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Research Article

Platelet Reactivity of Aspirin and Clopidogrel in Coronary Artery Disease Patients with or Without Diabetes Mellitus

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ABSTRACT

Background: Antiplatelet combination Aspirin (ASA) and Clopidogrel (CPG) has become part of therapeutic standard in patients with coronary artery disease (CAD). Thus, a decline in antiplatelet response due to platelet functional problem may be occured among CAD patients with diabetes mellitus (DM). Objective: To evaluate ASA platelet reactivity (Aspirin Reactivity Unit=ARU) and CPG (Platelet Reactivity Unit=PRU) among CAD patients with vs without DM who take a combination therapy of ASA 100 mg and CPG 75 mg/day. Methods: The study was done at Cardiology Outpatient Clinic - Dr. Soetomo Teaching Hospital Surabaya. A cross sectional observational study was carried out and an unpair qualitative analysis study was used to interprete the data. Platelet functional test was performed by VerifyNow, which was very specific for ASA (Aspirin Reactivity Unit = ARU) and CPG (Platelet Reactivity Unit = PRU). Results: A total of 20 patients were included in the study with the proportion of 10 CAD patients with DM and 10 CAD patients without DM. For ARU, about 4 CAD patients (40%) with DM and 3 CAD patients (30%) without DM seemed to have a resistance on ASA (p=1.000). For PRU, there were High On-Treatment Platelet Reactivity (HPR) on CAD patients with DM compared to CAD patients without DM (77.8% vs 11.1%; p = 0.015). Conclusion: Eventhough the number of CAD patient with DM who showed resistance to ASA was slightly higher compared to CAD patient without DM, there was no statistical difference between two groups. But for CPG, there were very significant higher HPR on CAD patient with DM than without DM.

Keywords: Aspirin, Clopidogrel, Type 2 Diabetes Mellitus, Coronary Artery Disease, Platelet Reactivity

INTRODUCTION

Coronary Artery Disease (CAD) is a sort of heart disease caused by abnormalities in the coronary arteries. Generally, Diabetes Mellitus (DM) has a very important impact on atherosclerosis. According to its natural history, the process of type II DM is accompanied by increased of platelet hyperactivity, thromboxane level in the blood vessels that facilitates the aggregation of platelets, coagulation factors and platelet function abnormalities, as well as the increased of intracellular calcium. In another side, there is decrease—in the production of nitric oxide, platelet turnover following platelet structural damage caused by hyperlipidemia, and fibrinolysis proccess that finally contribute to an increase in thrombotic process and the development of atherosclerosis[1]. Platelet aggregation plays an important role in the development of ischemia in CAD patients and thromboischemic complication after Percutaneous Coronary Intervention (PCI). Platelet activation mediates the inflammation at blood vessels' walls that leads to vessel damage after platelet aggregation and the generation of thrombin[2]. Aspirin (ASA) and Clopidogrel (CPG) are major antiplatelet agents in atherosclerosis. Both of these drugs are often together given because theit better effectiveness. This study aimed to evaluate the reactivity of ASA and CPG in patients receiving both ASA and CPG. This is the first study to investigate ASA and CPG Resistance conducted in Indonesian patients with or without diabetes. The number of patients who consume either ASA, CPG or both in our country is very high. Hence, it is interesting for our health care team to initiate pilot study to investigate this phenomenon.

MATERIAL AND METHODS

This was an analytic observational study with cross sectional design. Selection of study subjects began in May 2014 - July 2014 with consecutive sampling. Inclusion criterias were CAD patients with and without type 2 DM, age > 20 years old, had received 100 mg ASA therapy and 75 mg CPG daily for 7 days, and willing to participate in research as written in the informed consent. Then, the exclusion criterias were patients with history of gastrointestinal disorders, platelet count <100,000 / uL, taking NSAIDs, anticoagulants and antiplatelet other than ASA and CPG for 10 days before (because their counter effects in platelet agregation). Patients with complication of DM such as gangrene and DM nephropathy**,** serum creatinine > 2 mg / dl, and heart

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Table 1: Patients Demography

Variables	Total Patients with CAD (n=20) with DM (n=10)	without DM (n=10)
Sex		
Male	5 (50%)	7 (70%)
Female	5 (50%)	3 (30%)
Age (Years)		
35-40	1 (10%)	1 (10%)
41-50	1 (10%)	3 (30%)
51-60	4 (40%)	4 (40%)
61-70	3 (30%)	1 (10%)
71-80	1 (10%)	1 (10%)
Mean	55,6±11,3	58,4±7,5
BMI (kg/m2)	22.8 ± 9.7	25.3 ± 10,7
Comorbids		
Hypertension	1 (80%)	4 (40%)
Hyperlipidemia	1 (60%)	1 (10 %)
Hypertension +Dyslipidemia	7 (70 %)	5 (50 %)
None	1 (10 %)	0 (0 %)
Hypertension+ Dyslipidemia		
Risk Factors		
Smoking	2 (20%)	2 (20%)
Drugs Used		
CPG	10 (100%)	10 (100%)
Statin	8 (80%)	7 (70%)
ACEI	8 (80%)	7 (70%)
ARB	4 (40%)	2 (20%)
CCB	6 (60%)	5 (50%)
Nitrate	7 (70%)	6(60%)
ß-Blocker	10 (10%)	8 (80%)
Diuretic	4 (40%)	1 (10%)
OAD	4 (40%)	0%
Insulin	6 (60%)	0%

failure were not included in the study. The parameters of ARU (Aspirin Reactivity Unit) and PRU (Platelet Reativity Unit) had been applied in the study by using VerifyNow. The patients' blood sample was inserted into a vacuum tube containing platelet agonist (arachidonic acid in 3.2% sodium citrate) as its platelet function would be further evaluated. The values obtained in the form of infrared absorption resulted from the process of platelet agglutination. If the value of ARU > 550, it was said to be resistant to ASA. High On-Treatment Platelet Reactivity (HPR) was defined by the value of PRU > 208 and Normal On-Treatment Platelet Reactivity (NPR) PRU value ≤ 208. This research had passed the ethnical clearance from the ethic committee of Dr. Soetomo Teaching Hospital Surabaya. Distributions of variables were assessed for normality using Shapiro-Wilk test. ASA CPG resistance difference between CAD patients with and without diabetes type 2 were analyzed using chi square test. If the chi square test requirements are not met, Fisher exact test was done. The result is statistically significant is p value more than 0.05.

RESULTS

Table 1 shows the demographic data of patients. There were 12 men and 8 women with a mean age of 57.2 ± 11.5 years in CAD group with type 2 DM and 53.4 ± 9.8 years in the CAD group without type 2 DM. The average BMI in the CAD group with type 2 DM was 22.8 ± 9.7 and 25.3 ± 10.7 in the group without type 2 DM. In both groups, hypertension was the most comorbidities, 8 patients in the CAD group with type 2 DM and 9 in the CAD group without type 2 DM. The number of patients with hyperlipidemia was equal between two groups. Figure 1, 2 and Table 2 present ARU from 10 CAD patients with DM (mean 497.00 ± 126.044) and 10 CAD patients without DM (mean 469.20 ± 109.381). There were aspirin resistance (ARU > 550) among 4 CAD patients with DM and 3 CAD patients without DM. Thus, no significant difference found between groups (p=1.000). Figure 1, 2 and Table 3 present PRU from 9 CAD patients with DM and 9 CAD patients without DM. There were High Platelet Reactivity on CPG (PRU > 208) among 8 CAD patients with DM (mean 228.44 ± 68.672) and 2 CAD patients without DM (mean 145.33 ± 42.971). Thus, statistically significant difference found between groups (p=0.015).

DISCUSSION

From the study, it can be analyzed that 4 CAD patients with type 2 DM and 3 CAD patients without type 2 DM show Aspirin resistance. Thus, based on statistical analysis using Fisher exact Test, it can be said that the difference is not statistically significant between two groups (p = 1.000). This non-significancy may be caused by other factors or comorbidities such as age, Body Mass Index (BMI), smoking, and genetic[3]. The average age in CAD group with DM is 57.2 ± 11.2 years old, while in CAD group without DM was 53.4 ± 9.8 years old. In the study of ASA resistance conducted by Vatury[3] to 583 patients who received a dose of 75-325 mg per day, an increase in the value of ARU > 550 was shown in those aged > 75 years old (p = 0.007). Contrary, there was non-significant increase in the value of ARU among those aged < 75 years old (p = 0.40). The influence of age on ASA resistance might be due to the higher level of microvascular disturbances in the advanced age which lead to the decrease of nitric oxide synthesis and disruption of platelet response to vasoactive substances[4,5]. In both groups of this study, hypertension and dyslipidemia still be two dominant risk factors. High plasma glucose level would lead to the increase level of Reactive Oxygen Species (ROS) and Advanced Formation Glycosylation End Product (AGE Product) at endothelial blood vessels which finally cause endothelial damage and inflammation of blood vessels. The inflammation of blood vessels will trigger the synthesis of nitric oxide, causing vasodilatation[6]. ASA resistance occurs more often among women with the statistical results of 34.4% vs. 17.3% (p = 0.001)[7]. Another study

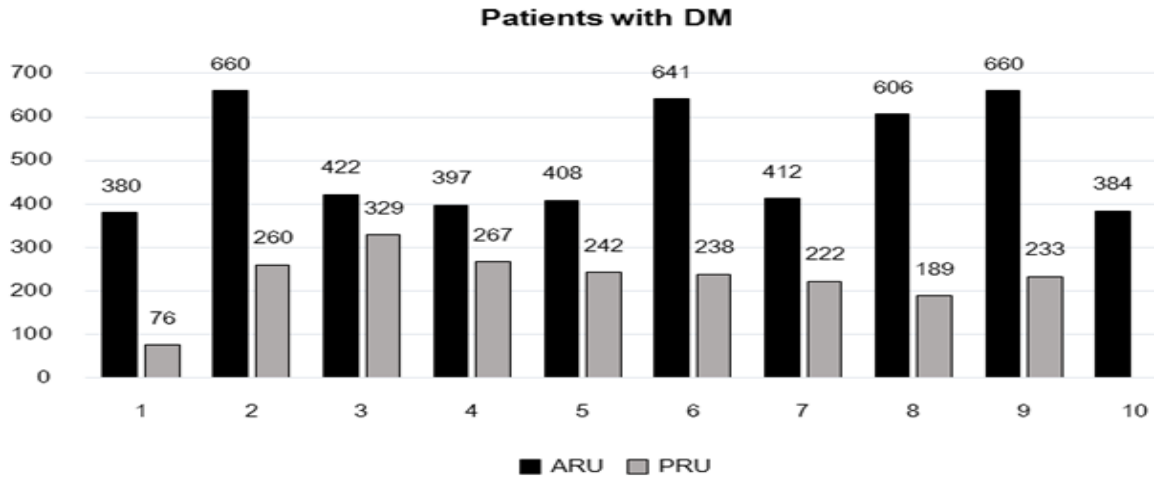

Figure 1: Value of ARU and PRU among 10 CAD patients with DM (note: Patients no. 10: PRU not available)

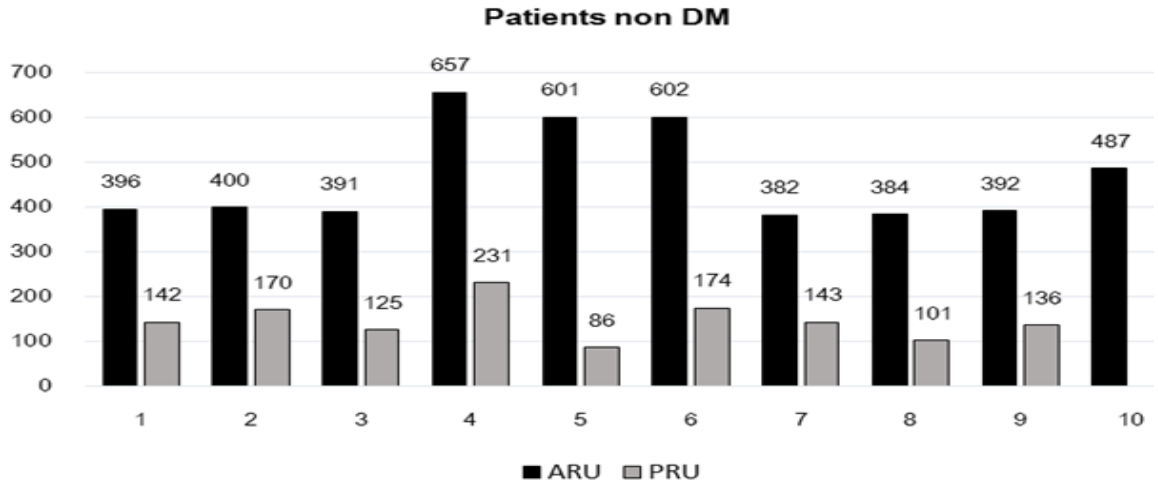

Figure 2: Value of ARU and PRU among 10 CAD patients without DM

NPR = normal platelet reactivity if $PRU \leq 208$

HPR = high platelet reactivity if $PRU > 208$

Table 2: Statistical Analysis of ARU among CAD Patients with DM and without DM

ARU / n = 10	DM	without DM
Mean ± SD	497.00 ± 126.044	469.20 ± 109.381
Sum of Patients Aspirin Resistance (ARU > 550)	4 (40%)	3 (30%)
Aspirin Sensitive (ARU < 550)	6 (60%)	7 (70%)

Incidence of aspirin sensitivity among CAD patients with DM and without DM were not statistically significant (p=1.000)

conducted by Lev[8] supported the finding that women tend to have ASA resistance than men (23.4% vs. 7.8%; p = 0.01). This phenomenon might be due estrogen protective effect against atherosclerosis. Besides, women are also more prone to disruption of vasodilation response to acetylcholine. In this study, the numbers of women who develop ASA resistance are less than male (3 vs 4 patients). In CAD patients group without type 2 DM who develop ASA resistance are those who smoke. In another side, the group of CAD patients with type 2 DM who smoke doesn't show any ASA resistance. Unfortunately, the number of smoker in this study is not enough to detect the significant difference, but smoking plays role in ASA resistance. In acute conditions, smoking increases platelet thrombus formation at arterial wall. Inhibition of platelet COX by ASA may not be sufficient in preventing acute conditions. An increase of platelet thrombus formation is due to the elevated aggregation response to thrombin. The acute increase of platelet thrombus formation after smoking may be associated with the higher levels of epinephrine to increase platelet aggregation although ASA therapy has been given[9] to both groups in accordance with the CAD guidelines including the provision of β-blockers, statins, and ACEI[10]. In both of groups, some of CAD patients with type 2 DM were treated with a combination of antidiabetic drugs (OAD) and insulin. Insulin acts as inhibitor of platelet aggregation. It owns a protective effect against thrombus formation and release of vasoactive and chemotactic mediators which contribute to the pathophysiology of

Table 3: Statistical Analysis of PRU among CAD Patients with DM and without DM

PRU / n = 9	DM	without DM
Mean ± SD	228.44 ± 68.672	145.33 ± 42.971
Sum of Patients (%) HPR (PRU > 208)	7 (77.8%)	1 (11.1%)
NPR (PRU ≤ 208)	2 (22.2%)	8 (88.9%)

Incidence of HPR among CAD patients with DM and without DM was statistically significant difference (p=0.015)

thrombosis and atherosclerosis. A research conducted by Ariturk[11] had described that ASA resistance occurred in OAD group by 4.7%, and 8.6% in the insulin group. There was no statistically significant difference between the OAD and insulin groups (p = 0.359). ASA resistance may also be caused by genetic variation. A study that supports individual variation in respond to ASA had been conducted by Shen[12] on Heredity and Performed type Intervention (HAPI) Heart study. A research of 745 patients, with the proportion of 400 men and 345 women, was organized using the TAT (Thrombocytes Aggregation Test) with the dose of 81 mg / day ASA after patients taking ASA for 14 days. Thus, as many as 21% of patients showed ASA resistance, 30% of them were women and 16% of them were men. It might be caused by fewer barriers of platelet aggregation in women. ASA resistance may also be due to genetic polymorphism. Genetic polymorphisms in conjunction with ASA resistance is most commonly caused by several factors, or can also be caused by other hemostatic genetic variation (polymorphism in blood coagulation and fibrinolytic factors). A series of single nucleotide polymorphisms (SNPs) in the genes of prostaglandin endoperoxide synthase 1 (PTGS1) includes A842G, C22T [R8W], G128A [Q41Q], C644A [G213G], and C714A [L237M] associated with the response to ASA[13]. At the cellular level of platelets, ASA translocation to cytoplasm in Multidrug Resistance is influenced by the presence of protein-4 (MRP-4). An increase in MRP-4 will lead to the decrease of intracellular availability of ASA. The increase of MRP-4 is individualy varied and influenced by some diseases, such as DM. MRP-4 is generally elevated among patients after revascularization using coronary artery bypass grafting. Dipyridamole inhibits MRP-4. Therefore, it becomes the reason why ASA effects do increase after administration of dipyridamole[14]. In this study, platelet function has been assessed with the VerifyNow P2Y12 assay. Here we found that CAD patients with type 2 DM have higher platelet reactivity despite adequate CPG pretreatment compared to CAD patients without type 2 DM (Table 3) in agreement with previous research. Detailed studies have found that platelets from diabetics are generally more reactive and less responsive to antiplatelet[15,16]. Several mechanisms are involved in this platelet dysfunction. These include hyperglycemia, insulin resistance, and increased inflammatory status[17]. Hyperglycemia enhances platelet aggregation by inducing P-selectin expression, by activating protein kinase C (a mediator of platelet activation) and by glycating platelet surface proteins, with consequent decrease in membrane fluidity and amplification of platelet adhesion[18]. Hyperglycemia also induces an increase in non-enzymatic glycation of low-density lipoprotein (glycLDL) which renders platelets more susceptible to oxidative stress. Additionally, glycLDL may induce platelet dysfunction by increasing the intracellular calcium concentration and inhibiting platelet membrane sodium–potassium-ATP activity[19,20]. Moreover, diabetic patients with poor glycemic control have increased platelet reactivity despite dual antiplatelet therapy. Increased plasma fibrinogen is significantly associated with a lower response to CPG in patients with DM, possibly due to a direct interaction of fibrinogen with the glycoprotein IIb/IIIa receptor. Furthermore, in diabetic patients increased production of platelet agonists, such as epinephrine and thrombin receptor agonist peptide, may explain the higher levels of platelet activation through different signaling pathways besides those depending on the $P2Y_{12}$ receptor[15]. Platelets are the target of insulin, which interacts with its own receptor on the surface of the platelet.This results in suppression of cAMP, inhibition of $P2Y_{12}$ signalling and reduced platelet reactivity, however platelets of diabetic patients are victims of the insulin resistance phenomenon that characterises diabetes mellitus type 2 and results in decreased sensitivity to insulin. The end result is up-regulation of the $P2Y_{12}$ pathway and increased platelet reactivity[17,20]. Chronic hyperglycemia can result in reactive oxygen species (ROS) production directly via glucose metabolism and auto-oxidation, and indirectly through the formation of advanced glycation end products and their receptor binding. ROS may then activate signaling molecules within endothelial cells, including PKC and nuclear factor-kB (NF-kB), resulting in transcription of redox sensitive genes which switch to proinflammatory and prothrombotic phenotypes. ROS may also induce structural and functional changes in coagulative proteins. Finally, ROS may lead to the formation of 8-iso-PGF2a, a non-enzymatic oxidation product of circulating LDL and arachidonic acid, which mediates vasoconstriction and platelet hyperreactivity. Not surprisingly, improved glycemic control is associated with a significant reduction in both lipid peroxidation and platelet activation in vivo[19]. Other mechanisms linked to HTPR include increased exposure to ADP, increased cytosolic level of calcium, and increased platelet turnover[17]. Thus, in patients with DM a global hyperreactive platelet status is present, which may explain low responsiveness even after higher maintenance doses of antiplatelet drugs[21].

CONCLUSION

From the results of research on ASA resistance in CAD patients with and without type 2 DM that was conducted in Cardiology Outpatient Clinic Dr. Soetomo Public Hospital Surabaya from May to August 2014, it can be

concluded that there is no statistically significant difference related to ASA resistance among patients with and without type 2 DM. Diabetic patients tend to have higher platelet reactivity compared to non-diabetic patient. This study suggests that the use of platelet function test may help to identify patients with HPR despite adequate CPG pre-treatment. Patients with DM undergoing PCI need to get a more aggressive antiplatelet regimen to decrease the incidence of events after PCI.

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