



# Does Microalbuminuria Affect Resistin and Cardiometabolic Risk Factors in Hypertensive Non-Diabetic Females?

## *Non-Diyabetik Hipertansif Kadınlarda Mikroalbuminüri, Rezistin ve Kardiyometabolik Risk Faktörlerini Etkiler mi?*

Sena Ulu, Gül Gürsoy\*, Berrin Demirbaş\*\*, Yaşar Acar, Ahmet Cimbek, Hayriye Cankar Dal, Murat Bayram

Ankara Education and Research Hospital, Department of Internal Medicine, Ankara, Turkey

\*Kafkas University Faculty of Medicine, Clinic of Internal Medicine, Kars, Turkey

\*\*TOBB Hospital, Clinic of Endocrinology and Metabolism, Ankara, Turkey

### Abstract

**Aim:** Hypertension, obesity, insulin resistance and lipid levels are risk factors for cardiovascular disease. The association of cardiovascular risk with C-reactive protein and homocysteine has been debated for decades. Resistin and microalbuminuria are presumed to be associated with diabetes mellitus, insulin resistance and cardiovascular disease. The objective of our study was to investigate the relationship of microalbuminuria with antropometric and metabolic parameters, C-reactive protein, homocysteine and resistin in non-diabetic hypertensive females.

**Methods:** We conducted a randomized study including 37 female non-diabetic hypertensives without microalbuminuria and 47 female non-diabetic hypertensive patients with microalbuminuria. We made comparisons of antropometric and metabolic parameters, C-reactive protein, homocysteine, insulin resistance index and resistin between the groups.

**Results:** C-reactive protein, homocysteine, resistin, insulin levels and homeostasis model assessment of insulin resistance were higher in hypertensives with microalbuminuria than in hypertensives without microalbuminuria (all  $p < 0.05$ ).

**Conclusion:** We found that microalbuminuria may have an influence on C-reactive protein, homocysteine and resistin levels in non-diabetic hypertensives. We also think that insulin and insulin resistance may also be related with microalbuminuria in non-diabetic hypertensive female patients. (*The Medical Bulletin of Haseki 2014; 52: 172-6*)

**Key Words:** Albuminuria, hypertension, obesity, c-reactive protein, homocysteine, resistin, blood glucose, lipids

### Özet

**Amaç:** Hipertansiyon, obezite, insülin rezistansı ve lipid seviyeleri kardiyovasküler hastalık için risk faktörleridir. C-reaktif protein, homosistein ile kardiyovasküler risk arasındaki ilişki uzun zamandır tartışılmaktadır. Rezistin ve mikroalbuminürinin diyabetes mellitus, insülin rezistansı ve kardiyovasküler hastalık ile bağlantılı olduğu kabul edilmektedir. Çalışmamızda non-diyabetik hipertansif kadınlarda mikroalbuminüri ile antropometrik ve metabolik parametreler, C-reaktif protein, homosistein ve rezistin ilişkisini araştırmayı planladık.

**Yöntemler:** Otuz yedi mikroalbuminürisi olmayan ile 47 mikroalbuminürisi olan non-diyabetik hipertansif kadın hastayı kapsayan randomize bir çalışma gerçekleştirdik. Gruplarımızda antropometrik ve metabolik parametreleri, C-reaktif protein, homosistein, insülin rezistans indeksi ve rezistin seviyelerini kıyasladık.

**Bulgular:** Mikroalbuminürisi olan hipertansiflerde olmayanlara oranla C-reaktif protein, homosistein, rezistin, insülin seviyeleri ve insülin rezistans indeksi yüksek bulundu (all  $p < 0,05$ ).

**Sonuç:** Non-diyabetik hipertansiflerde mikroalbuminürinin C-reaktif protein, homosistein ve rezistin seviyeleri üzerine etkisi olabileceğini bulduk. Non-diyabetik hipertansif kadınlarda insülin ve insülin rezistansı da mikroalbuminüriden etkilenebileceğini de düşünmekteyiz. (*Haseki Tıp Bülteni 2014; 52: 172-6*)

**Anahtar Sözcükler:** Albüminüri, hipertansiyon, obezite, c-reaktif protein, homosistein, rezistin, kan şekeri, lipidler

## Introduction

Microalbuminuria (MA) is the level of urinary albumin excretion that is above normal limit but below the usual limit of detection by qualitative testing (1). As it is a sign of future cardiovascular disease (CVD) risk in the general population and in diabetics, it is now believed to be a risk factor for atherosclerosis (2). It is also considered that increased albumin excretion even below the conventional MA range is associated with an increased likelihood of cardiovascular mortality (3).

As for patients with diabetes mellitus (DM), for patients with hypertension, MA has been thought to be an independent risk factor for CVD and premature cardiovascular mortality (2,4,5). It has also been shown to be a predictor for future development of hypertension among normotensives (4).

Inflammatory biomarkers of vascular changes and endothelial dysfunction have been studied as possible markers of atherosclerotic burden, mediators of vascular damage or both (4). C-reactive protein (CRP) is a large pentameric protein produced by the liver in response to signals from intra-abdominal fat stores. It is suspicious that it is pathogenic itself, but has been linked with impaired endothelial function. CRP has been demonstrated to be correlated with the degree of global cardiometabolic risk related with adiposity (6).

Plasma homocysteine (Hcy) level has also been considered to be a marker of endothelial dysfunction and a predictor of CVD (7,8). However, studies examining the association between plasma Hcy and MA have suggested that this association may be explained by other factors such as preexisting CVD, diabetes, hypertension, and reduced kidney function (9).

It has been demonstrated that adipocytokines may have roles in insulin resistance, diabetes, atherosclerosis and CVD (10-13). The role of resistin, which is one of those adipocytokines, in insulin resistance, obesity, type 2 DM (T2DM), CVD and hypertension is still being investigated (14-20). Some studies have shown the relationship of MA with insulin resistance, obesity, and dyslipidaemia in patients with diabetes and essential hypertension (2,16,21-23).

Bearing in mind these complex relationship of MA with insulin resistance, obesity, hypertension, lipid disorders, cardiovascular risk factors and resistin, we investigated the association of antropometric and metabolic parameters with CRP, Hcy and resistin in non-diabetic hypertensive females either with or without MA.

## Methods

### Patients

A total of 84 female non-diabetic hypertensives, 37 without MA and 47 with MA aged between 30 and 80

years were recruited from the internal medicine outpatient clinic at Ankara Education and Research Hospital from January 2011 to May 2011. As resistin serum and mRNA levels were significantly higher in females than in males at all ages, we examined only females in order to obtain an homogenous group.

Subjects with male gender, DM, glucose intolerance, hyperlipidemia, conditions which may effect metabolic parameters (such as current or a history of thyroid dysfunctions), chronic diseases, infection, and coronary artery disease were excluded.

After detailed physical examination, body weight and height were measured in all subjects. Waist circumference was measured with the participant fasting and in a standing position midway between the costal margin and the iliac crest, whereas hip was measured at the greatest circumference around the buttocks, using a non-stretch tape measure. Waist to hip ratio (WHR) was calculated. Body mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared ( $\text{kg}/\text{m}^2$ ). Percentage body fat was estimated by using Tanita body composition analyser TBF -300 after the subjects rested 30 minutes.

Blood samples were withdrawn after a 12-hour overnight fast, at 08.30 a.m. for fasting plasma glucose (FPG), serum total and high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), CRP, Hcy, free insulin (FI) and resistin levels. Another blood sample was taken for postprandial plasma glucose (PPPG) 2 hours after breakfast.

Systolic and diastolic blood pressures (SBP and DBP) were measured after 5 minutes of rest in the semi-sitting position using a sphygmomanometer. Blood pressure was determined at least three times at the level of the right upper arm, and the mean value was used in the analysis. Patients, who were taking antihypertensive drugs or patients with a mean blood pressure levels of  $\geq 140/90$  mmHg, were considered hypertensive and were included in the study.

Microalbuminuria was examined in spot urine. Patients with a MA level of  $< 30$  mg/dL were classified as MA negative and those with a MA level of  $\geq 30$  mg/dL, as MA positive.

Insulin resistance was assessed from the fasting plasma insulin by the homeostasis model assessment of insulin resistance (HOMA-IR) using the following formula: ( $\mu\text{unit}/\text{mL}$ ) x fasting plasma glucose ( $\text{mmol}/\text{L}$ )/22.5.

This study was performed according to the Helsinki declaration 2008. The local ethics committee approved this study and all the subjects gave written informed consent.

### Laboratory Methods

Plasma glucose, total cholesterol, TG and HDL-C concentrations were determined by enzymocalorimetric

spectrophotometric method in a Roche/Hitachi molecular PP autoanalyser. Low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald equation ( $\text{LDL-C} = \text{Total cholesterol} - \text{HDL-C} - \text{TG}/5$ ). Insulin was measured by means of DRG Diagnostics (DRG Instruments GmbH, Germany) ELISA kits. For the measurement of MA, the nephelometric method was used. High-sensitivity CRP was measured by immunofluorometric assays using a Beckman Cutler device. Hcy concentrations were determined according to the HPLC method using an Agilent 1100 device.

For the measurements of resistin, after fasting, blood samples were drawn, blood was put into a dry tube and were centrifuged at 5000 g/min for 10 minutes. Serum was then separated and put into another dry tube before storing at  $-80^{\circ}\text{C}$ . Serum resistin levels were assayed by a commercial resistin ELISA kit.

#### Statistical Analysis

Calculations were performed using SPSS version 11.5 (Customer ID 30000105 930). Data are presented as mean $\pm$ SD. Student t-test was used to compare the groups in a parametric way. A p value of  $<0.05$  was considered statistically significant.

#### Results

This study was performed on 84 female non-diabetic hypertensive patients. Thirty seven of them were not

having MA and 47 were having MA. The groups were compared for demographic characteristics and laboratory findings (Table 1).

The patients with MA had higher CRP, Hcy, FI, HOMA-IR and resistin levels than did patients without MA ( $p<0.05$ ). There was no significant difference between the groups in age, BMI, WHR, percentage fat, FBG, PPBG, total cholesterol, LDL-C, HDL-C, and TG levels.

#### Discussion

In our study, we aimed to investigate the effects of MA on obesity, levels of glucose, lipids, Hcy, resistin and CRP as well as insulin resistance in non-diabetic hypertensive female patients. In non-diabetic hypertensive patients who had MA, only CRP, Hcy, FI, HOMA-IR and resistin were higher than in those without MA, but there was no difference in obesity markers, blood glucose and lipid levels between the groups.

High-sensitivity CRP is an acute phase reactant and a marker of inflammation. It has also been accepted to be a marker of cardiovascular risk (24-26). Significant correlation between the levels of MA and CRP has been demonstrated (27-29). In patients with hypertension, a casual risk factor for CVD, MA implies a higher expression of existing microvascular damage. In our hypertensives, who had increased transcapillary albumin escape rate, we

**Table 1.** Findings of hypertensive patients who were not having and having microalbuminuria

	Microalbuminuria negative n=37	Microalbuminuria positive n=47	p
Age (year)	54.70 $\pm$ 8.26	60.00 $\pm$ 12.67	NS
BMI (kg/m <sup>2</sup> )	30.01 $\pm$ 4.87	30.66 $\pm$ 4.96	NS
WHR (cm/cm)	0.85 $\pm$ 0.08	0.88 $\pm$ 0.10	NS
Fat ratio (%)	36.13 $\pm$ 8.58	36.41 $\pm$ 8.49	NS
FBG (mg/dl)	87.32 $\pm$ 9.93	85.42 $\pm$ 10.53	NS
PPBG (mg/dl)	171.02 $\pm$ 23.21	169.22 $\pm$ 23.12	NS
T. Chol (mg/dl)	207.81 $\pm$ 63.13	219.89 $\pm$ 58.68	NS
LDL-C (mg/dl)	135.60 $\pm$ 31.80	132.90 $\pm$ 23.00	NS
HDL-C (mg/dl)	48.46 $\pm$ 14.88	47.68 $\pm$ 15.00	NS
TG (mg/dl)	166.90 $\pm$ 22.80	159.00 $\pm$ 12.80	NS
CRP (mg/dl)	1.63 $\pm$ 1.30	3.64 $\pm$ 1.10	$<0.05$
Hcy ( $\mu\text{mol/ml}$ )	15.43 $\pm$ 6.50	17.00 $\pm$ 7.06	$<0.05$
FI ( $\mu\text{U/ml}$ )	7.94 $\pm$ 5.01	12.28 $\pm$ 7.21	$<0.05$
HOMA-IR	2.15 $\pm$ 1.17	2.61 $\pm$ 1.60	$<0.05$
Resistin (ng/ml)	16.70 $\pm$ 7.34	28.50 $\pm$ 7.10	$<0.005$

BMI: Body mass index, WHR: Waist-hip ratio, FBG: Fasting blood glucose, PPBG: Post prandial blood glucose, T.Chol: Total cholesterol, LDL-C: Low density lipoprotein cholesterol, HDL-C: High density lipoprotein cholesterol, TG: Triglyceride, CRP: C-reactive protein, Hcy: Homocysteine, FI: Fasting insulin, HOMA-IR: Homeostasis model assessment insulin resistance index, Data are presented as mean  $\pm$  SD. NS: nonsignificant.

found higher levels of CRP, an indicator of inflammation, than in patients without MA. It is obvious that handling hypertensives with MA needs more attention.

Microalbuminuria and plasma Hcy levels have both been considered to be markers of endothelial dysfunction and shown to be predictors of CVD (8,30). Besides, kidney function is critical in Hcy clearance; recent studies on animals have shown that high Hcy levels induced renal injury and MA (9). In a study including diabetic and non-diabetic patients, minimal hyperhomocysteinemia was found in microalbuminuric patients compared with normoalbuminuric ones (7). In vivo and in vitro, it has been documented that Hcy could induce insulin resistance by directly regulating the expression and secretion of resistin from adipose tissue (31,32). Since vascular smooth muscle cell (VSMC) migration was shown to be a key event in vascular disease, it was thought that resistin was involved in Hcy-induced VSMC migration. This study also demonstrated that Hcy promoted VSMC migration through a paracrine or endocrine effect of adipocyte-derived resistin, which may provide an evidence of the adipose tissue and vascular tissue interaction in metabolic disorders (33). In concordance with later studies, we also demonstrated higher HOMA-IR and also Hcy levels as well as resistin levels in our microalbuminuric hypertensive patients than normoalbuminuric ones.

As metabolic syndrome has been recently accepted to represent a cluster of cardiometabolic risk factors, including central obesity, insulin resistance, glucose intolerance, dyslipidemia, hypertension, hyperinsulinemia and MA, we sought the association amongst some of these parameters. Like the studies which concluded that insulin resistance could significantly predict development of MA in type 2 diabetic patients and normal population (21,34), we demonstrated the association of MA with insulin resistance. But we were not able to show the correlation between MA and obesity and its indexes such as BMI, WHR and fat percentage unlike the studies in which this correlation was found (21,22). We may explain this controversy by the fact that all our hypertensive patients were obese with BMI above 30 kg/m<sup>2</sup>.

Studies evaluating MA in diabetic, hypertensive patients and in normal population have shown that subjects with MA were significantly more likely to have dyslipidemia than patients without MA (35,36). Moreover, high TG, high total cholesterol and LDL-C, and low HDL-C were found to be significantly associated with MA (22). Our hypertensive patients, either with or without MA, had TG, total cholesterol and LDL-C and HDL-C levels beyond normal limits. This may be the reason why we could not find any statistically significant difference between our hypertensive groups in lipid levels.

In studies investigating the association of MA with glucose levels, there were conflicting results (22,37,38). Like some of these studies, we were not able to demonstrate any correlation between fasting or postprandial glucose levels and MA in a hypertensive group.

In conclusion, our results may clarify the association of MA with insulin resistance and resistin in non-diabetic hypertension, suggesting that MA is associated with a cluster of metabolic disturbances. As MA has also been shown to be related to some cardiovascular risk factors such as CRP and Hcy; according to our opinion, screening and early recognition of MA in these patients would be the first strategy and working for the regression to normoalbuminuria would be the second one. However, since our study was conducted with females, it would not be appropriate to generalize our results to all the population, hence, larger studies, including male subjects as well, are needed.

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