

Original Article

Antiplatelet Drugs Resistance and Correlation in Patients Presenting with Acute Coronary Syndrome

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Background: Antiplatelet agents are the mainstay treatment for patients with cardiovascular diseases. However, a substantial proportion of these patients still develop new cardiovascular events while receiving such antiplatelet therapy. Antiplatelet drugs resistance may contribute to the failure of treatment. **Objective:** We determine the prevalence and clinical significance of antiplatelet drug resistance among patients presenting with acute coronary syndrome (ACS) while receiving aspirin alone or in combination with clopidogrel. The study further investigated possible factors prediction antiplatelet resistance. **Materials and Methods:** A total of 49 ACS patients who had been taking aspirin or with clopidogrel for at least 7 days were enrolled in this study. The light-transmittance aggregometry (LTA) was used to determine antiplatelet drug responsiveness. The antiplatelet drug resistance was defined as the maximum aggregation $\geq 60\%$ when stimulated by $10 \mu\text{mol/L}$ of adenosine diphosphate (ADP). **Results:** Antiplatelet drug resistance was observed in 3 of patients (6.7%). The level of cardiac markers, both CKMB ($p = 0.045$) and Troponin-T ($p = 0.030$), of these 3 patients were significantly higher than in those with antiplatelet responsiveness. Severe angina (≥ 2 episodes within 24 hour) and high risk by Thrombolysis in myocardial infarction (TIMI) risk score had a tendency to be associated with antiplatelet resistance. However, there was no significant difference in cumulative occurrence of recurrent ACS and death in 28 days between patients who responded to or resisted antiplatelet drugs. **Conclusions:** Antiplatelet resistance, assessed by the LTA, is a predictor for the severity of ACS in term of higher cardiac injury but not associated with short-term cardiovascular outcomes both in recurrence and mortality.

Key Words : ● Antiplatelet resistance ● Aspirin ● Clopidogrel ● Acute coronary syndrome

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Introduction

Aspirin and clopidogrel is the most commonly used antiplatelet drugs worldwide for prevention of cardiovascular and cerebrovascular diseases. However, some patients develop recurrent cardiovascular events despite receiving these antiplatelet agents.¹ Therefore,

antiplatelet resistance could be one of the contributing factors to the clinical treatment failure.

In meta-analyses and systematic reviews^{2,3}, patients with biochemically-defined antiplatelet resistance are more likely to have cardiovascular events. Several trials showed an increased risk of cardiovascular death, myocardial infarction, unstable angina and stroke in aspirin-resistant patients^{1,4}. Concordantly, among coronary artery disease patients who underwent percutaneous coronary intervention (PCI) with stent,

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the clopidogrel resistance significantly predicted the occurrence of cardiovascular events^{5,6}.

We conducted a cross-sectional study to explore the incidence of laboratory-defined antiplatelet resistance among patients with acute coronary syndrome while receiving aspirin monotherapy or in combination with clopidogrel. Furthermore, factors predicting antiplatelet drug resistance and the clinical outcomes of patients with antiplatelet resistance were explored.

Materials and Methods

Patients

Acute coronary syndrome (ACS) patients in Department of Internal Medicine, Maharaj Nakorn Chiang Mai Hospital between June 2010 - December 2010 were recruited. The diagnosis of ACS based on the ACC/AHA 2007 guidelines⁷. Patients must take aspirin as a single antiplatelet agent or in combination with clopidogrel (Plavix[®]) as dual antiplatelet therapy for at least one week before admission. The patients were excluded if they were treated with glycoprotein IIb/IIIa inhibitor, thrombolytic agents, anticoagulant or non-steroidal anti-inflammatory drugs (NSAIDs) within 2 weeks prior to enrollment. The patients who had platelet count less than $100 \times 10^9/L$ or more than $600 \times 10^9/L$, the patients who had a history of inherited and acquired platelet dysfunction or ones who had significant kidney and liver failure were also excluded from the study. All the patients signed an institutional review board-approved informed-consent form.

Laboratory studies and data collection

The clinical characteristics collected included sex, age and cardiovascular risk factors (hypertension, diabetes mellitus, dyslipidemia). The dosage, type and duration of antiplatelet treatment were recorded. The laboratory data collected included hemoglobin, peak level of serum cardiac troponin T and creatinine kinase-MB (CK-MB). The ACS was characterized

by clinical presentation of unstable angina, non-ST segment elevation myocardial infarction (NSTEMI) and ST segment elevation myocardial infarction (STEMI) group and the severity of ACS is defined by Thrombolysis in myocardial infarction (TIMI) risk score.

Light-transmittance aggregometry (LTA)

Venous blood samplings were obtained on the first day that patients presented with ACS before receiving additional antiplatelet or anticoagulant therapy. The platelet function was assessed by the light-transmittance aggregometer within 4 hours after the blood drawn using $10 \mu\text{mol/L}$ of ADP as platelet agonist. The antiplatelet drug resistance was defined as platelet aggregation appearing over 60%.

Outcome measurement

The primary endpoint was the prevalence of aspirin resistance among CAD patients presenting with ACS while receiving aspirin or dual antiplatelet therapy. The secondary endpoints were the cardiovascular outcomes between the patients with different antiplatelet as well as factors predicting for aspirin resistance.

Statistical analysis

Baseline characteristics were expressed using descriptive statistics. The independent *t* test and chi-square test were used to compare between groups in which the data involved continuous and categorical variables, respectively. The variables with $p < 0.1$ in univariate analysis were analyzed in multivariate model to verify the independent factors predicting cardiac outcomes and aspirin resistance. The following variables were analyzed including age, sex, cardiovascular risk factors and dosage of antiplatelet agents. A two-sided test was used to indicate statistical significance at the *p* value of < 0.05 . The statistical analyses were implemented by SPSS software version 16 (SPSS for windows, Rel. 16.0, 2007 Chicago:SPSS Inc.).

Results

Prevalence of antiplatelet resistance

Of 49 enrolled patients with a median age of 72 years (range 40-84), 29 (59.2 %) were male (table 1). Antiplatelet resistance was demonstrated in 3 patients (6.7%). There were no significant difference in baseline characteristics including gender, age, hemoglobin level, presentation of ACS and atherosclerotic risk upon admission among the patients with different status of antiplatelet response.

The association between platelet aggregation status and clinical presentation

The platelet aggregation measured by LTA after the addition of 10 $\mu\text{mol/L}$ of ADP was shown in table 2. The patients who were on aspirin plus clopidogrel had significantly lower maximum platelet aggregation

($p = 0.007$). Moreover, there was a tendency of higher platelet aggregation among the patients age over 60 years old and having intermediate to high TIMI risk score.

Clinical outcomes

The cardiac enzymes were recorded in all patients. Significant higher serum CK-MB ($p = 0.045$) and cardiac Troponin T ($p = 0.030$) were observed among patients with antiplatelet resistance. The cumulative incidence of adverse cardiovascular events including recurrent MI, death from cardiovascular diseases within 28 days after hospitalization was 6.1%, all were antiplatelet responsive patients. However, there was no significant difference in term of cardiovascular outcomes from the patients with antiplatelet resistance.

Table 1. Demographic characteristics among antiplatelet drugs resistant and sensitivpatients

Parameter		Antiplatelet drugs	Antiplatelet drugs	p-value
		sensitive, N = 46 (%)	resistance N = 3 (%)	
Gender	Male	28 (96.6)	1 (3.4)	0.347
	Female	18 (90)	2 (10)	
Age (year)		69.15 \pm 9.60	73.00 \pm 3.46	0.092
Hypertension	Yes	40 (93)	3 (7)	0.504
	No	6 (100)	0 (0)	
Diabetes	Yes	18 (90)	2 (10)	0.347
	No	28 (96.6)	1 (3.4)	
Dyslipidemia	Yes	35 (94.6)	2 (5.4)	0.713
	No	11 (91.7)	1 (8.3)	
Hemoglobin (g/dL)		12.06 \pm 1.77	12.53 \pm 2.99	0.210
ACS presentation	UA/NSTEMI	40 (95.2)	2 (4.8)	0.355
	STEMI	6 (85.7)	1 (14.3)	
TIMI Risk group	Low	1 (100)	0 (0)	0.664
	Intermediate	9(100)	0 (0)	
	High	36 (92.3)	3 (7.7)	

ACS = acute coronary syndrome; UA = unstable angina; STEMI = ST-segment elevation myocardial infarction; NSTEMI = non ST-segment elevation myocardial, infarction; TIMI risk score = Thrombolysis in myocardial infarction risk score [age \geq 65 years, at least 3 risk factors for CAD (family history, diabetes, hypertension, hypercholesterolemia, current smoking), prior known coronary stenosis \geq 50%, ST deviation, 2 angina events in the last 24 hours, use of aspirin in the last 7 days; and elevated serum cardiac markers: low (0-2), intermediate (3-4) and high (5-7)]

Table 2. Association between antiplatelet resistance by light-transmittance aggregometry and clinical presentation

	Parameter	Mean Maximum aggregation (%)	
		ADP 10 $\mu\text{mol/L}$	p value
Age (year)	40-60	24.7 \pm 15.0	0.08
	60-80	31.3 \pm 17.2	
	> 80	49.0 \pm 7.8	
Aspirin dose	< 100 mg/day	31.4 \pm 17.1	0.76
	\geq 100 mg/day	29.9 \pm 17.2	
Dual antiplatelet	Yes	22.0 \pm 15.0	0.007
	No	35.6 \pm 16.2	
TIMI Risk group	Low	8 \pm 0	0.35
	Intermediate	28 \pm 12.1	
	High	31 \pm 17.8	

Dual antiplatelet = aspirin + clopidogrel; TIMI Risk = Thrombolysis in myocardial infarction risk [age \geq 65 years, at least 3 risk factors for CAD (family history, diabetes, hypertension, hypercholesterolemia, current smoking), prior known coronary stenosis \geq 50%, ST deviation, 2 angina events in the last 24 hours, use of aspirin in the last 7 days; and elevated serum cardiac markers: low (0-2), intermediate (3-4) and high (5-7)]

Discussion

In this cohort, the prevalence of antiplatelet resistance among the patients diagnosed with ACS was 6.7%. This was in concordance with previous reported studies using the same definition of antiplatelet drug resistance by LTA with ADP as the platelet agonist. Gum et al, reported incidence of aspirin resistance was 5.2% when aggregation \geq 70% with 10 $\mu\text{mol/L}$ of ADP⁴. We choose ADP as the platelet agonist as being able to demonstrate the inhibition of platelet aggregation by both aspirin or clopidogrel.

There was a variability of the results among methods used to classify aspirin resistance. Gurbel et al⁹ reported that the prevalence of aspirin resistance ranged from 0-6% by LTA but was higher (1-27%) when PFA-100 was used. The variation in the prevalence of antiplatelet resistance among between studies could be related to the difference in platelet function assays. Muir et al⁸ assessing the prevalence of aspirin resistance by LTA, PFA-100 and thromboxane B2 metabolite level found that there was poor correlation between LTA and

PFA-100. In the same population, the prevalence of aspirin resistance ranged 1.7-4.7% by LTA but was 20.3-22.1% by PFA-100.

The maximum platelet aggregation was evaluated to determine the *in vitro* responsiveness of antiplatelet agents. We found that the platelet aggregation was significantly lower among patients receiving dual antiplatelets therapy (aspirin and clopidogrel) than aspirin monotherapy ($p = .007$). This may reflect a better efficacy of dual antiplatelet therapy in platelet function inhibition. We could not demonstrate the effect of difference doses of aspirin on the magnitude of platelet aggregation. Unlike a prior study, Mirkhel et al¹⁰ reported that aspirin dosage correlated with platelet responsiveness. The patients receiving low dose aspirin (81 mg/day) had higher prevalence of aspirin non-response rate (12.1%) compared to 5.3% in those receiving higher aspirin dose (325 mg/day). Our result is consistent with a meta-analysis by the Antithrombotic Trialists' Collaboration, which concluded that an aspirin dose of 75-150 mg/day was

at least as effective as higher doses.

There was a trend of higher platelet aggregation in patients with intermediate to high TIMI risk scores which was correlated to the findings in some of the previous reports^{12,13} as well as the findings from Acikel et al¹⁴. They studied in patients with NSTEMI and showed that aspirin-resistant patients had significantly higher TIMI risk scores compared to those with aspirin-sensitivity. This finding could be explained from the increase of platelet reactivity in patients with CAD¹⁵ that the increased platelet reactivity correlated with the disease severity.

We sought the clinical factors attributing to the antiplatelet response. Although, we could not demonstrate any predicting factors of antiplatelet resistance, all 3 antiplatelet resistant patients had severe angina (> 2 episodes in 24 hour) with high risk TIMI score and receiving only aspirin monotherapy. In correlation with our observation that patients receiving aspirin monotherapy had higher platelet aggregation, the dual antiplatelet therapy with aspirin and clopidogrel would minimize the degree of antiplatelet resistance which may partly contribute to the recurrent ACS.

The significantly higher level of cardiac markers among patients with antiplatelet resistance would imply the more severity of ACS among patients with antiplatelet resistance. This finding is in concordance with Acikel et al¹⁴, which revealed that patients who resisted to aspirin had higher cardiac myonecrosis marker than aspirin sensitive patients. Borna et al¹³ demonstrated that the incidence of aspirin resistance were higher among patients with STEMI than with NSTEMI and non-cardiac chest pain (83.3, 26.0, and 9.1% respectively). Antiplatelet resistance was strongly correlated with more disease severity. LTA could function as a point-of-care test surrogate marker for high risk ACS patients.

In contrast to some studies that showed the

correlation between the antiplatelet resistance and the adverse cardiac outcomes,^{5,15,16} differences we did not observe any difference in cumulative events of recurrent ACS within 28 days or death between the two groups.

It may be explained by the small population of patients as well as the short follow up period in our study. The differences in cardiac outcomes between studies might also be the heterogeneity of reperfusion therapy in which included thrombolytic agents, percutaneous coronary intervention, coronary artery bypass graft surgery, and unrelated to antiplatelet response.

This study has potential limitations. The enrollment was based on the history of aspirin therapy, but compliance was could not be assessed. Blood salicylate level could be a way to address issue on the compliance and metabolism of aspirin in each patients. The concomitant use of proton pump inhibitor which may interfere the platelet aggregation during clopidogrel therapy was not recorded. The relatively small patient population and short follow up time in this cohort would reduce the chance of observing the true difference in the clinical outcomes.

Conclusions

Antiplatelet resistance, assessed by the LTA, is a predictor for the severity of ACS in term of higher cardiac enzymes but not associated with the poor cardiovascular outcomes.

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การศึกษาความสัมพันธ์ของการดื้อต่อยาต้านเกล็ดเลือดต่อการดำเนินโรค ในผู้ป่วยหลอดเลือดหัวใจอุดตัน

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บทคัดย่อ ยาต้านเกล็ดเลือดเป็นการรักษาหลักของผู้ป่วยโรคหัวใจและหลอดเลือด อย่างไรก็ตาม ผู้ป่วยบางส่วนถึงแม้ได้รับการรักษาด้วยยาเหล่านี้แล้วแต่ก็ยังมีอาการกลับเป็นซ้ำของโรคซึ่งสาเหตุส่วนหนึ่งอาจเกิดมาจากภาวะดื้อต่อยาต้านเกล็ดเลือด **วัตถุประสงค์** เพื่อหาอุบัติการณ์และความสัมพันธ์ของภาวะดื้อต่อยาต้านเกล็ดเลือดต่อการดำเนินโรคหลอดเลือดหัวใจอุดตันเฉียบพลัน (acute coronary syndrome) และเพื่อหาปัจจัยที่เกี่ยวข้องกับการดื้อต่อยาต้านเกล็ดเลือด **วัสดุและวิธีการ** ผู้ป่วยโรคหลอดเลือดหัวใจอุดตันเฉียบพลันที่ได้รับยาแอสไพรินหรือแอสไพรินร่วมกับโคลพิโดเกรลมาอย่างน้อย 7 วัน ได้เข้าร่วมการศึกษา โดยมีผู้ป่วยทั้งสิ้น 49 ราย ผู้ป่วยที่ถูกนิยามว่ามีภาวะดื้อต่อยาต้านเกล็ดเลือดได้แก่ ผู้ที่มีผลการตรวจเลือดด้วยเครื่อง light-transmittance aggregometer แล้วพบการเกาะกลุ่มของเกล็ดเลือดมากกว่าร้อยละ 60 หลังจากกระตุ้นด้วย adenosine diphosphate (ADP) 10 ไมโครโมลต่อลิตร **ผลการศึกษา** ผู้ป่วยมีภาวะดื้อต่อยาต้านเกล็ดเลือด ร้อยละ 6.7 ผู้ป่วยในกลุ่มนี้มีระดับของเอนไซม์ CKMB และ Troponin-T สูงกว่าผู้ป่วยที่ไม่มีการดื้อต่อยาต้านเกล็ดเลือดอย่างมีนัยสำคัญ ($p = 0.045$ และ $p = 0.030$ ตามลำดับ) สำหรับผู้ป่วยที่ได้รับยาต้านเกล็ดเลือด 2 ชนิดมีอัตราการเกาะกลุ่มกันของเกล็ดเลือดที่ต่ำกว่าผู้ป่วยที่ได้รับแอสไพรินเพียงชนิดเดียวอย่างมีนัยสำคัญ ($p = 0.007$) โดยผู้ป่วยที่มีประวัติเจ็บอกตั้งแต่สองครั้งภายใน 24 ชั่วโมงและมี TMI risk score ที่สูงมีแนวโน้มที่จะเกิดภาวะดื้อต่อยาต้านเกล็ดเลือด นอกจากนี้ภาวะดื้อต่อยาต้านเกล็ดเลือดนี้ไม่มีความสัมพันธ์กับการเกิดโรคหลอดเลือดหัวใจอุดตันเฉียบพลันซ้ำหรือการเสียชีวิตใน 28 วัน **สรุป** ภาวะดื้อต่อยาต้านเกล็ดเลือดที่ตรวจโดยเครื่อง light-transmittance aggregometry จะพยากรณ์ความรุนแรงของโรคหลอดเลือดหัวใจอุดตันเฉียบพลันโดยมีระดับเอนไซม์ของกล้ามเนื้อหัวใจที่สูงกว่า แต่ไม่มีความสัมพันธ์กับการกลับเป็นซ้ำของโรคหรืออัตราการเสียชีวิต

Key Words : ● Antiplatelet resistance ● Aspirin ● Clopidogrel ● Acute coronary syndrome

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