Aspirin Resistance in Thai Patients with Chronic Stable Angina

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Objective: *To determine the prevalence, clinical profile and risk factors of aspirin resistance in Thai patients with chronic stable angina.*

Material and Method: *The patients were prospectively recruited from the consecutive patients diagnosed chronic stable angina at Siriraj Hospital during March 2011 to February 2012. Ten milliliter of blood samples were cautiously drawn from the antecubital vein of the patients to determine the hemoglobin, platelet count and platelet aggregation test performed by light transmittance aggregometry using platelet-rich plasma. Platelets were stimulated with 0.5 mg/ml of arachidonic acid and 10 mM adenosine diphosphate. Platelet aggregation was expressed as the maximal percent change in light transmittance from baseline. Aspirin resistance was defined as the mean platelet aggregation of* $\geq$ *70% with 10 mM ADP and the mean platelet aggregation of* $\geq$ *20% with 0.5 mg/ml of arachidonic acid.*

Results: *One-hundred and fifty seven patients diagnosed chronic stable angina were enrolled in the present study. There were 34 patients (21.6%) demonstrating aspirin resistance. The clinical characteristic of these patients included male 58.8% with mean age of 66 years, body mass index 27.5 kg/m^2, diabetes mellitus 52.9%, smoking 8.8%, hypercholesterolemia 70.6% and proton pump inhibitor use 23.5%. Multivariate analysis demonstrated none of the risk factors including age, female, body mass index, diabetes mellitus, hypercholesterolemia, smoking and proton pump inhibitor (PPI) use had a statistically significant association with aspirin resistance.*

Conclusion: *Our study demonstrated that the prevalence of aspirin resistance in Thai patients with chronic stable angina was 21.6%. No significant association was demonstrated between age, female, body mass index, diabetes mellitus, hypercholesterolemia, smoking, proton pump inhibitor (PPI) use and aspirin resistance.*

Keywords: *High on-treatment platelet reactivity, Aspirin resistance, Aspirin non-responsiveness, Aspirin, Platelet aggregation*

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Atherosclerotic coronary heart disease is still a leading cause of death in the world including Thailand. Platelet is the major factor regarding to the pathophysiology of atheromatous plaque progression and acute coronary syndromes[1]. Aspirin is an effective agent for preventing vascular events in high-risk populations including acute coronary syndrome, stroke and peripheral vascular disease[2,3]. Antithrombotic Trialist's Collaboration did a meta-analysis, comprising 135,000 patients, showed a 25%-30% reduction in cardiovascular events including MI, stroke, or death with aspirin therapy in high risk patients[4].

However, 10%-20% of the patients treated with aspirin, still experience a recurrent vascular event within 5 years. This may be partly related to a condition, namely, aspirin resistance[5-10]. This term has been used to describe the inability of aspirin to inhibit platelet aggregation as demonstrated by platelet function tests. In addition, it has also been used to describe the occurrence of cardiovascular events despite aspirin use. The mechanisms of aspirin resistance are poorly defined but are likely to be multifactorial[7,11,12].

However, there is no information regarding the prevalence, clinical significance and clinical outcomes of aspirin resistance in Thai patients with chronic stable angina pectoris. We propose this study

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to determine the prevalence, clinical profiles and possible risk factors of aspirin resistance in these patients.

Material and Method

The patients were consecutively recruited from Siriraj Hospital between March 2011 and February 2012. All patients diagnosed chronic stable angina as defined by symptoms of chest discomfort or dyspnea on exertion and documented coronary artery stenosis $\geq$ 70% on cardiac catheterization[13]. The patients had been taking aspirin 81-300 mg/dL for at least 7 days before enrollment. Informed consent was obtained from every patient. The exclusion criteria included history of abnormal bleeding, taking other anti-platelet agents (ticlopidine, dipyridamole, cilostazol and NSAIDs) and administration of unfractionated heparin or low molecular weight heparin within 24 hours before enrollment, malignancy, platelet count < 150,000 or > 450,000/mm^3 and hemoglobin < 8 g/dL.

Blood samples and assay procedure

10 ml of blood samples were cautiously drawn from the antecubital vein of the patients. The blood then was collected in 3.2% sodium citrate tube for platelet aggregation test and EDTA tube for analysis of hemoglobin and platelet count. The hemoglobin and platelet count were analyzed by automated Hematology Analyzer. Platelet aggregation test was performed by light transmittance aggregometry using platelet-rich plasma and platelet count was adjusted to between 200 x 10^3 and 300 x 10^3/mm^3. Platelets were stimulated with 0.5 mg/ml of arachidonic acid (AA) and 10 mM adenosine diphosphate (ADP). Aggregation was performed with a Luminaggregation Module Series 1008. The extent of aggregation was defined as the maximal light transmission at 6 min after addition of the agonist, with platelet-poor plasma used as reference. Intra-assay coefficient of variation of the test was less than 10%. The technicians who performed the platelet aggregation test did not know about detailed information of the study population.

Aspirin resistance was defined as the mean platelet aggregation of $\geq$ 70% with 10 mM ADP and the mean platelet aggregation of $\geq$ 20% with 0.5 mg/ml of arachidonic acid[14].

Statistical analysis

The baseline characteristics of the patients were described using descriptive statistics, including means, standard deviation, median, minimum and maximum or frequencies and percentage. The normality of distribution of the variables was examined with the Kolmogorov-Smirnov. The association of normally distributed variables was determined through use of the Student's t-test to compare mean and Chi-square test to compare proportion between two groups. For non-normally distributed variables, the Mann-Whiney U test or Fisher exact test was used, in the cases of continuous or dichotomous variables respectively. The variables found to be significantly associated with the aspirin resistance from the previous study and in our univariate analysis were entered in a multivariables enter (conditional), multiple logistic regression model. For all tests performed, a two-tailed p-value < 0.05 was considered as denoting statistical significance. The statistical software SPSS, version 18.0 was employed for all the analyses performed.

Results

One-hundred and fifty seven patients diagnosed chronic stable angina pectoris were consecutively recruited for the present study. From these patients, 76.7% were men. The mean age of all study patients (n = 157) was 62 years. Most patients (95.9%) took 81 mg/day of aspirin. The others took 162-300 mg/day. The increased body mass index (BMI), diabetes mellitus and hypercholesterolemia were found be more common, even though not statistically significant, in patients with aspirin resistance compared with those with aspirin responsiveness. The other baseline characteristics were shown in Table 1. The distribution of platelet aggregation in response to 0.5 mg/ml of arachidonic acid (AA) and 10 mM adenosine diphosphate (ADP) after receiving aspirin therapy was also shown in Fig. 1 and 2.

From the present study, there were 34 patients (21.6%) demonstrating aspirin resistance diagnosed by measuring platelet aggregation in response to 10 mM adenosine diphosphate (ADP) and 0.5 mg/ml arachidonic acid (AA) after receiving aspirin therapy.

Multivariate analysis to determine the risk factors for aspirin resistance in the present study patients was performed as shown in Table 2. The variables entered into the model included: age, female, BMI, diabetes mellitus, hypercholesterolemia, smoking and proton pump inhibitor (PPI) use. It was found that none of the risk factors had a statistically significant association with the aspirin resistant.

Discussion

In this prospective study, the authors found

Table 1. Baseline characteristics of the study patients (n = 157)

	Aspirin resistance (n = 34)	Aspirin responsiveness (n = 123)	p-value
Age (yr)	66 ± 9.5	64 ± 10.7	0.32
Male sex	20 (58.8)	77 (62.6)	0.67
Body mass index (kg/m^2)	27.56 ± 3.79	25.99 ± 4.84	0.08
Smoking	3 (8.8)	17 (13.8)	0.57
Diabetes mellitus	18 (52.9)	45 (36.6)	0.09
Hypertension	27 (79.4)	94 (76.4)	0.71
Hypercholesterolemia	24 (70.6)	74 (60.2)	0.27
Prior MI	8 (23.5)	26 (21.1)	0.76
Prior PCI	11 (32.4)	28 (22.8)	0.25
Prior CABG	1 (2.9)	10 (8.1)	0.46
Prior stroke	0	7 (5.7)	0.35
CHF	8 (23.5)	30 (24.4)	0.92
PPI use	8 (23.5)	26 (21.1)	0.76
CKD	7 (20.6)	37 (30.1)	0.28
Creatinine clearance (mL/min)	63.38 ± 24.5	60.95 ± 28.67	0.65
Chronic liver disease	0	3 (2.4)	1.0
Medication			
Beta blocker	31 (91.2)	97 (78.9)	0.10
ACEI	11 (32.4)	41 (33.3)	0.91
ARB	10 (29.4)	36 (29.3)	0.99
Statin	29 (82.1)	101 (85.3)	0.66
Platelet count (x 10^3/ml)	225 ± 67	228 ± 65	0.82
Hemoglobin (g/dl)#, median (Min, Max)	12.55 (8.2-36.8)	13.1 (7.2-44)	0.16

Data are expressed as number (%) and mean ± standard deviation
ACEI = angiotensin converting enzyme inhibitor, ARB = angiotensin receptor blocker,
CABG = coronary artery bypass graft, CHF = congestive heart failure, CKD = chronic kidney disease,
MI = myocardial infarction, PCI = percutaneous coronary intervention, PPI = proton pump inhibitor
* CKD was defined as creatinine clearance < 60 mL/min
# Comparison of hemoglobin between aspirin resistance and aspirin responsiveness was done by Mann-Whitney U test due to abnormal distribution

that the prevalence of aspirin resistance in Thai patients with chronic stable angina was 21.6%. Previous studies reported the prevalence of this condition varied from 1-60%[8,15]. This variation may be due to different laboratory assay for defining aspirin resistance and study population. Gum et al, using light transmittance aggregometry, reported that the prevalence of aspirin resistance was only 5.5%[12]. This result was much different from the present study despite that the methods of platelet aggregation test and criteria for defining this condition were similar. There are some reasons to explain this difference. First, it may be related to the difference of study population. The present study enrolled only the patients with chronic stable angina whereas Gum et al evaluated the patients with various cardiovascular diseases including chronic stable angina, old MI or stroke. Second, the possible risk factors for aspirin resistance such as increased age, diabetes mellitus and smoking in our patients were more prevalent compared with the study of Gum et al. Finally, the sample size may partly explain this different result. The number of study population of Gum et al was 325 whereas the number of our population was only 157.

Previous studies reported some possible risk factors for aspirin resistant including increased age, increased BMI, smoking, hypercholesterolemia, congestive heart failure, using proton pump inhibitors and dose of aspirin[9,10,16-18]. However, by using univariate and multivariate analysis, the present study demonstrated that none of the risk factors described above were significantly associated with aspirin resistance. This may be related to the different baseline characteristics of our study population. Finally, we could not determine the relation between the dose of

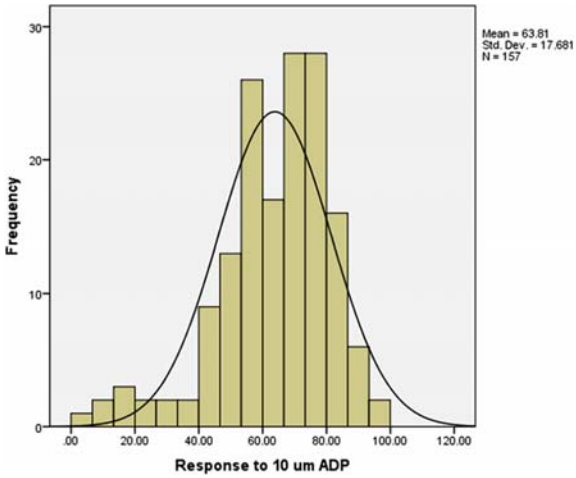

Fig. 1A Distribution of platelet aggregation in response to 0.5 mg/ml of arachidonic acid (AA) after receiving aspirin therapy

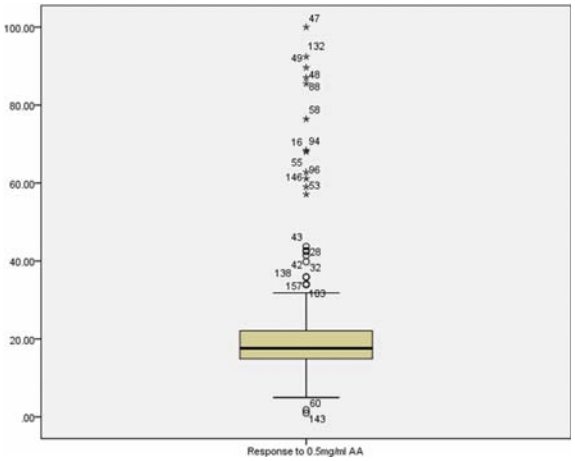

Fig. 1B Median and interquartile range of platelet aggregation in response to 0.5 mg/ml of arachidonic acid (AA) stimulation after receiving aspirin therapy

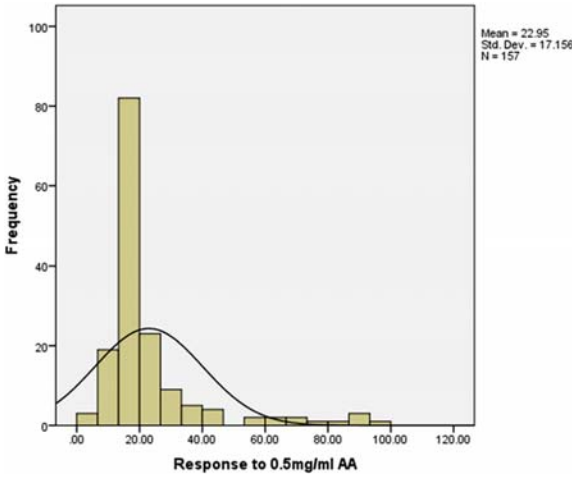

Fig. 2A Distribution of platelet aggregation in response to 10 mM adenosine diphosphate (ADP) after receiving aspirin therapy

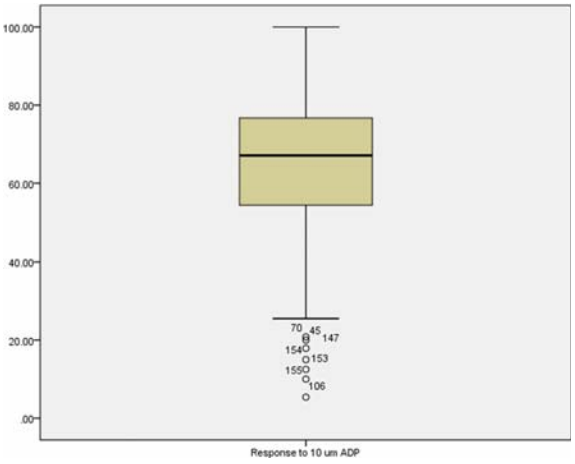

Fig. 2B Median and interquartile range of platelet aggregation in response to 10 mM adenosine diphosphate (ADP) after receiving aspirin therapy

Table 2. Multivariate analysis of risk factors for aspirin resistance

Factor	n	Aspirin resistant n (%)	Crude OR (95% CI)	Adjusted OR (95% CI)	p-value
Age	157	34/157 (21.6)	1.02 (0.98-1.06)	1.02 (0.98-1.06)	0.34
Female	60	14/60 (23.3)	1.17 (0.54-2.54)	0.79 (0.33-1.9)	0.60
BMI	157	34/157 (21.6)	1.07 (0.99-1.16)	1.07 (0.99-1.17)	0.11
DM	63	63/157 (40.1)	1.95 (0.92-4.20)	1.77 (0.79-3.94)	0.16
Hyper-CH	98	98/157 (62.4)	1.59 (0.70-3.61)	1.37 (0.58-3.20)	0.47
Smoking	20	20/157 (12.7)	0.60 (0.17-2.19)	0.74 (0.19-2.91)	0.66
PPI use	34	34/157 (21.6)	1.15 (0.47-2.83)	1.13 (0.44-2.87)	0.80

* BMI = body mass index, DM = diabetes mellitus, Hyper-CH = hypercholesterolemia, PPI = proton pump inhibitor

aspirin use and aspirin resistance because most patients (95.9%) used aspirin at the dose of 81 mg/day.

Conclusion

The prevalence of aspirin resistance in the present study was 21.6%. No significant association was demonstrated between age, female, body mass index, diabetes mellitus, hypercholesterolemia, smoking, proton pump inhibitor (PPI) use and aspirin resistance.

Limitation

There are some limitations to the present study. Aspirin use was assessed by discussion with the patient without confirmation by pill counts or salicylate level. Platelet aggregation studies were performed only at a baseline; therefore, different variable response to aspirin may exist. Laboratory variability in the isolation of platelet rich plasma may influence the measurement of platelet aggregation. Finally, the study population may be not large enough to demonstrate the significant risk factors for aspirin resistance.

Acknowledgement

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Potential conflicts of interest

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ภาวะดื้อต่อการออกฤทธิ์ของแอสไพรินในผู้ป่วยคนไทยที่เป็นหลอดเลือดหัวใจตีบเรื้อรัง

เรวัตร พันธุ์กิ่งทองคำ, ประดิษฐ์ ปัญจวีณิน, ยิ่งยง ชินธรรมมิตร์, ดำรัส ตรีสุโกศล, ชุณหเกษม โชตินัยวัตรกุล, วิวรรณ ทังสุบุตร, ณัฐวุฒิ วงษ์ประภารัตน์, ดารารัตน์ การะวี, สุมาลี ชันแสง

วัตถุประสงค์: *เพื่อศึกษาความชุก ลักษณะทางคลินิก รวมทั้งปัจจัยเสี่ยงของภาวะดื้อต่อการออกฤทธิ์ของแอสไพรินในผู้ป่วยคนไทยที่เป็นโรคหลอดเลือดหัวใจตีบเรื้อรัง*

วัสดุและวิธีการ: *การศึกษานี้คัดเลือกผู้ป่วยที่ได้รับการวินิจฉัยว่าเป็นโรคหลอดเลือดหัวใจตีบชนิดเรื้อรังที่โรงพยาบาลศิริราชในช่วงเดือน มีนาคม พ.ศ. 2554 ถึงเดือน กุมภาพันธ์ พ.ศ. 2555 ผู้ป่วยที่เข้าการศึกษาจะได้รับยาแอสไพรินขนาด 81-325 มก.ต่อวัน เป็นเวลาอย่างน้อย 7 วันก่อนจะทำการตรวจการทำงานของเกล็ดเลือด สำหรับวิธีการตรวจการทำงานของเกล็ดเลือดจะใช้หลักการ light transmittance aggregometry ร่วมกับการใช้สาร arachidonic acid ขนาด 0.5 มก.ต่อมล. และสาร adenosine diphosphate ขนาด 10 ไมโครโมล เป็นตัวกระตุ้น เกณฑ์การวินิจฉัยภาวะดื้อต่อการออกฤทธิ์ของแอสไพรินจะใช้ค่าเฉลี่ยของการเกาะกลุ่มของเกล็ดเลือดมากกว่าหรือเท่ากับร้อยละ 70 ถ้าใช้สาร ADP เป็นตัวกระตุ้น ร่วมกับค่าเฉลี่ยของการเกาะกลุ่มของเกล็ดเลือดมากกว่าหรือเท่ากับร้อยละ 20 ถ้าใช้สาร arachidonic acid เป็นตัวกระตุ้น*

ผลการศึกษา: *ผู้ป่วยที่เข้าเกณฑ์การศึกษามีจำนวน 157 ราย พบว่ามีภาวะดื้อต่อการออกฤทธิ์ของแอสไพรินจำนวน 34 ราย (ร้อยละ 21.6) โดยผู้ป่วยจะมีอายุเฉลี่ย 66 ปี เป็นเพศชายร้อยละ 58.8 ค่าเฉลี่ยดัชนีมวลกายเท่ากับ 27.5 กก.ต่อตารางเมตร เป็นโรคเบาหวานร้อยละ 52.9 สูบบุหรี่ร้อยละ 8.8 เป็นโรคโคเลสเตอรอลในเลือดสูงร้อยละ 70.6 และร้อยละ 23.5 ใช้ยากลุ่ม proton pump inhibitor จาก Multivariate analysis ไม่พบว่า อายุ เพศ ค่าดัชนีมวลกาย โรคเบาหวาน โรคโคเลสเตอรอลในเลือดสูง การสูบบุหรี่ และ ใช้ยากลุ่ม proton pump inhibitor มีความความสัมพันธ์กับภาวะดื้อต่อการออกฤทธิ์ของแอสไพริน*

สรุป: *จากการศึกษานี้พบว่าความชุกของภาวะดื้อต่อการออกฤทธิ์ของแอสไพริน ในผู้ป่วยคนไทยที่เป็นหลอดเลือดหัวใจตีบเรื้อรังมีจำนวนร้อยละ 21.6 และไม่พบว่า อายุ เพศ ค่าดัชนีมวลกาย โรคเบาหวาน โรคโคเลสเตอรอลในเลือดสูง การสูบบุหรี่ และการใช้ยากลุ่ม proton pump inhibitor มีความสัมพันธ์กับภาวะดื้อต่อการออกฤทธิ์ของแอสไพรินอย่างมีนัยสำคัญ*