation coefficient, r	
	Osteocalcin	HOMA_IR
Waist circumference	0.079	0.104
BMI	0.028	0.032
Systolic BP	0.151	−0.062
Diastolic BP	0.089	−0.045
Fasting plasma glucose	−0.366*	0.554*
Total cholesterol	0.061	−0.059
Triglyceride	0.009	0.369*
HDL cholesterol	−0.070	−0.217*
LDL cholesterol	0.091	−0.059
Insulin	−0.193	0.922*
HOMA-IR	−0.305*	–
Osteocalcin	–	−0.305*

* $p < 0.05$ by Spearman's rho correlation.