Platelet function analysis with two different doses of aspirin

İki farklı aspirin dozu ile platelet fonksiyon analizi

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Objectives: We aimed to compare the level of platelet inhibition using the platelet function analyzer (PFA)-100 in patients receiving low and medium doses of aspirin.

Study design: On a prospective basis, 159 cardiology outpatients (83 men, 76 women; mean age 60.9±9.9 years) taking 100 mg/day or 300 mg/day aspirin at least for the previous 15 days were included. Of these, 79 patients (50%) were on 100 mg and 80 patients (50.3%) were on 300 mg aspirin treatment. Blood samples were collected between 09:30 and 11:00 hours in the morning. Platelet reactivity was measured with the PFA-100 system. Incomplete platelet inhibition was defined as a normal collagen/epinephrine closure time (<165 sec) despite aspirin treatment.

Results: Baseline clinical and laboratory characteristics of the patient groups taking 100 mg or 300 mg aspirin were similar. The overall prevalence of incomplete platelet inhibition was 22% (35 patients). The prevalence of incomplete platelet inhibition was significantly higher in patients treated with 100 mg of aspirin (n=24/79, 30.4%) compared with those treated with 300 mg of aspirin (n=11/80, 13.8%) (p=0.013). In univariate analysis, female sex (p=0.002) and aspirin dose (p=0.013) were significantly correlated with incomplete platelet inhibition. In multivariate analysis, female sex (OR: 0.99; 95% CI 0.9913-0.9994; p=0.025) and aspirin dose (OR: 3.38; 95% CI 1.4774-7.7469; p=0.003) were found as independent factors predictive of incomplete platelet inhibition.

Conclusion: Our findings suggest that treatment with higher doses of aspirin can reduce incomplete platelet inhibition especially in female patients.

Key words: Aspirin/therapeutic use; blood platelets/drug effects; dose-response relationship, drug; drug resistance; platelet aggregation/drug effects; platelet function tests/methods.

Amaç: Düşük ve orta dozda aspirin kullanan hastalarda platelet fonksiyon analizi (PFA-100) ile platelet inhibisyon derecelerin kıyaslanması planlandı.

Çalışma planı: En az son 15 gündür 100 mgr/gün veya 300 mgr/gün aspirin kullanan 159 poliklinik hastası (83 erkek, 76 kadın; ort. yaş 60.9±9.9) ileriye dönük olarak çalışmaya alındı. Bu hastaların 79'u (%50) 100 mgr, 80'i (%50.3) ise 300 mgr dozda aspirin almaktaydı. Hastalardan kan örnekleri sabah 09:30-11.00 saatleri arasında alındı. Platelet reaktivitesi PFA-100 sistemiyle ölçüldü. Yetersiz platelet inhibisyonu, aspirin tedavisine rağmen PFA-100 sisteminde kolajen/epinefrin kapanma zamanının normal bulunması (<165 sn) olarak tanımlandı.

Bulgular: Günde 100 mgr ve 300 mgr aspirin kullanan hasta gruplarında tüm klinik ve laboratuvar özellikler benzer idi. Yetersiz platelet inhibisyonu sıklığı tüm hastalar içinde %22 (35 hasta) bulundu. Yetersiz platelet inhibisyonu sıklığı 100 mgr aspirin kullanan hastalarda (n=24/79, %30.4) 300 mgr kullananlara (n=11/80, %13.8) göre anlamalı derecede daha yüksek idi (p=0.013). Tekdeğişkenli analizde, kadın cinsiyet (p=0.002) ve aspirin dozunun (p=0.013) yetersiz platelet inhibisyonu ile anlamlı ilişki gösterdiği görüldü. Çokdeğişkenli analizde de, kadın cinsiyetin (OR: 0.99; %95 GA 0.9913-0.9994; p=0.025) ve aspirin dozunun (OR: 3.38; %95 GA 1.4774-7.7469; p=0.003) yetersiz platelet inhibisyonunu öngörmede bağımsız faktörler olduğu görüldü.

Sonuç: Çalışmamızın sonuçları, yüksek doz aspirinin özellikle kadın hastalarda yetersiz platelet inhibisyonunu azaltabileceğini göstermektedir.

Anahtar sözcükler: Aspirin/terapötik etki; trombosit/ilaç etkisi; doz-yanıt ilişkisi, ilaç; ilaç direnci; trombosit agregasyonu/ilaç etkisi; trombosit fonksiyon testi/yöntem.

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Many clinical trials have demonstrated that acetylsalicylic acid (aspirin) is effective for both primary and secondary prevention of myocardial infarction (MI), stroke, and cardiovascular death[1-3] and in the acute management of MI, unstable angina, and embolic stroke.[3-5] According to the Antithrombotic Trialists' Collaboration,[3] there is no difference in effectiveness between medium (160-325 mg/day) and low (75-150 mg/day) doses of aspirin regimens. High doses (500-1,500 mg/day) are effective but more gastrotoxic.[6]

Although formal diagnostic criteria are lacking, aspirin resistance generally describes the failure of the drug to produce an expected biological response or to prevent atherothrombotic events. The ASPECT study assessed platelet responsiveness to three different, frequently used aspirin doses (81, 162, and 325 mg/day) by various assays in patients with stable coronary artery disease and demonstrated dose-related antiplatelet effects of aspirin especially in patients with diabetes mellitus.[7,8] In this study, we aimed to compare the level of platelet inhibition using the platelet function analyzer (PFA)-100 in patients receiving low (100 mg/day) and medium (300 mg/day) doses of aspirin.

PATIENTS AND METHODS

Patients and study protocol. This prospective study included 159 cardiology outpatients (83 men, 76 women; mean age 60.9±9.9 years) taking 100 mg or 300 mg enteric-coated aspirin at least for the previous 15 days. Of these, 79 patients (50%) were on 100 mg and 80 patients (50.3%) were on 300 mg aspirin treatment. Exclusion criteria were as follows: lack of aspirin therapy, use of drugs containing aspirin or clopidogrel, ticlopidine or non-steroidal drugs in the last seven days, heparin or low-molecular-weight heparin administration within 24 hours before enrolment, a family or personal history of bleeding disorder, platelet count of $<150 \times 10^3$ /μl or $>450 \times 10^3$/μl, hemoglobin <8 g/dl, collagen/ADP closure time >100 sec in PFA-100 analysis, history of myeloproliferative syndrome, major surgical procedure within one month before enrolment, or presence of malignant paraproteinemia. Patients were questioned carefully for their compliance with aspirin use. The study was approved by the local ethics committee, and all patients gave informed consent before enrolment.

Laboratory analysis. Blood samples were collected between 09:30-11:00 a.m. on the day of the last aspirin dose from the patients by a clean puncture of an antecubital vein and with minimal hemostasis. Platelet reactivity was measured with the PFA-100 system (Dade Behring, Marburg, Germany). In our laboratory, the normal range of collagen/epinephrine (CEPI) closure time is between 95 and 164 seconds. In this study, the mean calculated interassay coefficient of variation was 0.6% (range 0.0% to 1.1%), and incomplete inhibition of platelets determined by the PFA-100 was defined as a normal collagen/epinephrine closure time despite aspirin treatment (<165 sec).

Statistical analysis. Continuous variables were expressed as mean±standard deviation. All continuous variables were checked with the Kolmogorov-Smirnov normality test to show their distributions. Continuous variables showing normal and abnormal distributions were compared using the unpaired Student's t-test and Mann-Whitney U-test, respectively. For categorical variables, the chi-square test was used. *P* values of less than 0.05 were considered statistically significant. The SPSS statistical package (version 9.0) was used for statistical analyses. The factors affecting aspirin resistance were assessed in a univariate analysis. A multivariate logistic regression model was used to assess the independent predictors of aspirin resistance. The alternative test hypothesis was built as two-sided for each statistical analysis. The tests were independent so the experiment-wise Type I error did not exceed an alpha level of 0.05.

RESULTS

Seventy-seven patients (48.4%) were taking aspirin for primary prevention and 82 patients (51.6%) for secondary prevention. Baseline clinical and laboratory characteristics of the patient groups taking 100 mg or 300 mg aspirin were similar (Table 1). Patients treated with 100 mg of aspirin (n=24/79, 30.4%) exhibited a significantly higher rate of incomplete platelet inhibition compared with patients treated with 300 mg of aspirin (n=11/80, 13.8%) (p=0.013; Table 1). The overall prevalence of incomplete platelet inhibition was 22% (35 patients).

In univariate analysis, female sex (p=0.002) and aspirin dose (p=0.013) were significantly correlated with incomplete platelet inhibition (Table 2). Statins did not change the prevalence of incomplete inhibition at different doses (Table 2). In multivariate analysis, female sex (OR: 0.99; 95% CI 0.9913-0.9994; p=0.025) and aspirin dose (OR: 3.38; 95% CI 1.4774-7.7469; p=0.003) were found as independent factors predictive of incomplete platelet inhibition.

DISCUSSION

In our study, the overall prevalence of incomplete platelet inhibition (22%) was consistent with previ-

Table 1. Baseline clinical and laboratory features and PFA-100 results of patient groups receiving 100 mg and 300 mg of aspirin

	Aspirin 100 mg/day (n=79)			Aspirin 300 mg/day (n=80)			
	n	%	Mean±SD	n	%	Mean±SD	*p*
Age (years)			61.5±10.3			60.3±9.4	0.437
Sex (Females)	42	53.2		34	42.5		0.205
Hypertension	50	63.3		49	61.3		0.764
Diabetes mellitus	18	22.8		26	32.5		0.215
Hyperlipidemia	53	67.1		46	57.5		0.253
Coronary artery disease	37	46.8		45	56.3		0.268
Statin	35	44.3		37	46.3		0.739
ACE inhibitors	21	26.6		18	22.5		0.709
Beta-blocker	25	31.7		33	41.3		0.122
Calcium channel blockers	19	24.1		15	18.8		0.558
Hemoglobin (g/dl)			13.7±1.6			13.7±1.7	1.0
PFA-100 results							
Platelet count (x10^9/Kmm^3)			244,350			235,760	0.872
Incomplete platelet inhibition	24	30.4		11	13.8		**0.013**
CEPI closure time (sec)			229±76			259±65	**0.009**
CEPI closure time >300 sec	37	46.8		50	62.5		0.056

ACE: Angiotensin converting enzyme; CEPI: Collagen/epinephrine; PFA: Platelet function analyzer.

ous data (24-28%)[9-11] and there were significant correlations between incomplete inhibition of platelets and aspirin dose and female gender. In the ASPECT study, Gurbel et al.[7] investigated the effect of different doses of aspirin (81, 162, and 325 mg/day) on platelet responsiveness to aspirin in 120 patients with stable coronary artery disease using light transmittance aggregometry, VerifyNow, PFA-100, and levels of urinary 11-dehydro-thromboxane B2. Statistically significant differences were observed between different aspirin dose groups with respect to aspirin resistance measured by the PFA-100.[7] Our study was designed to measure the effectiveness of two widely used aspirin regimens (100 mg *vs*. 300 mg) by the PFA-100 and we found that 300 mg was more effective to inhibit platelets. Incomplete inhibition of platelets was also more prominent in female patients treated with 100 mg of aspirin. In a study using the VerifyNow test, a higher incidence of incomplete platelet inhibition was found in patients taking lower doses of aspirin (≤100 mg) and in female patients.[12] Gum et al.[13] examined patients taking 325 mg aspirin and found that aspirin-resistant patients were more likely to be women in the optical platelet aggregation test; however, they did not find the same correlation by the PFA-100 analysis. In a large primary prevention trial among women, 100 mg of aspirin did not lower the risk for MI or death from cardiovascular causes; it lowered the risk for stroke without affecting the risk for MI, but in a subgroup analy-

Table 2. Univariate analysis of clinical and laboratory parameters associated with incomplete platelet inhibition

	Platelet inhibition (n=124)			Incomplete platelet inhibition (n=35)			
	n	%	Mean±SD	n	%	Mean±SD	*p*
Age (years)			61.3±10.1			59.6±9.1	0.372
Sex (Female)	51	41.1		25	71.4		**0.002**
Hypertension	76	61.3		23	65.7		0.696
Diabetes mellitus	36	29.0		8	22.9		0.528
Hyperlipidemia	73	58.8		26	74.3		0.116
Coronary artery disease	69	55.7		13	37.1		0.058
Aspirin dose							**0.013**
300 mg/day	69	55.7		11	31.4		
100 mg/day	55	44.4		24	68.6		
Statin	56	45.2		16	45.7		0.845
Hemoglobin (g/dl)			13.5±1.6			13.5±1.7	0.883
Platelet count (x10^9/Kmm^3)			255,700±64550			260,580±60,240	0.768

sis, 100 mg of aspirin lowered the incidence of cardiovascular events among women aged 65 years or older.[14] The reasons for any sex-based differences in the efficacy of aspirin in preventing cardiovascular events are unclear and require further research. The menopausal status and the use of hormone replacement therapy after menopause have been shown to have no effect on these differences.[14]

In a subgroup analysis of the ASPECT study, DiChiara et al.[8] demonstrated that diabetic patients with coronary artery disease had a higher prevalence of incomplete platelet inhibition during therapy with 81 mg and that increasing the dose of aspirin in diabetic patients reduced platelet resistance. Recently in the JPAD trial, low-doses of aspirin were not effective for primary prevention of atherosclerotic events in patients with type 2 diabetes mellitus.[15] In another study, incomplete platelet inhibition was reported to be more prevalent among smokers.[16] In our study, we did not find any relationship between smoking status or diabetes mellitus and incomplete platelet inhibition. Despite reports favoring statins in reducing aspirin-resistant platelet aggregation in patients with coronary heart disease,[17] our findings did not show any benefit of varying statin doses in decreasing the prevalence of incomplete platelet inhibition.

Study limitations. In this study, we evaluated incomplete platelet inhibition using the PFA-100 system. There is no consensus on the most appropriate method to measure aspirin resistance and none of the aspirin assay tests is known to be superior in identifying aspirin resistance and incomplete platelet inhibition.[9] Compared with platelet aggregometry, which is considered the gold standard to evaluate platelet function, the PFA-100 system offers important advantages because it is simple, cheap, and provides rapid results.[18] Reproducibility of PFA-100 measurements and determination of plasma aspirin levels were other issues which were not undertaken in our study.

We concluded that treatment with higher doses of aspirin could reduce biochemical aspirin resistance especially in female patients.

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