Relationship between Ischemia Modified Albumin and Metabolic Syndrome

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ABSTRACT

The aim of the study was to evaluate the association between ischemia modified albumin, inflammation markers, and lipids in patients with metabolic syndrome. In this case-control study, serum ischemia modified albumin, uric acid, lipid profile, insulin, fasting and non-fasting glucose, HbA1c, fibrinogen and CRP were measured in 47 patients with metabolic syndrome and 31 controls. International Diabetes Federation criteria were used for metabolic syndrome diagnosis. Ischemia modified albumin, uric acid, fibrinogen, total cholesterol, LDL, triglycerides and HOMA-IR were higher and HDL was lower in the patient group adjusted for body mass index and age. Significant correlations were observed between IMA and total cholesterol, triglycerides, HDL and LDL, fasting glucose, postprandial glucose, HOMA-IR, HbA1c, fibrinogen and CRP. We observed significant correlations also between CRP and fasting glucose, postprandial glucose, HOMA-IR, HbA1c, total cholesterol, triglycerides, HDL, LDL, uric acid and fibrinogen. Uric acid was also found as positively correlated with triglyceride, fasting glucose, HbA1c, HOMA-IR and negatively correlated with HDL. In conclusion, this case-control study has shown that higher levels of IMA and uric acid are strongly related to components of the metabolic syndrome. Both IMA and uric acid are independent risk factors for cardiovascular disease, so these easily measured parameters may add prognostic information to risk prediction. New investigations need to be done.

Keywords: C-reactive protein, fibrinogen, ischemia modified albumin, metabolic syndrome, uric acid

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INTRODUCTION

Metabolic syndrome is a cluster of cardiovascular risk parameters, in which insulin resistance is referred as the common denominator of this syndrome.[1] The International Diabetes Federation has defined the risk factors for the development of metabolic syndrome as high visceral obesity, hypertriglyceridemia, low HDL levels, hypertension and impaired glucose tolerance.[2] The criteria clustered confer the risk of mortality and morbidity.[3] Because the molecular basis of all this complex pathology has not been solved yet, early and practical diagnostic marker research is still under investigation.[4]

Elevated levels of uric acid are considered to increase blood pressure by acting on the renal interstitium.[5] In a large epidemiological study, hyperinsulinemia has been reported as associated with high levels of serum uric acid.[6] Accordingly, due to the relationship of hyperurisemia with insulin resistance, hypertension, obesity and dyslipidemia, uric acid was proposed to be a component of MetS.[7,8]

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Insulin resistance and atherosclerosis share a common inflammatory basis,[9] and CRP, the inflammatory biomarker, corresponds with both of them.[10] Adipose tissue produces proinflammatory cytokines such as tumor necrosis factor-α and interleukin-6 which can influence insulin resistance and increase hepatic CRP production.[11,12]

Ischemia modified albumin (IMA), the new biomarker has been demonstrated in patients with acute myocardial ischemia[13] and pulmonary embolism.[14] It was investigated for its diagnostic sensitivity in patients with acute chest pain compared with ECG and cardiac Troponin T, and was concluded to be solely used for diagnosis.[15] It is produced within minutes of the onset of ischemia that modifies the N-terminal end of albumin. However, the mechanism still remains unclear. Hence this study investigated the correlation between serum IMA, CRP, uric acid and other risk factors of metabolic syndrome.

MATERIALS AND METHODS

Study design:

This case control study was conducted with 78 subjects selected from outpatients in the Department of Endocrinology, at Atatürk Training and Research Hospital during May and July 2010. The study was approved by the local ethical committee of the hospital and was held in accordance with the Declaration of Helsinki on human experimentation. All participants signed a written informed consent.

The patients aged between 18 and 60 and defined as having MetS according to the definition of the International Diabetes Federation (IDF)[2] were included in the study. The age and gender matched control group was selected from the apparently healthy laboratory staff. Exclusion criteria were renal insufficiency, acute febrile disease, heart failure, thyroid function abnormality, hypoalbuminemia (<3.4 g/dL) and determined malignancy.[16]

Central obesity was assessed using waist circumference that is independently associated with MetS components. Waist circumference was measured midway between the lowest rib and the top of the iliac crest following a normal expiration. Height was measured to within 0.5 cm and weight to within 0.1 kg. Body mass index (BMI) was calculated as weight (kg)/height (m^2).

Definition of MetS:

The biological thresholds, defined by the IDF criteria were: 1) waist circumference ≥102 cm (male adults) and ≥88 cm (female adults); 2) fasting plasma glucose ≥100 mg/dl; 3) blood pressure of ≥130/85 mm Hg; 4) triglycerides ≥150 mg/dl; and 5) high-density lipoprotein-cholesterol (HDL-C) <40 mg/dl (male adults) and <50 mg/dl (female adults). Prescription drug use was estimated for lipid-modifying agents, anti-hypertensives, and anti-hyperglycemic medications. They were diagnosed as having MetS with the presence of at least three parameters.

Biochemical measurements:

After an overnight fast, the venous blood samples were drawn from 78 subjects (47 MetS patients and 31 healthy controls) for measurement of serum glucose, insulin, TG, HDL-C, uric acid, CRP, HbA1c, ferritin, iron, IMA and plasma fibrinogen concentrations. All analyses were performed in the Biochemistry Laboratory of Atatürk Training and Research Hospital.

Analysis methods:

Glucose (hexokinase), lipid parameters (enzymatic), CRP (immunoturbidimetric), uric acid (uricase), fibrinogen (Clauss method) and LDL cholesterol were estimated using Friedewald equation.[17]

Insulin was quantified using the Immulite 2000 (Diagnostic Products Corporation, Los Angeles, CA, USA) insulin assay with a solid-phase, two-site, chemiluminescent enzyme-labelled immunometric assay. Insulin resistance index was calculated by the homeostasis model assessment of insulin resistance (HOMA-IR) as (fasting insulin IU/L) x (fasting glucose mmol/L/22.5).

Measurement of IMA depends on the mechanism that human serum albumin no longer binds metal ions such as cobalt and copper ions due to the ischemic tissue changes and the advantage is the change occurs within minutes of the ischemia. The present quantitative colorimetric

assay method developed by Bar et al.,[18] depends on the mechanism for scanning the amount of the reduced cobalt to albumin binding capacity. Only for IMA measurement sera were stored at -20˚C for one month until analysis. IMA was quantified spectrophotometrically with a method which depended on the determination of a variant form of albumin with a reduced affinity for cobalt binding which was developed by Bar-Or et al.[18] The absorbances were obtained at 470 nm (Shimadzu Corporation, Kyoto, Japan) and were expressed as ABSU (absorbance unit).

Statistical analysis

All statistical analyses were conducted with SPSS, version 15.0 (SPSS Inc., Chicago, IL, USA). Normality of quantitative variables was verified by Kolmogorov-Smirnov test. The continuous variables of the study groups were compared using the Student *t*-test. A P value <0.05 (two-tailed) was considered significant. Categorical variables were determined with X^2 test. Results were expressed as mean ± standard deviation (SD). Pearson and Spearman correlation coefficient tests were used for the estimation of associations between MetS parameters and cardiovascular risk related parameters with IMA. Univariate linear regression analysis was used for differences of the parameters measured among groups, and covariates were selected as age and BMI according to the results of the correlation analyses.

RESULTS

The demographic and biochemical parameters have been presented in Table 1. Among

TABLE 1. Baseline characteristics of study participants.

	Control (n=31)	Patient (n=47)	*P* value
Gender (male/female)	8/23	13/34	0.857
Age (years)	34.16 ± 9.21	36.53 ± 8.55	0.257
Height (m)	166.77 ± 7.73	166.21 ± 8.71	0.766
Weight (kg)	62.54 ± 11.58	91.18 ± 15.89	<0.001
BMI (kg/m^2)	22.53 ± 3.60	33.00 ±5 .20	<0.001
Waist circumference (cm)	73.47 ± 7.58	101.55 ± 11.11	<0.001
Waist/Hip ratio	0.77 ± 0.06	0.90 ± 0.09	<0.001
SBP (mm Hg)	115.57 ± 10.38	144.81 ± 29.2	<0.001
DBP (mm Hg)	73.79 ± 8.99	83.67 ± 16.5	0.009
Insulin (µU/ml)	4.58 ± 2.24	15.37 ± 7.69	<0.001
Iron (mg/dL)	86.71 ± 30.40	66.11 ± 24.33	0.001
Total Cholesterol (mg/dL)	167.39 ± 21.34	208.53 ± 42.98	<0.001
Triglyceride (mg/dL)	83.13 ± 34.82	197.55 ± 84.19	<0.001
HDL Cholesterol (mg/dL)	56.52 ± 9.22	41.49 ± 8.65	<0.001
LDL Cholesterol (mg/dL)	94.39 ± 18.55	126.87 ± 36.19	<0.001
Fasting glucose (mg/dL)	86.78 ± 6.333	116.38 ± 37.380	<0.001
Postprandial glucose (mg/dL)	90.53 ± 9.938	133. 21 ± 58.282	<0.001
HbA1c (%)	5.31 ± 0.32	6.58 ± 1.63	<0.001

HOMA-IR: indicates homeostatis model assessment of insulin resistance. Data are expressed as mean ± SD. Student' s t-test was used for comparison. Significant values are written in bold font.

patients, 63.8% were obese (n=30; BMI ≥30) and 34% were overweight (n=16; BMI ≥25) and in the control group only one subject was obese and 5 of them were overweight according to the definition by World Health Organization.[19]

While using the threshold of HOMA-IR>2.5, the prevalence of insulin resistance was 72.3% in the MetS group.

Patients had significantly higher IMA (Fig 1), uric acid and fibrinogen levels compared with controls adjusted by BMI and age (Table 2). No significant differences were determined for CRP and ferritin values.

Correlation analysis resulted in significant positive association of CRP with age (r= .283, p=.012), IMA (r=.429, p<0.001), fasting glucose (r=.485, p<0.001), non-fasting glucose (r=.408, p<0.001), BMI (r=.615, p<0.001), HbA1c (r=.542, p<0.001), Chol (r=.339, p=.002), LDL (r= .282, p=.012), TG (r=.526, p<0.001), uric acid (r=.349, p=0.002), fibrinogen (r=.395, p<0.001), IMA (r=.429, p<0.001), insulin (r=.489, p<0.001), HOMA-index (r=.537, p<0.001), weight (r=.600, p<0.001), hip (r=.528, p<0.001), waist/hip (r=.557, p<0.001) and negatively associated with HDL (r= -.469, p<0.001) and iron (r= -.294, p=.009) in all groups (Table 3). Waist circumference significantly correlated with insulin resistance (r=.768, p<0.001).

DISCUSSION

Uric acid an oxidative stress marker, fibrinogen a procoagulant and inflammation associated marker, and IMA an oxidative stress and inflammation marker were found as higher in the patient group vs healthy controls. The results presented show that CRP associates to a comparable degree with insulin resistance, proinflammatory, and prothrombotic parameters. Subjects with older age and with higher BMI, glucose, HbA1c, LDL, Chol, TG, uric acid, fibrinogen, IMA and HOMA-index tend to have higher CRP values in all groups (p<0.05) similar to a previous study.[20] Waist circumference determined showed a high association with insulin resistance similar to a study performed with Asian people.[21]

IMA was found to be related with hypercholesterolemia, fasting glucose, CRP and with oxidative stress markers in studies.[23,23] They found higher IMA levels in the patient group of diabetes and hypercholesterolemia vs control similar to our study, from which they concluded that IMA might take part in oxidative stress and atheromatous plaque formation.[22] In another study, IMA was found as useful for early diagnosis of acute myocardial ischemia when compared with ECG and troponin.[14] In an experimental model, IMA levels elevated acutely due to the mesenteric ischemia in a rat model.[24]

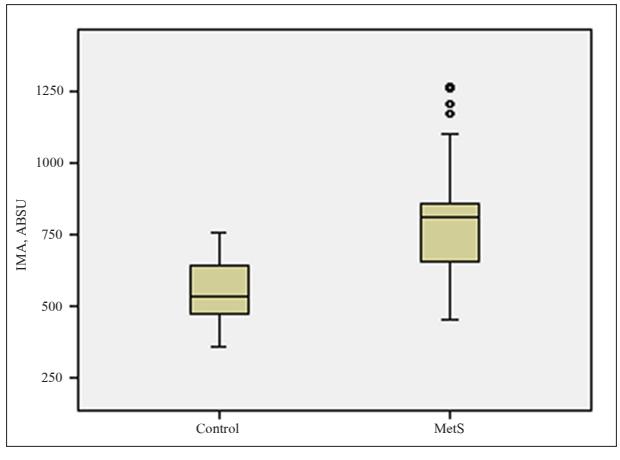

Fig 1. Ischemia Modified Albumin levels of control and MetS group.

TABLE 2. Analysis of covariance assessing the MetS components as dependent variables, groups as fixed factors, and age, BMI as covariates.

	Control (n=31)	MetS (n=47)	F	*P*
IMA, ABSU	0.551 ± 0.103	0.800 ± 0.186	18.932	**<0.001**
CRP, mg/dl	0.29 ± 0.41	0.93 ± 0.82	1.462	0.231
UA, mg/dl	3.90 ± 1.17	5.19 ± 1.48	4.048	**0.048**
HOMA-IR	0.95 ±0.48	4.36 ± 2.95	10.538	**0.002**
Fibrinogen, mg/dl	280±38	329±70	4.100	**0.047**

Data are mean ± SD. Significant values are written in bold font.

TABLE 3. Correlation between serum CRP, ischemia modified albumin, uric acid concentrations and cardiovascular risk parameters.

	CRP		IMA		Uric acid	
	Correlation coefficient	*P* value	Correlation coefficient	*P* value	Correlation coefficient	*P* value
Age, years	0.283	0.012	0.022	0.847	-0.131	0.254
IMA, ABSU	0.429	<0.001			0.120	0.295
Fasting glucose, mg/dl	0.485	<0.001	0.458	<0.001	0.295	0.009
Postprandial glucose, mg/dl	0.408	<0.001	0.396	<0.001	0.141	0.219
HOMA-IR	0.537	<0.001	0.457	<0.001	0.433	<0.001
Fibrinogen, mg/dl	0.395	<0.001	0.296	0.008	0.163	0.155
HbA1c, %	0.542	<0.001	0.491	<0.001	0.261	0.021
Total cholesterol, mg/dl	0.339	0.002	0.299	0.008	0.116	0.314
Triglyceride, mg/dl	0.526	<0.001	0.387	<0.001	0.482	<0.001
HDL cholesterol, mg/dl	-0.469	<0.001	-0.468	<0.001	-0.618	<0.001
LDL cholesterol, mg/dl	0.282	0.012	0.324	0.004	0.098	0.395
Uric acid, mg/dl	0.349	0.002	0.120	0.295		
Waist/Hip ratio	0.557	<0.001	0.414	<0.001	0.517	<0.001
BMI, kg/m^2	0.615	<0.001	0.513	<0.001	0.476	<0.001
Fibrinogen, mg/dl	0.395	<0.001	0.296	0.008	0.163	0.155

Data are analyzed using Spearman's rank order correlation.

Ectopic fat storage (visceral fat) is an important component of insulin resistance which might be the result of the activity fail of the cells for normal hormonal respond. The obese visceral fat associated with the MetS is often called as "low grade" chronic inflammation that plays a major role in the pathophysiological consequences. The macrophage infiltration of adipose tissue has been detected in a study.[25] High glucose levels, consequently high HOMA-IR levels were associated with CRP levels in our MetS group.

Uric acid may work as an antioxidant or prooxidant. Higher than the upper reference limit, uric acid is associated with the prooxidative events.[26] The uric acid increase is found as associated with vascular inflammation and sudden cardiac death and together with the inflammation marker CRP, both are risk factors associated with the MetS.[26] In patients with type 2 diabetes, although lipid and glucose levels remained similar to the baseline values, long term follow up (at least 7 years) showed an increasing trend of mortality especially at CRP values higher than 10 mg/L.[27] In this study, hyperuricemia was found to be associated with high BMI, CRP and low HDL in the patient group.

Fibrinogen, an important coagulation protein, is associated with the risk factors of coronary artery disease. Consequently, reducing the lifestyle risk factors (smoking cessation, regular exercise) reduces the levels of fibrinogen.[28] Long term increase in plasma fibrinogen level of 1 g/L was reported to be associated with a doubling of risk of CVD outcomes.[29] A positive association of fibrinogen with BMI, CRP, low HDL, and triglyceride was established.[30] In this study, fibrinogen was found to be higher in the MetS group vs control, although no associations were established in patients with other risk parameters which might be due to the medications they use. However, in the control group, fibrinogen associated positively with CRP, age and total cholesterol.

In conclusion, this case-control study has shown that higher levels of IMA and uric

acid are strongly related to components of the metabolic syndrome. Both IMA and uric acid are independent risk factors for cardiovascular disease, so these easily measured parameters may add prognostic information to risk prediction. A major limitation of our study is the small number of subjects. New investigations need to be done.

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