RESEARCH ARTICLE

Correlation between peripheral immature platelet level and coronary immature platelet levels in coronary artery disease: an observational study in cardiac referral centre in East Java, Indonesia

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Abstract

Objective: To investigate the correlation between peripheral and coronary immature platelet, and factors that may predict coronary immature platelet levels.

Method: The cross-sectional, observational, analytical study was conducted at the Cardiovascular Diagnostic and Intervention Centre of Dr Soetomo General Academic Hospital, Surabaya, Indonesia, from November 2017 to January 2018, and comprised patients of either gender with coronary artery disease. Peripheral and coronary blood samples were retrieved during coronary catheterisation. Immature platelet fraction was acquired by examining whole blood samples analysed through automated flow cytometry. Relationship between peripheral and coronary immature platelet fractions and counts were analysed using parametric correlation test, followed by linear regression analysis model of variables that influenced coronary immature platelet fraction. The statistical analysis was carried out using SPSS Statistics for Windows, Version 25.0 (IBM Corp, Armonk, NY, USA).

Results: Of the 70 patients, 55(78.6%) were males and 15(21.4%) were females. The overall mean age was 57±5.32 years. There were 35(50%) patients with a history of smoking, and 34(48.6%) had hypertension and dyslipidaemia. Mean peripheral immature platelet fraction was 3.86±1.84% and mean coronary immature platelet fraction was 3.63±1.7%. There was a robust positive and significant correlation ($r=0.882$; $p<0.001$) between immature platelet levels in peripheral and coronary blood. Peripheral immature platelet and glycated haemoglobin >7.5 were independent predictors of coronary immature platelet ($p=0.001$).

Conclusion: There was a strong correlation between immature platelet levels in peripheral and coronary blood.

Key Words: Haemoglobin, Platelets, Coronary vessels, Heart.

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Introduction

Coronary artery disease (CAD) is a chronic disease that has a significant role in terms of morbidity, mortality and healthcare costs in both developed and developing countries. The 2016 heart disease and Stroke Statistics update of the American Heart Association (AHA) reported that the overall death rate from CAD was 102.6 per 100,000 people[1]. In Indonesia, CAD was the second leading cause of death in 2012, accounting for 138,400, or 9% of all deaths.[2]

One theory states that platelet reactivity, which is the main driving force in thrombus formation in CAD, is related to immature platelets[3]. Immature platelets are young platelets rich in granules containing residues of messenger ribonucleic acid (mRNA) megakaryocytes that allow protein biosynthesis, have a larger size, and can express cyclooxygenase-2 (COX-2)[3, 4]. Various studies have proven the relationship between peripheral immature platelets and the incidence of CAD, and have suggested an increase in immature platelets in patients with comorbidities, such as diabetes mellitus (DM), and history of smoking.[5-7]

Platelets have an important role in atherogenesis and thrombus formation. This immature platelet affects the response to antiplatelet drugs, and the potential association with clinical outcomes in patients with cardiovascular disease [8]. A study showed that immature platelets were independent predictors of cardiovascular death in patients with acute coronary syndrome (ACS) who had undergone percutaneous coronary intervention (PCI) for 12 months[3]. Immature platelets, which can be calculated as immature platelet fraction (IPF), are considered a new biomarker for stratification of the risk of major cardiovascular events in patients with CAD [9]. IPF is also associated with platelet reactivity and is a better predictor of the response of aspirin and thienopyridine, which are part of the main therapies for the prevention of re-occlusion in CAD[4, 10]. In patients with stable CAD treated with aspirin and clopidogrel, a higher proportion

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of immature platelets strongly correlated with reduced response to both drugs[11]. Another study found that circulating immature platelet, using IPF as an indicator, was also strongly associated with reduced response to prasugrel in acute myocardial infarction (AMI) patients.[12]

However, IPF is not specific to CAD, as it can be influenced by many conditions that alter platelet use. People with DM are also known to have higher platelet activity, aggregation and adhesion[7, 13]. Studies have reported immature platelets from peripheral blood samples that had not been standardised. To our knowledge, no study has yet explored intra-coronary IPF in CAD. The current study was planned to fill the gap in literature by exploring the relationship between intra-coronary and peripheral immature platelet levels in patients with CAD, and to identify the factors that may predict coronary immature platelet levels.

Patients and Methods

The cross-sectional, observational, analytical study was conducted at the Cardiovascular Diagnostic and Intervention Centre of Dr Soetomo General Academic Hospital, Surabaya, Indonesia, from November 2017 to January 2018, and comprised patients of either gender with coronary artery disease.

After approval from the institutional ethics review committee, the sample size was calculated using EpiInfo[14]. The sample was raised using consecutive sampling technique. Those included were stable CAD patients based on the 2013 European Society of Cardiology (ESC) guidelines, who underwent elective cardiac coronary catheterisation[15]. Written informed consent was obtained from all those included. Those excluded were patients with thrombocytopenia.

Peripheral and coronary blood samples were obtained during the coronary catheterisation procedure. Blood samples were then directly sent to the clinical pathology laboratory at the same centre using pneumatic tubes. IPF was acquired by examining whole blood samples analysed through automated flow cytometry (Sysmex XE-2100, Sysmex, Kobe, Japan).

Demographic data, cardiovascular risk factors and list of medications were retrieved from the medical record. History of smoking was defined as those who had smoked >100 cigarettes in their lifetime. Hypertension (HTN) was defined as resting blood pressure >140/90mmHg at two visits, or those on medication. Dyslipidaemia was defined as serum low-density lipoprotein (LDL) concentration >3.0mmol/l, or those on medication. DM was defined as having been clinically diagnosed before, or those on medication. Glycated haemoglobin (HbA1c) was divided into <7.5% and ≥7.5% categories, and scored 0 and 1, respectively.

The statistical analysis was carried out using SPSS Statistics for Windows, Version 25.0 (IBM Corp, Armonk, NY, USA).For descriptive analysis, continuous data was presented as mean ± standard deviation for normally distributed data, and median with interquartile range (IQR) for non-normally distributed data. Categorical data was presented as frequencies and percentages. For bivariate analysis, chi-square test was used for categorical data, and independent t-Test or Mann-Whitney U test for continuous data. Analysis of variance (ANOVA) was used as appropriate. The relationship between peripheral and coronary IPF and immature platelet count (IPC) levels was analysed using a Pearson parametric correlation test and a linear regression analysis model. Variables with p<0.25 in univariate analysis were included in multivariate regression model. P<0.05 was considered significant.

Results

Of the 70 patients, 55(78.6%) were males and 15(21.4%) were females. The overall mean age was 57 (±5.32) years (range: 26-78 years). There were 35(50%) patients with a history of smoking, and 34(48.6%) each had HTN and dyslipidaemia (Table 1).

Table-1: Patient characteristics.

Variable	Male (n = 55)	Female (n = 15)	P
Age (years)	58.72(±7.96)	58.83(±4.31)	0.961
Risk Factor			
Hypertension	26 (47.27%)	8 (53.3%)	0.429
Dyslipidaemia	26 (47.27%)	8 (53.3%)	0.370
History of smoking	34 (61.8%)	1 (6.7%)	0.000
Diabetes Mellitus	14 (25.4%)	6 (40.0%)	0.704
Medication history			
Aspilet use	37 (52.9%)	11(15.9%)	0.654
Clopidogrel use	41 (59.4%)	11(15.9%)	0.837

Mean peripheral IPF was 3.86±1.84% and mean coronary IPF was 3.63±1.7%. There was a strong correlation (r=0.882; p<0.001) between peripheral and coronary IPF (Figure 1). Subgroup analysis showed that HbA1c >7.5% was a predictor for a higher platelet turnover, marked by a significant increase of IPF mean level in both peripheral (4.56±2.05 vs 3.39±1.70; p=0.042) and coronary (4.62±2.24 vs 3.76±1.95; p=0.045) values (Figure 2).

Peripheral IPF and HbA1c level >7.5% were significantly correlated with coronary IPF (Tables 2-3).

After all linear regression assumptions were fulfilled, the

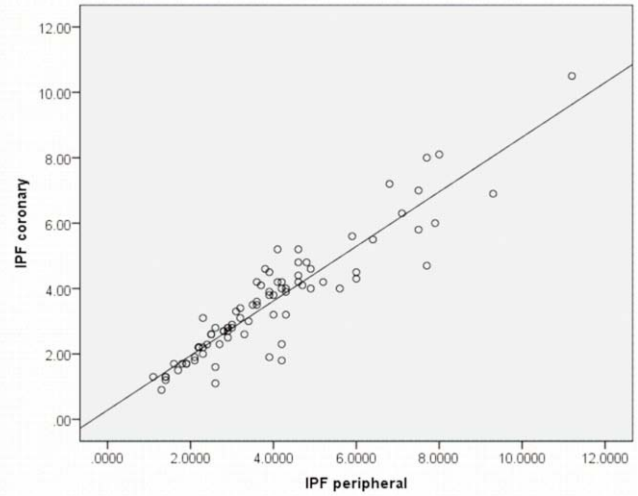

Figure-1: Scattered plot between coronary and peripheral immature platelet fraction (IPF) showing a significantly strong positive correlation.

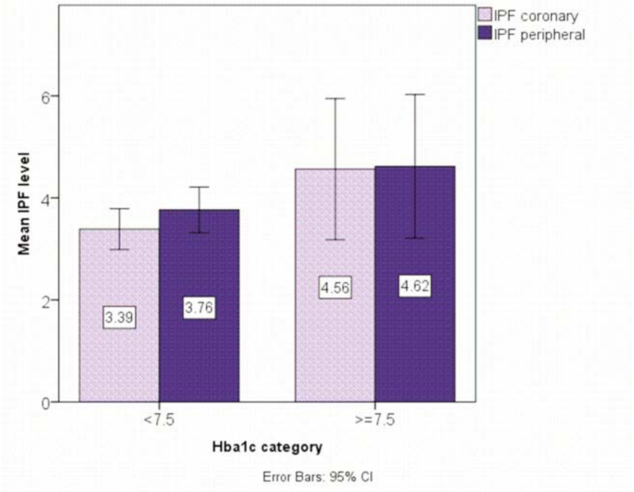

Figure-2: Comparison of mean peripheral and intra-coronary immature platelet fraction (IPF) levels between low and high glycated haemoglobin (Hba1c) levels.

Table-2: Univariate analysis based on IPF tertiles.

Variable	1st tertile IPF (<2.6)	2nd tertile IPF (2.6-4.1)	3rd tertile IPF (>4.1)	P
Age	60.41	56.82	59.37	0.305
Risk Factor				
Hypertension				
● Yes	15 (37.1%)	14 (11.4%)	12 (29.3%)	0.852
● No	14 (32.6%)	14 (32.6%)	15 (34.9%)	
Dyslipidaemia				
● Yes	15 (36.6%)	15 (36.6%)	11 (26.8%)	0.606
● No	14 (32.6%)	13 (30.2%)	16 (37.2%)	
History of smoking				
● Yes	14 (31.8%)	13 (29.5%)	17 (38.6%)	0.426
● No	15 (37.5%)	15 (37.5%)	10 (25.0%)	
Diabetes mellitus				
● Yes	12 (38.7%)	10 (32.3%)	9 (29.0%)	0.848
● No	17 (32.1%)	18 (34%)	18 (34%)	
Medication history				
Aspilet use	18 (30.5%)	20 (33.9%)	21 (35.6%)	0.478
Clopidogrel use	21 (33.9%)	20 (32.3%)	21 (33.9%)	0.862
Laboratory findings				
Blood glucose	127 (IQR 41)	132 (IQR 40)	118 (IQR 42)	0.464
Hba1c	5.8 (IQR 1.7)	5.6 (IQR 1.9)	5.5 (IQR 1.6)	0.894
Serum creatinine	1.24 (IQR 0.66)	1.07 (IQR 0.31)	1.14 (IQR 0.26)	0.358
Coronary platelet count	264.89 (±70.41)	276.10 (±78.1)	236.04 (±105.53)	0.209
Peripheral platelet count	289.34 (±65.74)	277.21 (±67.50)	236.26 (±124.58)	0.075

IPF: Immature platelet fraction, IQR: Interquartile range.

Table-3: Independent variables subjected to multivariate regression analysis of coronary IPF.

Model	Unstandardized Coefficients				Collinearity Statistics	
	B	Std. Error	t	Sig.	Tolerance	VIF
1 (Constant)	.197	.156	1.261	.211		
Peripheral IPF	.848	.036	23.232	.000	.977	1.023
HbA1c_category	.452	.208	2.177	.032	.977	1.023

IPF: Immature platelet fraction, HbA1c: Glycated haemoglobin, VIF (Varieance Inflation Factor).

formula for coronary IPF was:

Coronary IPF = 0.197 + 0.848 x Peripheral IPF + 0.452 x (Hba1c level category) (R2 87.7%; p=0.000).

Discussion

The current study showed that CAD patients were mostly males (78.6%), and the minimum age was as low as 26 years. The data supported the finding that CAD could manifest in patients aged <40 years.[16]

Various modifiable risk factors of CAD were found in the current study, including HTN, cholesterol, smoking and DM. HTN directly affects the endothelial lining of blood vessels, resulting in endothelial dysfunction and atherosclerotic plaque. Cholesterol is known as a major component of atherosclerosis. Smoking can increase the risk of developing CAD[17]. DM is associated with abnormalities of lipid metabolism, obesity, systemic HTN and increased thrombogenesis.[18]

Hyperglycaemia is known to increase the risk of a cardiovascular event, with antiplatelet effect attenuation as one of the possible mechanisms. The current findings showed that uncontrolled hyperglycaemia (Hba1c >7.5%) increased IPF. A previous study showed a significant positive correlation between HbA1c level and IPC[19]. Hyperglycaemia can escalate platelet activation by the induction of surface adhesion protein P-selectin expression, platelet glycating by reducing membrane fluidity that increases platelet adhesion, activating protein kinase C (PKC) through osmotic effects of glucose, and various activation mediators. Insulin deficiency's role in platelet dysfunction through insulin receptor substrate (IRS) is associated with increased intracellular calcium concentration, leading to increased platelet degranulation and aggregation. Other factors related to insulin resistance (IR) that are not IRS-dependent are impaired responses to nitric oxide (NO) and prostacyclin (PGI2), thereby increasing platelets reactivity.[20-22]

In univariate and multivariate analysis, the current study found that peripheral IPF and IPC strongly correlated with coronary IPF and IPC. This result has important clinical value as no research has analysed this critical relationship. The results successfully proved that high immature platelet levels in the periphery also illustrated high immature platelet levels in the coronary. The clinical implication is that a more accurate estimation of coronary immature platelet level can be calculated from peripheral immature platelet and Hba1c level category using the linear regression formula (R 87.7%) so that the ACS risk and the process of atherosclerosis in the coronary artery could be predicted with more precision.

Immature platelets have a relationship with the effectiveness of antiplatelet therapy. CAD patients with high immature platelet levels have low antiplatelet effectiveness[23]. Antiplatelet therapy in the form of aspilet and clopidogrel can experience resistance associated with high immature platelet levels in circulation. This resistance is due to the short half-life of aspilet (2-6 hours) and clopidogrel (8 hours). If the platelet lifetime is shorter, such as in immature platelet conditions (<1 day), then many new platelets will be produced by the spinal cord, resulting in more unbound platelet, and reducing the efficacy of the antiplatelet effect of aspilet and clopidogrel.[8]

Through the theoretical basis of half-life and bonding to platelets, anti-platelet agents are recommended to have a longer half-life with reversible bonds. In contrast to the association between reticulated platelet (RP) levels and response to prasugrel, no significant association was found between RPs and ticagrelor.[24]

Ticagrelor has reversible bonding properties, and it can bind to platelets and then be able to release the bonds if new platelets are produced. It also has a longer half-life (12 hours), so, given twice a day, it can keep the platelets bound for 24 hours.[25]

The current study had several limitations. It had a cross-sectional design and used consecutive sampling without randomisation. The study also did not use an immature platelet correlation test with antiplatelet effectiveness. Besides, the study was conducted in 2017, and changes could have emerged in the intervening 7 years. However, the strength of the study is that it showed for the first time that there was a strong correlation between the coronary and peripheral platelet turnover process, and the actual coronary IPF could be calculated with a simple formula to get a more precise result of platelet turnover in CAD population. A fresh study with larger sample size is recommended to validate the findings.

Conclusion

There was a strong correlation between coronary and peripheral IPF and IPC. Coronary IPF in CAD patients was determined using HbA1c level, and could be calculated with a simple formula to get a more accurate condition of the platelet turnover process in the coronary artery.

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Conflict of Interest: None.

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