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Prevalence of clopidogrel nonresponder in patients with coronary artery disease

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Siriswangvat S, Sansanayudh N, Panomvana Na Ayudhya D. Prevalence of clopidogrel nonresponder in patients with coronary artery disease. Chula Med J 2009 May - Jun; 53(3): 155 - 68

Introduction : *The current guidelines recommend dual antiplatelet of aspirin and clopidogrel for patients who have acute coronary syndrome (ACS) or those undergoing percutaneous coronary intervention (PCI).*

Objective : *To determine the frequency of clopidogrel nonresponder in patients with coronary artery disease (CAD).*

Setting : *Division of Cardiology, Phramongkutklao Hospital.*

Research design : *A prospective study.*

Patients : *All consecutive patients with angiographic diagnosis of CAD were recruited. The study consisted of patients who had taken clopidogrel 75 mg/day for at least 5 days and those who received loading dose of clopidogrel 300 mg and aspirin 300 - 325 mg before undergoing PCI. All patients were treated with aspirin 81 - 325 mg/day at least 7 days prior to the study. Exclusion criteria included previous treatment with proton pump inhibitors (PPIs) within 2 weeks and serum creatinine > 1.5 mg/dl.*

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Methods : *The effect of clopidogrel on platelet aggregation was measured by light transmission aggregometry using ADP 20 μM as the agonist. Clopidogrel nonresponder was defined as ADP 20 μM-induced maximal platelet aggregation (MPA) > 50%.*

Results : *There were 85 patients enrolled during August 2008 to January 2009. Overall, 19% were scheduled for elective PCI patients and 81% were stable CAD. We found that 34% of the patients were clopidogrel nonresponders. Average value of MPA after ADP 20 μM stimuli was 38.57 ± 20.25%.*

Conclusion : *Our study has found the prevalence of clopidogrel nonresponder, 34%, as tested by ADP 20 μM-induced maximal platelet aggregation in CAD patients.*

Keywords : *Clopidogrel, Nonresponder, Maximal platelet aggregation, Resistance.*

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บทนำ : *แนวทางการรักษาในผู้ป่วยกล้ามเนื้อหัวใจขาดเลือดเฉียบพลันหรือผู้ป่วยที่ได้รับการถ่างขยายหลอดเลือดหัวใจในปัจจุบันแนะนำให้ใช้ยาต้านการแข็งตัวของเกล็ดเลือดสองชนิดร่วมกันคือ แอสไพรินและโคลพิโดเกรล*

วัตถุประสงค์ : *เพื่อศึกษาความชุกของการไม่ตอบสนองต่อยาโคลพิโดเกรลในกลุ่มผู้ป่วยโรคหัวใจและหลอดเลือด*

สถานที่ที่ทำการศึกษา : *แผนกหัวใจและหลอดเลือด โรงพยาบาลพระมงกุฎเกล้า*

รูปแบบการวิจัย : *การศึกษาแบบไปข้างหน้า*

ผู้ป่วยที่ทำการศึกษา : *ผู้ป่วยโรคหัวใจและหลอดเลือด ซึ่งประกอบด้วยผู้ป่วยโรคหัวใจและหลอดเลือดที่ได้รับยาโคลพิโดเกรล 75 มิลลิกรัมต่อวัน ติดต่อกันอย่างน้อย 5 วัน และ ผู้ป่วยที่ได้รับการเตรียมถ่างขยายหลอดเลือดหัวใจ ซึ่งจะได้รับโคลพิโดเกรล 300 มิลลิกรัมร่วมกับแอสไพริน 300-325 มิลลิกรัม ก่อนการถ่างขยายหลอดเลือดหัวใจ ผู้ป่วยแต่ละคนจะได้รับแอสไพริน 81-325 มิลลิกรัม อย่างน้อย 7 วันก่อนเข้าวิจัย โดยต้องไม่ได้รับยาลดกรดในกระเพาะกลุ่ม proton pump inhibitors (PPIs) ภายใน 2 สัปดาห์ก่อนเข้าการวิจัย และมีค่าครีแอทินินไม่เกิน 1.5 mg/dl*

วิธีการศึกษา : *การประเมินผลของโคลพิโดเกรลจะวัดค่าการเกาะกลุ่มของเกล็ดเลือดหลังจากถูกกระตุ้นด้วย ADP 20 μM ด้วยวิธี light transmission aggregometry การศึกษานี้ได้จำกัดความการไม่ตอบสนองต่อยาโคลพิโดเกรล หมายถึงเมื่อถูกกระตุ้นด้วย ADP 20 μM แล้วมีค่าการเกาะกลุ่มของเกล็ดเลือดมากกว่าร้อยละ 50*

ผลการศึกษา : *จากการรวบรวมผู้ป่วยจำนวน 85 คนในช่วงระหว่างเดือนสิงหาคม 2551 ถึง มกราคม 2552 ร้อยละ 19 เป็นผู้ป่วยกลุ่มเตรียมถ่างขยายหลอดเลือดหัวใจ และร้อยละ 81 เป็นผู้ป่วยโรคหัวใจ และหลอดเลือดที่ได้รับโคลพิโดเกรลติดต่อกันมากกว่า 5 วัน พบผู้ที่ไม่ตอบสนองต่อยาโคลพิโดเกรลร้อยละ 34 ค่าเฉลี่ยของการเกาะกลุ่มของเกล็ดเลือดมีความแปรปรวนค่อนข้างมากอยู่ในช่วงร้อยละ 38.57 $\pm$ 20.25*

สรุปผลการศึกษา : *การศึกษานี้พบความชุกของการไม่ตอบสนองต่อยาโคลพิโดเกรลในกลุ่มผู้ป่วยโรคหัวใจและ หลอดเลือด ซึ่งวัดด้วยค่าการเกาะกลุ่มของเกล็ดเลือดเมื่อถูกกระตุ้นด้วย ADP 20 μM พบได้ร้อยละ 34*

คำสำคัญ : *โคลพิโดเกรล, ไม่ตอบสนองต่อยา, การเกาะกลุ่มของเกล็ดเลือด*

ACC/AHA 2007 guidelines for the management of patients with unstable angina/non - ST-elevation myocardial infarction (NSTEMI)[1] and ACC/AHA/SCAI 2005 guideline update for percutaneous coronary intervention (PCI)[2] recommended a combination of aspirin and clopidogrel in acute coronary syndrome (ACS) for patients undergoing PCI. Yusuf *et al.* (2001)[3] found that a combination of dual antiplatelets reduced the number of patients who experienced adverse cardiovascular outcomes by 20% over aspirin alone. Nevertheless, approximately 10% of patients taking dual antiplatelets experienced further atherothrombotic events during their follow-up periods.[4]

There was wide variability in individual responsiveness to oral antiplatelet therapy. There has been no standard criterion for the diagnosis of clopidogrel nonresponder or resistance. In 2007, a systematic review of 25 studies that included 3,688 clopidogrel-treated patients who underwent PCI found that nonresponsiveness was common (mean prevalence 21%; 95% CI 17–25%) and associated with an increased risk of adverse cardiovascular outcomes (pooled OR 8.0; 95% CI 3.4–19.0).[5] In the literature review, the definition of antiplatelet response is not consensual and usually based on the change in adenosine 5'-diphosphate (ADP)-induced maximal platelet aggregation before and after the initiation of clopidogrel, such as absolute difference in maximal platelet aggregation (ΔMPA) < 10%[6, 7], inhibition of maximal platelet aggregation [1 – (MPA$_{treatment}$ / MPA$_{baseline}$)] x 100 (IPA) <10%[8]; and ADP-induced MPA > 50%.[6, 9, 10]

The causes of clopidogrel resistance have not yet been defined but they are probably multi-factorial. These included inadequate dosing, individual variability in absorption[11, 12] and drug-drug interaction (e.g. Clopidogrel–Omeprazole) interfering with the pharmacokinetic effects of clopidogrel.[13] Among the genetic polymorphisms that have been studied, CYP2C19 is a major determinant of clopidogrel metabolic activation.[14, 15] Genetic polymorphism of *CYP2C19* is different among ethnic populations, *CYP2C19*2* and *CYP2C19*3* are commonly found in Asian populations.[16] The frequency of *CYP2C19* poor metabolizers who lack of this enzyme was accounted for 6.54-13.2% in Thai population.[17-19] Also P2Y$_{12}$, a pharmacodynamic receptor, was found involved in the efficacy of clopidogrel.[20] These genetic polymorphism may be the important contributor to different frequencies of clopidogrel resistance found among various populations. Moreover, cellular factors such as platelet turnover, increase platelet exposure to ADP, reduce cytochrome activity and up regulation of purinergic signaling (P2Y$_1$ and P2Y$_{12}$) may also play a role in clopidogrel response variability.[4] In addition, clinical factors which have been reported to have major roles in reducing the response profiles to clopidogrel were diabetes,[6] acute coronary syndrome and body mass index (BMI).[21, 22]

Despite these previous works, there have been no data regarding the prevalence of nonresponsiveness to clopidogrel in Thai population. The aim of this study was, therefore, to evaluate the frequency of clopidogrel nonresponder in coronary artery disease (CAD) patients.

Patients

This prospective study was conducted in adult CAD patients recruited from the Division of

Cardiology, Phramongkutklao Hospital. The recruited patients were either stable CAD patients who had been taking clopidogrel 75 mg/day once daily for at least 5 days or those who were scheduled for elective PCI and received loading dose (LD) of clopidogrel 300 mg and aspirin 300-325 mg before undertaking PCI. All patients received aspirin therapy with a daily dose of 81-325 mg at least 7 days prior to the study.

As for the patients who were scheduled for elective PCI, their platelet function was assessed 24 hour after receiving LD of clopidogrel 300 mg and aspirin 300-325 mg (after an overnight fasting). As for the stable CAD group, the platelet function was measured before taking clopidogrel 75 mg/day (after an overnight fasting). The study was performed according to the Declaration of Helsinki and after an approval from the Institutional Ethics Committee (IEC) of Phramongkutklao Hospital. All patients were extensively informed and they provided their written consents before being recruited into the study.

The exclusion criteria included taking of the following drugs: proton pump inhibitors (PPIs), ketoconazole, erythromycin, rifampicin within the period of 2 weeks earlier; warfarin within the 1 month; any use of glycoprotein (GP) IIb/IIIa before PCI procedure; use of nasogastric (NG) tube feeding; having high risk of bleeding; platelets count < 100,000/mm^3; S_{Cr} > 1.5 mg/dl; AST/ALT > 3 fold of upper limit and active gastrointestinal ulcer.

Sample size calculation

A sample size calculation was based on Bliden KP *et al.* (2007)[10] which used the similar patient groups with equivalent definition of clopidogrel nonresponder. Therefore, for a 2-sided alpha value of 0.05 and 10% for a difference of clopidogrel nonresponders, we estimated that 66 patients should be recruited. We expected that 25% of patients might be lost of follow-up, so the minimum target number of included patients was 83.

Platelet aggregation analysis

Their blood samples were collected in tubes containing 3.2% trisodium citrate for assessment of platelet aggregation. Aggregation induced by 20 μM ADP (Helena laboratory, beaumont, Texas) was assessed in platelet-rich plasma (PRP) using the light transmission aggregometry method in a 4-channel aggregometer (Aggrecoder PA-3210 model, Kyoto Daichi, Kagaku Co., Ltd, Kyoto, Japan). PRP was obtained as a supernatant after centrifugation of citrated blood at 1000 rpm for 10 min. The isolated PRP was kept at room temperature before use. Platelet poor plasma (PPP) was obtained by a second centrifugation of the blood fraction at 3500 rpm for 10 min. The platelet count in PRP was adjusted to the range of 200,000 – 300,000/mm^3 by dilution with PPP when it was out of range. Light transmission was adjusted to 0% with PRP and to 100% for PPP in each measurement. Platelet aggregation was assessed within 4 hours from blood sampling. The curves were recorded for 10 minutes and platelet aggregation was determined as the percent of maximal platelet aggregation in light transmittance from baseline using PPP as reference. Clopidogrel nonresponsiveness was defined as maximal platelet aggregation by ADP 20μM > 50 % (MPA > 50%).

Statistical analysis

Continuous variables are reported as mean

± standard deviation (SD) while categorical variables are presented as frequencies and percentages. Independent t-test was used to compare the characteristics and clinical data of clopidogrel nonresponder and clopidogrel responder. Comparison of genders, health behaviors, groups of patients, clinical risk factors and co-medication between the 2 groups were done by Chi-square test or Fisher's exact test. The Kolmogorov–Smirnov test was used to test for normality. Correlations were calculated by Pearson test. A p value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS v 13.0.

Results

A total of 85 consecutive patients were enrolled from August 2008 to January 2009. Baseline demographics and clinical characteristics as well as laboratory data of the patients are described in table 1.

Table 1. Baseline demographic and clinical characteristics according to platelet activity.

	Overall (n = 85)	Clopidogrel Nonresponder (n = 29)	Clopidogrel Responder (n = 56)	*P* value
Age (yrs)	62.82 ± 10.59	65.48 ± 11.62	61.45 ± 9.84	0.096
Male, n (%)	66 (77.6)	20 (69)	46 (82.1)	0.167
BMI (Kg/m^2)	25.15 ± 3.32	24.96 ± 2.83	25.24 ± 3.58	0.71
Groups of patients with, n (%)				
Elective PCI	16 (18.8)	4 (13.8)	12 (21.4)	0.393
Received clopidogrel ≥ 5 days	69 (81.2)	25 (86.2)	44 (78.6)	
Current Smoking, n (%)	2 (2.4)	0 (0)	2 (3.6)	0.545
Medical history, n (%)				
Diabetes mellitus	35 (41.2)	14 (48.3)	21 (37.5)	0.339
Hypertension	70 (82.4)	25 (86.2)	45 (80.4)	0.502
Dyslipidemia	83 (97.6)	29 (100)	54 (96.4)	0.545
Single vessel disease	13 (15.3)	6 (20.7)	7 (12.5)	0.352
Double vessel disease	20 (23.5)	6 (20.7)	14 (25.0)	0.657
Triple vessel disease	16 (18.8)	3 (10.3)	13 (23.2)	0.150
Non-STEMI	9 (10.6)	6 (20.7)	3 (5.4)	0.057
STEMI	8 (9.4)	3 (10.3)	5 (8.9)	1.000
Family history, n (%)	6 (7.1)	2 (6.9)	4 (7.1)	1.000
Baseline medications, n (%)				
Aspirin				
81 mg/day	20 (23.5)	7 (24.1)	13 (23.2)	0.995
100-162 mg/day	6 (7.1)	2 (6.9)	4 (7.1)	
300-325 mg/day	59 (69.4)	20 (69.0)	39 (69.6)	

Table 1. Baseline demographic and clinical characteristics according to platelet activity (continue).

	Overall (n = 85)	Clopidogrel Nonresponder (n = 29)	Clopidogrel Responder (n = 56)	*P* value
Beta-blockers	68 (80)	26 (89.7)	42 (75)	0.154
ACEI	48 (56.5)	17 (58.6)	31 (55.4)	0.821
Angiotensin receptor blockers	16 (18.8)	3 (10.3)	13 (23.2)	0.241
Lipid lowering agents				
Simvastatin	25 (29.4)	9 (31)	16 (28.6)	0.813
Atorvastatin	36 (42.4)	12 (41.4)	24 (42.9)	0.896
Rosuvastatin	15 (17.6)	6 (20.7)	9 (16.1)	0.596
Pravastatin	3 (3.5)	1 (3.4)	2 (3.6)	1.000
Calcium channel blockers	22 (25.9)	8 (27.6)	14 (25)	0.796
Nitrates	56 (65.9)	18 (62.1)	38 (67.9)	0.594
α-blockers	7 (8.2)	3 (10.3)	4 (7.1)	0.686
Diuretics	30 (35.3)	13 (44.8)	17 (30.4)	0.186
Creatinine clearance (ml/min)	69.41 ± 26.38	63.95 ± 25.56	72.23 ± 26.57	0.171
Platelet count ($x10^3$ / mm^3)	250.78 ± 67.59	263 ± 83.27	244.45 ± 57.69	0.232

Data are expressed as mean ± SD or number of patients (%)

BMI = body mass index, PCI = percutaneous coronary intervention, STEMI = ST-elevated myocardial infarction, ACEI = Angiotensin converting enzyme inhibitors

Overall, the average age was 63 ± 11 years; 78% were male; 41% were diabetic patients; 19% were scheduled for elective PCI; and, 81% were stable CAD. The mean MPA after ADP 20 μM stimuli was 38.57 ± 20.25%, which highly varied and followed a normal bell-shaped distribution (Figure 1). Platelet activity was also divided into two groups: platelet activity after 24 hour LD of 300 mg clopidogrel; platelet activity after maintenance dose (MD) of clopidogrel 75 mg once daily were 38.24 ± 16.11% and 38.65 ± 21.20%, respectively (Table 2). The rates of clopidogrel nonresponder in both groups are described in table 2. There were no significant differences between platelet activity in two groups (p = 0.944).

The incidence of clopidogrel nonresponsiveness is accounted for 34% (95%CI 24-44%). Nonresponders are somewhat more likely to have history with NSTEMI (20.7% *vs.* 5.4%; p = 0.057) compared with responders, while other demographic data in both groups were similar. In addition, there was a trend for a correlation between age and ADP-induced MPA (r = 0.248; p = 0.022). In univariate analysis, none of baseline demographic or clinical data were significantly associated with nonresponder to clopidogrel.

Platelet reactivity quartile cut points for the 25^{th}, 50^{th}, and 75^{th} percentiles of the study population were 23.05%, 36.00%, and 56.6% (p < 0.0001).

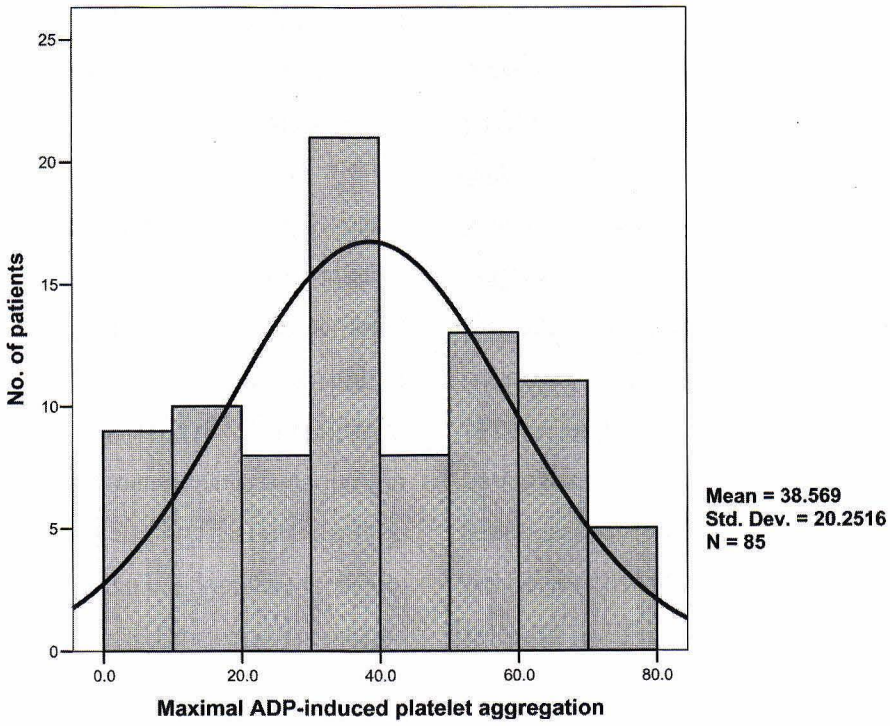

Figure 1. Interindividual distribution of ADP 20 μM-induced platelet aggregation in patients with scheduled elective percutaneous coronary intervention and stable coronary artery disease.

Table 2. Comparison of platelet activities between scheduled PCI patients (after 24 hour LD of 300 clopidogrel) and stable coronary artery disease patients (after MD of clopidogrel 75 mg OD).

Platelet activity	**Nonresponder (n = 29)**		**Responder (n = 56)**		**Overall (n=85)**	
(mean ± SD)	**% MPA**	**n (%)**	**% MPA**	**n (%)**	**% MPA**	**n (%)**
After 24 h LD of 300 mg clopidogrel	57.63 ± 4.24	4 (25)	31.78 ± 12.92	12 (75)	38.24 ± 16.11	16 (100)
After MD clopidogrel 75 mg/day ≥ 5 days	62.55 ± 7.37	25 (36)	25.06 ± 12.61	44 (64)	38.65 ± 21.20	69 (100)
Total	61.87 ± 7.18	29 (34)	26.50 ± 12.87	56 (66)	38.57 ± 20.25	85 (100)
P Value	0.208		0.109		0.944	

Discussion

Our study demonstrated that the prevalence of clopidogrel nonresponder (defined as MPA > 50%) in Thai patients with CAD was 34%. This finding agrees with previous studies which used the similar patient groups with equivalent definition of clopidogrel nonresponder. They reported clopidogrel nonresponder in the range of 22 - 62.5%.[9, 10] However, a recent study, using LD 600 mg clopidogrel with the same definition of clopidogrel nonresponsiveness found lower prevalence of clopidogrel nonresponder (14 - 15%).[6] The higher loading dose increased the efficacy of clopidogrel in inhibiting platelet aggregation.[6, 23, 24] In addition, using clopidogrel 600 mg reloading and increased MD to 150 mg for 4 weeks in clopidogrel nonresponder group found to decreased ADP-induced platelet aggregation significantly (83 ± 6% to 56 ± 14%; $p < 0.01$), and this result was maintained throughout 4 weeks.[25] Moreover, many studies have shown an association between less platelet aggregation inhibition and more recurrent cardiovascular events, such as more stent restenosis (8.6% *vs.* 2.3%)[26] and more cardiac death in patients with coronary stenting (18.2% *vs.* 2.9%; $p = 0.006$).[27]

In our study, the prevalence of clopidogrel nonresponder among patients receiving 300 mg LD clopidogrel was 25% (95% CI 10-50%) and 36% (95% CI 26-48%) in patients who had taken 75 mg MD of clopidogrel for at least 5 days. The difference was not statistically significant ($p = 0.393$). The higher prevalence of clopidogrel nonresponder in the latter group could due to patients' poor compliance associated with longer time of receiving clopidogrel. In addition, MD regimen might result in lower clopidogrel blood concentration as compared to LD regimen and in turn result in lower inhibition of platelet aggregation.

Drug-Drug interaction, including lipophilic statins, may also interfere with clopidogrel-induced antiplatelet effect. However, this observation is quite controversial as larger studies have shown the lack of interaction between lipophilic statins and clopidogrel.[28, 29] In addition, most studies did not show any negative clinical interaction with co-administration of these drugs.[30] In our study, we did not find any significant influence of either lipophilic or hydrophilic statins on platelet inhibition of clopidogrel ($p = 0.895$).

Another important factor which may have a major effect on clopidogrel responsiveness is the genetic polymorphism of *CYP2C19*, a major determinant for clopidogrel metabolizer. Genetic polymorphism of *CYP2C19,* which is poor metabolizer phenotype can be found in 2 - 5% in Caucasian, 11 - 23% in Asian[17] and 6.54 - 13.2% in Thai population.[17-19] A higher percentage of poor metabolizer could cause the active metabolite of clopidogrel to be decreased.

In addition, our study did not confirm the result from a previous study which identified diabetes mellitus (DM) as a risk factor for nonresponsiveness of clopidogrel.[22] In our study, the prevalence of clopidogrel nonresponder in diabetic patients was 40% (14/35) (95% CI 26-56%) while in non-diabetic patients was 30% (15/50) (95% CI 19-44%). Even though there was a trend for higher clopidogrel nonresponder in diabetic patients but this was not statistically significant ($p = 0.339$). Furthermore, from a univariate analysis, platelet activity between diabetic and non diabetic patients was not statistically

significant (p = 0.212). However, the reason that the difference did not reach the level of statistical significance may due to the small sample size of our study.

The platelet aggregation test that was used to evaluate the response of clopidogrel and concomitant doses of aspirin did not interfere the detection of prevalence of clopidogrel nonresponsiveness.[5] Moreover, in this study 20 μM ADP was used as agonist because Fitzgerald and Malee (2007)[31] had found that higher concentrations of ADP induced full and irreversible platelet aggregation that was insensitive to aspirin but was inhibited by at least 90% in the presence of a $P2Y_{12}$ antagonist.

The strength of this study lies in our study population who had not been treated with concomitant PPIs within the previous 2 weeks. A previous study demonstrated that PPIs users had significantly higher platelet aggregation,[13] and a recently study found an increased risk of death or rehospitalization for ACS who had been using concomitant PPIs, compared with those who did not use PPIs (29.8% *vs.* 20.8%, OR 1.25, 95%CI 1.11-1.41).[32] We expected higher prevalence of clopidogrel nonresponder if it is studied in similar ACS population who also took concomitant PPIs.

Limitations of this study included small sample size and one method of determining clopidogrel nonresponsiveness. Future studies are therefore recommended; firstly, to examine and compare different concentrations of ADP and methods for determining clopidogrel nonresponsiveness; secondly, to find out the association between platelet reactivity and genetic polymorphism of *CYP2C19* in Thai population; finally, to extend the study to other groups of population to identify the prevalence of clopidogrel nonresponder in different groups of patients.

In conclusion, our study has found a high prevalence of clopidogrel nonresponder, 34%, as tested by ADP 20 μM-induced maximal platelet aggregation in coronary artery disease patients. In the future, a large prospective study is needed to determine how these platelets responses in functional tests are associated with cardiovascular outcomes. Moreover, platelet function test might prove beneficial in tailoring individual antiplatelet medication especially in patients with known risk factor for nonresponsiveness.

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