The prevalence of aspirin resistance in patients with metabolic syndrome

Metabolik sendromlu hastalarda aspirine direnç sıklığı

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Objectives: Aspirin is recommended for primary prevention in patients with metabolic syndrome (MetS). In this study, we evaluated aspirin resistance in MetS patients.

Study design: The study included 32 patients (23 males, 9 females; mean age 60.7±11.4 years) with the diagnosis of MetS, according to the criteria of the International Diabetes Federation. Aspirin resistance was determined by the PFA-100 analysis (Platelet Function Analyzer). The results were compared with a control group of 30 patients (16 males, 14 females; mean age 61.6±7.3 years) without MetS. All the patients were taking aspirin at the time of the PFA-100 analysis.

Results: Overall, 21 patients (33.9%) were aspirin non-responders. The prevalence of aspirin resistance was 46.9% in the MetS group, and 20% in the control group. The difference between the two groups was statistically significant (p=0.033). Compared to aspirin responders, fasting blood glucose level was higher (102.0±14.6 mg/dl *vs.* 95.3±9.9 mg/dl; p=0.036) and waist circumference tended to be greater in nonresponders (97.4±14.1 cm *vs.* 89.7±15.0 cm; p=0.053). Multivariate logistic regression analysis showed that MetS (OR 0.28, 95% CI 0.09-0.88; p=0.029), fasting blood glucose (OR 0.95, 95% CI 0.91-0.99; p=0.045), uric acid (OR 0.46, 95% CI 0.28-0.76; p=0.002), gamma-glutamyl transferase (OR 1.04, 95% CI 1.00-1.08; p=0.043), high-sensitivity C-reactive protein (OR 1.07, 95% CI 1.01-1.12; p=0.015) levels and platelet count (OR 0.99, 95% CI 0.98-0.99; p=0.034) significantly affected aspirin resistance.

Conclusion: Our results show that a significant proportion of MetS patients will not benefit from aspirin use due to high aspirin resistance.

Key words: Aspirin; drug resistance metabolic syndrome X.

Amaç: Günümüzde aspirin metabolik sendromlu (MetS) hastalarda birincil korumada önerilmektedir. Bu çalışmada MetS'li hastalarda aspirine direnç araştırıldı.

Çalışma planı: Çalışmaya, Uluslararası Diyabet Federasyonu ölçütlerine göre MetS tanısı konan 32 hasta (23 erkek, 9 kadın; ort. yaş 60.7±11.4) alındı. Tüm hastalarda aspirine direnç PFA-100 (Platelet Function Analyzer) yöntemiyle araştırıldı. Sonuçlar, MetS bulunmayan 30 hastadan (16 erkek, 14 kadın; ort. yaş 61.6±7.3) oluşan kontrol grubuyla karşılaştırıldı. PFA-100 analizi sırasında tüm hastalar aspirin kullanmaktaydı.

Bulgular: Aspirine direnç her iki gruptan toplam 21 hastada (%33.9) görüldü. Metabolik sendrom grubunda direnç oranı %46.9 iken, kontrol grubunda bu oran %20 idi ve fark anlamlı bulundu (p=0.033). Aspirine direnç görülmeyenlerle karşılaştırıldığında, dirençli hastalarda açlık kan şekeri düzeyi anlamlı derecede yüksek bulunurken (102.0±14.6 mgr/dl ve 95.3±9.9 mgr/dl; p=0.036), bel çevresi ölçümleri de daha yüksek idi (97.4±14.1 cm *ve* 89.7±15.0 cm; p=0.053). Çokdeğişkenli lojistik regresyon analizinde aspirine direnç gelişimini anlamlı derecede etkileyen faktörler şunlardı: MetS (OR 0.28, %95 GA 0.09-0.88; p=0.029), açlık kan şekeri (OR 0.95, %95 GA 0.91-0.99; p=0.045), ürik asit (OR 0.46, %95 GA 0.28-0.76; p=0.002), gama-glutamil transferaz (OR 1.04, %95 GA 1.00-1.08; p=0.043), yüksek duyarlıklı C-reaktif protein (OR 1.07, %95 GA 1.01-1.12; p=0.015) ve trombosit sayısı (OR 0.99, %95 GA 0.98-0.99; p=0.034).

Sonuç: Bulgularımız, MetS'li hastaların önemli bir kısmının, aspirine direncin yüksek oranda olması nedeniyle aspirinden yarar görmeyeceklerini göstermektedir.

Anahtar sözcükler: Aspirin; ilaç direnci; metabolik sendrom X.

Received: April 11, 2008 *Accepted:* April 14, 2009

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Metabolic syndrome (MetS) is a complex disease that might be related to progressive cardiovascular atherosclerosis. Several guidelines recommend low-dose aspirin for primary prevention in MetS patients with a higher risk for cardiovascular disease.[1] Despite documented benefits of aspirin, a considerable proportion of patients do not benefit from aspirin. Aspirin resistance defined as failure to effectively inhibit thromboxane synthesis is associated with a higher risk for recurrent myocardial ischemia and cardiovascular death.[2] The incidence of aspirin resistance ranges between 8% and 45% in the literature.[3] In this study, we evaluated aspirin resistance in patients with and without MetS.

PATIENTS AND METHODS

Study design. The study included 62 patients (39 males, 23 females, age range 49 to 72 years). Thirty-two patients had the diagnosis of MetS, according to the criteria of the International Diabetes Federation,[4] whereas 30 patients did not have MetS. All the patients were taking aspirin (mean dose 151.6±85.4 mg/day). Exclusion criteria were as follows: hypersensitivity to aspirin; use of other nonsteroidal antiinflammatory drugs, other antiplatelet and anticoagulant drugs, or immunosuppressive or cytotoxic drugs; the presence of an acute or chronic inflammatory disease, myeloproliferative disorder, malignancy, renal, hepatic or thyroid disease, or acute coronary syndrome; platelet count lower than 100,000/mm^3 or higher than 450,000/mm^3, hematocrit level lower than 30% or higher than 52%, and left ventricular ejection fraction lower than 60%. Body mass index was calculated as weight (kg)/height squared (m^2). Abdominal obesity was defined as waist circumference ≥102 cm for men and ≥88 cm for women. Waist circumference was measured at the level of the umbilicus. Written consent was obtained from all patients and the study protocol was approved by our local ethical committee.

Laboratory measurements. By antecubital venipuncture, blood samples were obtained from each subject in the fasting state in the morning between 8 and 10 a.m., 2-4 hours after daily aspirin intake (range 150-300 mg). The first milliliters of blood were discarded to avoid spontaneous platelet activation. Citrated blood (0.129 M trisodium citrate in dilution 1:10) was used for PFA-100 analysis (Platelet Function Analyzer, Dade Behring, Germany) and 4.5 ml blood were collected in EDTA (ethylenediaminetetraacetic acid) tubes for platelet count and hematocrit determination. Mean platelet volume was measured using a Coulter S^+ resistive particle counting system. All analyses were performed within 1-2 hours after blood collection. Total cholesterol, HDL cholesterol and triglyceride levels were measured enzymatically on an Hitachi 911 autoanalyzer (Hitachi, Japan). LDL cholesterol level was determined using the Friedewald formula. Leukocyte and platelet counts were performed using a BCD autoanalyzer (Dade Behring, Germany).

The PFA-100 system. The PFA-100 system is a novel platelet function test that enables rapid, simple, and reproducible quantitative assessment of *in vitro* platelet aggregation and is used for identification of aspirin nonresponder status.[5-7] This test is based on a highly sensitive and specific *in vitro* system for the assessment of platelet aggregation in small samples of citrated whole blood.[8] It uses a disposable test cartridge coated with either collagen or epinephrine or with collagen and adenosine diphosphate (ADP). The instrument aspirates citrated whole blood under a constant vacuum condition at a high shear stress of 5000-6000 s^{-1} through a capillary tube and a precisely defined aperture in the membrane that mimics microcapillary system of human circulation. Time to complete occlusion of the aperture is defined as the closure time (CT). Normal reference ranges of closure time in our laboratory are 85-165 sec for collagen/epinephrine-coated membrane (EPI), and 71-118 sec for collagen/ADP-coated membrane (ADP), with the central 90% reference interval. Since aspirin has a prolonged EPI closure time in a dose-dependent fashion, aspirin resistance was defined as EPI closure time of <186 sec. All PFA-100 measurements were performed in duplicate.

Statistical analysis. Data were analyzed using the SPSS statistical software package (15.0 for Windows). Results were expressed as mean±standard deviation for continuous data or as percentages and numbers for categorical data. Continuous variables with normal distribution and unequal distribution were analyzed with the Student's t-test and Mann-Whitney U-test, respectively. Categorical data and proportions were analyzed by the chi-square test. Pearson correlation analysis was used to evaluate the relationship between variables. Binary logistic regression analysis was used for multivariate analysis. The odds ratios (OR) and 95% confidence intervals (CI) were calculated. Statistical level of significance was defined as $p<0.05$.

RESULTS

Overall, 21 patients (33.9%) were aspirin nonresponders. The prevalence of aspirin resistance was

Table 1. Demographic and clinical characteristics of the patients with and without metabolic syndrome

	Metabolic syndrome (n=32)			Control group (n=30)			
	n	%	Mean±SD	n	%	Mean±SD	*p*
Age (years)			60.7±11.4			61.6±7.3	0.711
Sex							0.212
Male	23	71.9		16	53.3		
Female	9	28.1		14	46.7		
Body mass index (kg/m^2)			27.9±7.4			22.8±1.9	**0.012**
Waist circumference (cm)			105.1±7.9			78.5±5.2	**0.013**
Hypertension	28	87.5		20	66.7		**0.050**
Diabetes mellitus	2	6.3		2	6.77		0.843
Current smokers	19	59.4		11	36.7		0.411
Hyperlipidemia	16	50.0		14	46.7		0.113
Aspirin dose (mg/day)			143.7±84.0			160.0±87.4	0.524
Systolic blood pressure (mmHg)			138.0±10.4			127.5±14.8	**0.011**
Known coronary artery disease	25	78.1		28	93.3		0.093
Laboratory measurements							
Uric acid (mg/dl)			6.2±1.4			4.4±0.9	**<0.001**
Gamma-glutamyl transferase (mg/dl)			43.6±27.7			21.6±9.1	**0.011**
High sensitivity CRP (mg/l)			8.1±7.1			4.4±1.3	**0.042**
Fibrinogen (mg/dl)			403.6±139.0			414.1±71.5	0.725
Mean platelet volume (fl)			10.6±1.0			9.8±0.7	**0.014**
Platelet count (x1000/Ul)			251.7±72.8			287.9±58.1	**0.043**
Total cholesterol (mg/dl)			190.7±48.2			211.9±29.7	0.094
LDL cholesterol (mg/dl)			147.9±52.6			131.4±27.9	**0.031**
HDL cholesterol (mg/dl)			40.0±8.3			45.5±8.0	**0.011**
Triglycerides (mg/dl)			184.6±77.3			150.5±26.0	**0.034**
Fasting blood glucose (mg/dl)			104.7±12.0			89.9±5.6	**0.012**
Medications							
Beta-blocker	12	37.5		10	33.3		0.732
ACE inhibitor	11	34.4		7	23.3		0.338
Angiotensin receptor blocker	11	34.4		9	30.0		0.713
Calcium-channel blocker	9	28.1		6	20.0		0.455
Oral antidiabetic	2	6.3		2	6.7		0.947
Statin	13	40.6		11	36.7		0.749
Fibrate	3	9.4		1	3.3		0.333
Aspirin resistance parameters							
Closure time for ADP (sec)			89.3±19.5			107.8±32.2	**0.012**
Closure time for epinephrine (sec)			205.5±74.7			247.1±58.2	**0.024**
Aspirin resistance	15	46.9		6	20.0		**0.033**

ADP: Adenosine diphosphate.

46.9% in the MetS group, and 20% in the control group. The difference between the two groups was statistically significant (p=0.033).

Patients with and without MetS did not differ significantly with regard to major risk factors such as age, gender, diabetes mellitus, hyperlipidemia, smoking, daily aspirin dose, fibrinogen, and total cholesterol levels. Compared to the control group, patients with MetS exhibited significantly higher values for mean platelet volume, gamma-glutamyl transferase, high-sensitivity C-reactive protein, uric acid, LDL cholesterol, triglyceride, fasting blood glucose levels, body mass index, waist circumference, and systolic blood pressure, and lower values for platelet count and HDL cholesterol level (Table 1). Closure times for EPI and ADP were also significantly lower in the MetS group (Table 1).

Distribution of MetS components in aspirin responders and nonresponders is shown in Table 2. There were no significant differences with respect to gender, systolic and diastolic blood pressures, HDL cholesterol and triglyceride levels between the two groups. However, fasting blood glucose level was higher (102.0±14.6 mg/dl *vs*. 95.3±9.9 mg/dl; p=0.036) and waist circumference tended to be greater in aspirin nonresponders (97.4±14.1 cm *vs*. 89.7±15.0 cm; p=0.053).

Table 2. Distribution of metabolic syndrome components in aspirin responders and nonresponders

	Aspirin responders	Aspirin nonresponders	*p*
Systolic blood pressure (mmHg)	133.2±15.3	135.2±12.1	0.592
Diastolic blood pressure (mmHg)	86.5±9.0	87.0±8.4	0.860
HDL cholesterol (mg/dl)	43.1±8.9	41.8±8.2	0.553
Triglycerides (mg/dl)	165.7±51.3	172.9±76.6	0.661
Fasting blood glucose (mg/dl)	95.3±9.9	102.0±14.6	**0.036**
Waist circumference (cm)	89.7±15.0	97.4±14.1	0.053
Gender (Male/Female)	15/26	8/13	0.907

Multivariate logistic regression analysis showed that MetS (OR 0.28, 95% CI 0.09-0.88; p=0.029), fasting blood glucose (OR 0.95, 95% CI 0.91-0.99; p=0.045), uric acid (OR 0.46, 95% CI 0.28-0.76; p=0.002), gamma-glutamyl transferase (OR 1.04, 95% CI 1.00-1.08; p=0.043), high-sensitivity CRP (OR 1.07, 95% CI 1.01-1.12; p=0.015) levels and platelet count (OR 0.99, 95% CI 0.98-0.99; p=0.034) significantly affected aspirin resistance.

Metabolic syndrome (3.6 fold) and increased levels of fasting blood glucose (1.1 fold), uric acid (2.2 fold), gamma-glutamyl transferase (1 fold), high-sensitivity CRP (1.1 fold), and platelet count (1 fold) significantly increased the risk for aspirin resistance.

In Pearson correlation analysis, closure time for EPI was negatively correlated with platelet count (r=-0.3, p=0.01), uric acid (r=-0.4, p=0.004), high-sensitivity CRP (r=-0.4, p=0.001), and age (r=-0.3, p=0.03).

DISCUSSION

Metabolic syndrome is a complex disease which is related to insulin resistance, abdominal obesity, atherogenic dyslipidemia, elevated blood pressure, elevated plasma glucose, and atherothrombotic vascular events.[1] Patients commonly manifest prothrombotic and proinflammatory states. It has been found that MetS is associated with a relative risk of 1.27 (95% CI 0.90-1.78; p=0.033) for all-cause mortality and 1.65 (95% CI 1.38-1.99; p=0.009) for cardiovascular disease.[9,10]

Aspirin is the cornerstone of antiplatelet therapy in cardiovascular medicine. Aspirin is recommended in primary prevention for MetS patients having a high risk for or known atherosclerotic cardiovascular disease, diabetes, a 10-year-risk of >20% for coronary heart disease, transient ischemic attack, stroke of carotid origin or >50% carotid stenosis.[1] However, antiplatelet effect of aspirin is not uniform in all patients.[11] Aspirin resistance is an important problem in these patients. Aspirin resistance is associated with a higher risk for recurrent myocardial ischemia and cardiovascular death, as well as recurrent cerebral ischemic attacks. Gum et al.[12] reported that aspirin resistance was associated with a 3.1-fold risk for serious vascular events. Incidence of aspirin resistance ranges between 8% and 45% depending on timing and technique of examination, time of the last aspirin intake, and aspirin dose, as well as heterogeneity of the patient population.[3]

In this study, we compared aspirin resistance in patients with and without MetS. The frequency of aspirin resistance was 46.9% in the MetS group and showed a statistically significant difference. The frequency of aspirin resistance in our study was higher than reported in previous studies. Kahraman et al.[13] reported the frequency of aspirin resistance as 21.9% in MetS patients without coronary artery disease. In our study, aspirin resistance was observed in almost half of the patients with MetS. The higher frequency of aspirin resistance in our study might be associated with the higher rate of coexistence of Mets and coronary artery disease.

There is a strong relationship between inflammation and atherothrombosis. High-sensitivity CRP is an important marker of inflammation. Antiplatelet therapy, especially aspirin, is commonly used for preventing atherothrombosis. However, research on the role of inflammation on aspirin efficiency is limited.[14-16] Ziegler et al.[14] reported that systemic inflammation had no effect on the results obtained by PFA-100. In contrast, Homoncik et al.[15] found a negative correlation between systemic inflammation and PFA-100 results. Similarly, we found a negative correlation between closure time for EPI and high-sensitivity CRP level, suggesting that aspirin responsiveness might be influenced by systemic inflammation.

Previous studies have demonstrated that diabetes mellitus can be related to aspirin resistance.[17-19] Diabetes mellitus and hyperglycemia are associated

with platelet activation.[20] This may explain, in part, why aspirin resistance occurs more frequently in diabetic patients.[21] Keating at al.[22] showed that platelet reactivity was affected by glucose in healthy subjects. Vaidyula at al.[23] found that CD40L-expressing platelets and monocyte tissue factor expression were increased in normal glucose/high insulin group in which glucose levels were ~100 mg/dl, whereas they did not increase in normal glucose/normal insulin group. In our study, fasting blood glucose levels were higher in patients with aspirin resistance. This finding was confirmed by the results of multivariate analysis showing that fasting blood glucose significantly affected aspirin resistance. In the MetS group, fasting blood glucose levels were also higher than controls. This may explain why aspirin resistance was more frequent in patients with MetS.

This study has some limitations. Inclusion of a small number of patients in both groups was the major limitation. Platelet function was evaluated with only one method. Aspirin resistance was defined only biochemically, but not clinically. It was not a clinical follow-up study.

In conclusion, compared to patients without MetS, aspirin resistance was significantly more frequent in patients with MetS in this study. Aspirin resistance is higher especially in MetS patients with high CRP and increased fasting blood glucose levels, so it will be better to scan this group of patients for aspirin resistance.

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