RESEARCH ARTICLE

Increased Platelet-derived Microparticles Counts is Correlated with Elevated Blood LDL Cholesterol in Acute Myocardial Infarction

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Abstract

BACKGROUND: Platelet-derived microparticles (PDMPs) and low-density lipoprotein (LDL) cholesterol are contributing factors to acute myocardial infarction (AMI). However, the association between LDL cholesterol and PDMPs in AMI has not fully discovered. This study assessed the correlation between these two parameters in patients diagnosed with AMI.

METHODS: This was an observational cross-sectional study involving 95 subjects with AMI. The blood measurement of PDMPs counts and LDL cholesterol levels were conducted concomitantly within 24 hours of admission. PDMPs count was analyzed by flow-cytometry method, meanwhile the LDL cholesterol was measured with enzymatic and colorimetric methods. For further analysis, subjects were further divided into LDL cholesterol level ≥130 mg/dL and <130 mg/dL. A statistical test was conducted for a correlative and comparative analyses.

RESULTS: A correlative analysis to assess the association between PDMPs counts and LDL cholesterol level depicted a low but significant positive correlation (r=0.231, *p*=0.024). Furthermore, mean PDMPs counts was significantly higher in subjects with LDL cholesterol level ≥130 mg/dL compared to LDL cholesterol level <130 mg/dL (12,499.59 (95% CI: 8,507.44-16,491.74) counts/µL *vs.* 9,267.23 (95% CI: 4,445.45-14,089.01) counts/µL; *p*=0.039).

CONCLUSION: There was a significant correlation between PDMPs counts and LDL cholesterol levels in AMI. A significantly increased PDMPs counts were found in subjects with LDL cholesterol level ≥130 mg/dL. Therefore, it is recommended to measure PDMPs in patients with high LDL cholesterol levels as both might be significant AMI biomarkers.

KEYWORDS: acute myocardial infarction, LDL-cholesterol, platelet microparticles, platelet activation

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Introduction

Acute myocardial infarction (AMI), defined as ischemic-related myocardial injury due to thrombosis caused by ruptured atherosclerotic plaque (1), is the most serious problem in the class of coronary artery disease. In the USA, more than 2.4 million people died due to this disease, while in Europe and Northern Asia, the number has even reached 4 million.(2) Overall, one third of all mortality cases are due to AMI.(2) The trend has actually greatly changed over the past 30-40 years resulting in shifting of worldwide burden to low and moderate-income countries.(3) It comprises more than 80% of total deaths of cardiovascular problem worldwide. With its high prevalence, AMI also has been proved to be not economically effective in cost spending for hospital and health-care providers.

Indonesia has been dealing with double attack of disease burdens. Along with communicable diseases that still reign over.(4) The incidence of non-communicable diseases

has also increased over time, mainly in cardiovascular issues. The rising prevalence of cardiovascular diseases in Yogyakarta region was evident as it ranked the third highest province in Indonesia, based on the Indonesian Basic Health Research in 2018.(4,5) In 2013, roughly 478,000 of Indonesian populations were diagnosed with coronary heart disease, with AMI dominating the proportion.(4)

AMI can be caused by many factors. One of them is the formation of atherosclerotic plaque that is vulnerable to disruption or erosion leading to coronary artery endothelium (type 1) damage.(2) Disrupted atherosclerotic plaque further expose the subendothelial milieu to the circulating platelets and cause thrombosis.(6) Platelet which constitutes of 70-80% of microparticles is an important component in blood homeostasis, both quantitatively and qualitatively, and taking an important role in angiogenesis, immunity and tissue regeneration. As part of microparticles, platelet will increase in number if there is activation of coagulation cascade or complement system, or under the influence of apoptotic signaling substance or shear forces.(7) These microparticles are formed through platelet activation in a high shear stress found in severe atherosclerosis.(8) Therefore, the presence of platelet-derived microparticles (PDMPs) has now been used as a predictor of AMI.

The deposition of cholesterol plays a vital factor in the pathogenesis of atherosclerosis. Furthermore, both the reactive oxygen species and reactive nitrogen species yield the oxidation of low-density lipoprotein (LDL) through the disruption of lipid's celluar function. The oxidized LDL cholesterol (ox-LDL) has a crucial role in the initiation and development of atherosclerosis through endothelial disruption, increased leukocyte adhesiveness and recruitment, induced leukocyte and monocyte adhesion molecules expression, all of which contribute to inflammation.(8-11) Although the pro-thrombotic characteristics of ox-LDL resulted from the interactions with platelet will further lead to the thrombus formation (9), the relationship of LDL cholesterol and PDMPs in cardiovascular diseases is still not fully understood. Therefore, it is necessary to assess the correlation between the two parameters in patients diagnosed with AMI.

Methods

Design Study and Subjects Recruitment

This was an observational cross-sectional study to assessed the correlation between the level of serum LDL cholesterol and the amount of PDMPs in subjects with AMI, both AMI with ST-segment elevation (STEMI) and non-STEMI. This study used a consecutive sampling to recruit subjects that were hospitalized in the Intensive Cardiac Care Unit of Dr. Sardjito Hospital, Yogyakarta, from January 2017 to January 2019. The inclusion criteria were subjects with AMI onset less than 24 hours, and subjects with the age range of 35-75 years old without existing comorbidities, such as chronic renal failure, congestive heart failure, cirrhosis, or prior heart valve diseases before admission. The exclusion criteria were subjects with simultaneous acute infection, sepsis, and acute stroke, thrombocytopenia, prior use of statins, prior use of antiplatelets, prior anemias and subjects that had the incomplete PDMPs and/or LDL cholesterol data.

The diagnosis of AMI, either STEMI and non-STEMI, was based on three criteria: typical anginal pain, electrocardiogram and troponin-I level. The initial treatment of subjects was in the discretion of attending cardiologists, based on hospital standard of care. Revascularization procedure, namely primary percutaneous coronary intervention or fibrinolysis, was performed in STEMI as indicated. The use heparin and other medical treatments were noted.

All subjects signed an informed consent to participate in this study. The Medical and Health Research Ethic Committee of our institution had approved the research protocol of this study (No. KE/FK/720/EC/2016).

Anthropometric Measurements

The age and sex of subjects were determined based on medical records. Body weight and height were measured during hospitalization with standardized weight/height measurement tool (ZT-120 Health Scale®, GEA Medical, Jakarta, Indonesia). Body mass index (BMI) was calculated based on ratio of body weight (kg) : height $(m)^2$.

Laboratory Measurements

The blood samples from subjects were obtained within 24 hours of admission. Routine hematological examination with an automated hematocytometer (Sysmex XN1000® hematology analyzer, Sysmex, Kobe, Japan) was performed to measure hemoglobin level, leucocyte and platelet counts. Blood glucose level examination was carried out on admission (random blood glucose) and during fasting within 24 hours admission (fasting blood glucose) by the hexokinase method (Cobas 6000® analyzer, Roche Diagnostic, Mannheim, Germany), using centrifugated (1,500 x g for 30 min in 4ºC) serum samples. The blood lipid examination, consisted of total cholesterol, LDL cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride

levels, was performed with enzymatic and colorimetric methods (Cobas 6000® analyzer, Roche Diagnostic), using centrifugated (1,500 x g for 30 min in 4ºC) serum samples.

The amount of PDMPs was analyzed by flow-cytometry method (FACS Calibur, Becton Dickinson, Maryland, USA) as described previously.(12) The PDMPs were determined if CD-41 FITC positive (Biolegend, San Diego, USA) and CD-62 PE positive (Biolegend) with the threshold size <1 µm and calculated after gated with standard formula.(12)

Statistics Analysis

The normality test was conducted for continuous data with log transformation if necessary. The correlation between PDMPs counts and LDL cholesterol levels was analyzed with the Spearman's correlation test, as well as for other continuous data. Subjects were divided into two groups based on LDL cholesterol level, namely ≥130 mg/dL and <130 mg/dL.(13) The mean PDMPs counts was compared between groups by using the Mann-Whitney test. The statistics with $p<0.05$ were considered significant. The SPSS software version 25.0 (IBM Corporation, Armonk, NY, USA) was used in this analysis.

Results

From 122 potential subjects, total 95 subjects were eligible for enrollment of this study (Figure 1). The characteristics of subjects were showed in Table 1. The mean age of subjects was 54.45 years old and mostly males (91.58%). Comorbidities were hypertension (47.49%) and diabetes mellitus (23.16%). There were 11 subjects (11.57%) who were diagnosed as non-STEMI and 84 subjects as STEMI (88.43%).

Subjects also received initial treatment within the first 24 hours. Sixty four percent subjects underwent primary percutaneous coronary intervention (PCI) and 17% had fibrinolysis as means of revascularization. The majority received heparin, angiotensin-converting enzymes (ACE)-inhibitors and beta-blockers in their initial treatments. All subjects were given high-intensity statins and anti-platelets (aspirin and clopidogrel) loads (Table 1).

Meanwhile, Table 2 showed subjects' characteristics based on the divided level of LDL cholesterol. Fifty subjects (52.63%) presented with LDL cholesterol level <130 mg/dL while 45 subjects (47.37%) had LDL cholesterol level ≥130 mg/dL. The mean age of subjects was slightly older in the former group with 55.06±1.34 years compared to the latter with mean age 53.06±1.19 years, although the differences were not significant. The proportion of male subjects was significantly bigger in subjects with LDL cholesterol <130 mg/dL. The proportion of hypertension and diabetes mellitus did not differ between two groups. The total cholesterol and triglyceride level were slightly higher in the group with LDL cholesterol level ≥130 mg/dL ($p<0.05$). The body weight and body mass index was insignificantly higher in subjects with LDL cholesterol level ≥130 mg/dL. There were no significant difference in initial treatment modalities between groups.

There was a significant positive correlation between the levels of LDL cholesterol and PDMPs count (Spearman correlation test: r=0.231; $p<0.05$). Thus, the increase of LDL cholesterol level was followed by the linear release of PDMPs from blood circulation (Figure 2). The correlation of other continuous variables were also significant for total cholesterol, triglyceride and platelet counts (Table 3).

We further investigated the difference of mean PDMPs counts between two different groups of the LDL cholesterol level cut-off of 130 mg/dL. As depicted from the histogram (Figure 3), the mean PDMPs counts was significantly higher in subjects with LDL cholesterol level ≥130 mg/dL as compared to those with LDL cholesterol level <130 mg/dL (mean: 12,499.59 (95% CI: 8,507.44–16,491.74) counts/µL *vs.* mean: 9,267.23 (95% CI: 4,445.45–14,089.01) counts/µL, $p=0.039$).

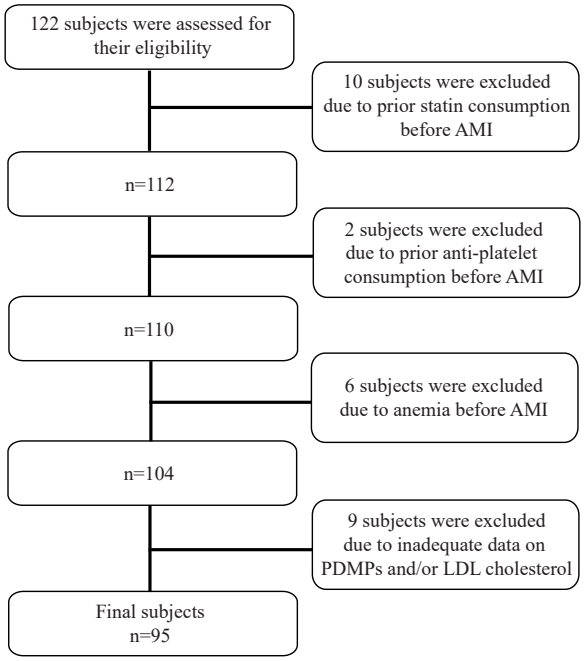

Figure 1. Flowchart for subjects recruitment.

Table 1. The characteristics of subjects with AMI.

Characteristics	Value (n=95)
Age (years), mean±SD	54.45±8.81
Sex	
Males, n (%)	87 (91.58)
Females, n (%)	8 (8.42)
Subjects with hypertension, n (%)	45 (47.49)
Subjects with diabetes mellitus, n (%)	22 (23.16)
Diagnosis of AMI	
STEMI, n (%)	84 (88.43)
Non-STEMI, n (%)	11 (11.57)
Anthropometric profile	
Body weight (kg), mean±SD	64.07±12.34
Body height (kg), mean±SD	162.45±7.06
Body mass index (kg/m^2), mean±SD	24.22±3.83
Lipid profile	
Total cholesterol (mg/dL), mean±SD	190.72±44.98
LDL cholesterol (mg/dL), mean±SD	131.00±41.46
HDL cholesterol (mg/dL), mean±SD	43.62±15.71
Triglyceride (mg/dL), mean±SD	128.90±69.26
Haemoglobin (g/dL), mean±SD	14.44±1.46
Leukocytes (x10^3/mm^3), mean±SD	13.88±3.79
Platelets (x10^3/mm^3), mean±SD	269.36±79.18
Random blood glucose (mg/dL), mean±SD	168.22±78.72
Fasting blood glucose (mg/dL), mean±SD	131.94±52.78
Received initial treatment	
Primary PCI, n (%)	60 (64)
Fibrinolysis, n (%)	16 (17)
Unfractionated heparin, n (%)	58 (61)
Low-molecular weight heparin, n (%)	30 (32)
ACE-inhibitor, n (%)	85 (89)
Angiotensin-receptor blocker, n (%)	8 (8.4)
Beta blocker, n (%)	80 (84)
Statin, n (%)	95 (100)
Aspirin, n (%)	95 (100)
Clopidogrel, n (%)	95 (100)

Discussion

This study indicated that among subjects with AMI, in the early hospital admission, PDMPs counts were significantly correlated with LDL cholesterol levels with positive correlation. Subjects with LDL cholesterol ≥130 mg/dL had significantly higher PDMPs counts as compared with their counterparts. This finding indicated the association between platelet activation and LDL cholesterol level during AMI.

Based on sex distribution, there was unequally higher proportion of males compared to females. However, this finding was in correspond with the previous studies that stated male as one of risk factors of suffering AMI in patients with earlier onset of diagnosis.(14,15) In our study, the mean age of female subjects were slightly older than male counterparts. The risk of AMI increases with ages in both sexes, with lowest risk for females. The gender gap is observed to persist throughout life. In general, males have approximately twice the risk of AMI as compared to females. The risk level of females corresponds to that in males about 10 to 15 years younger with a very low risk in young and middle-aged females.(16) The key reason behind this phenomenon is believed to be the cardioprotective effects of estrogen and progesterone towards the vascular activity, lipid profile and endothelial function.(16)

Hyperlipidemia, as one of the modifiable risk factors of AMI, is depicted by the increase of several laboratory parameters including LDL cholesterol level of ≥130 mg/dL, contributing to the formation and progression of atherosclerosis.(14) According to National Cholesterol Education Program (NCEP), the desirable LDL cholesterol limit is <130 mg/dL. There were multitudes of research that considered LDL cholesterol to predict the survival in life, especially in cardiovascular diseases. A prospective study of 67 AMI patients, dichotomized LDL cholesterol level into two groups, consisted of "optimal/near optimal" for LDL cholesterol <130 mg/dL and "high" for ≥130 mg/dL.(13) This dichotomous classification was proved to have superior specificity (73%; p<0.001) and accuracy (72%; p<0.001).(13) Therefore, in this research, the level of LDL cholesterol was classified into 2 groups with cut-off point of 130 mg/dL. Based on the statistical result, more subjects had LDL cholesterol <130 mg/dL compared to subjects with LDL cholesterol ≥130 mg/dL among AMI.

In 2013, the prevalence of high LDL cholesterol in serum of Indonesian population was 11.1%, meaning that the majority these population had normal level of LDL cholesterol. On the other side, a report from the USA stated that there were more than 71 million of American adults aged 20 or older had LDL cholesterol level greater than 130 mg/dL (33.5%). A study on the lipid profile of AMI patients found that there was significant decrease in LDL level in both STEMI and non-STEMI patients as compared to normal subjects.(17) By contrast, another study of 183 young adults who were experiencing AMI concluded that there was acceptable or optimal LDL level.(18)

The level of PDMPs was found to be significantly increased in the group of LDL cholesterol level ≥130 mg/dL, although our study had a higher proportion of AMI patients with the opposite group of LDL cholesterol level. Therefore, there is an increase of PDMPs released along with the elevated LDL cholesterol in AMI patients.

Table 2. Subjects' characteristics based on the divided level of LDL cholesterol.

Characteristics	LDL Cholesterol Level <130 mg/dL (n=50)	LDL Cholesterol Level ≥130 mg/dL (n=45)	*p*-value
Age (years), mean±SD	55.06±1.34	53.06±1.19	0.48
Sex			
Males, n (%)	49 (56.32)	38 (43.68)	0.01*
Females, n (%)	1 (12.5)	7 (87.5)	0.01*
Subjects with hypertension, n (%)	22 (48.90)	23 (51.10)	0.48
Subjects with diabetes mellitus, n (%)	11 (50)	11 (50)	0.77
STEMI subjects, n (%)	47 (55.95)	37 (44.04)	0.07
Anthropometric profile			
Body weight (kg), mean±SD	62.40±1.42	65.88±2.14	0.17
Body height (kg), mean±SD	162.73±0.90	159.91±2.68	0.30
Body mass index (kg/m^2), mean±SD	23.62±0.47	24.90±0.62	0.10
Lipid profile			
Total cholesterol (mg/dL), mean±SD	159.23±26.56	222.34±37.98	0.00*
HDL cholesterol (mg/dL), mean±SD	42.61±14.72	44.19±16.14	0.60
Triglyceride (mg/dL), mean±SD	112.36±60.32	144.17±72.38	0.01*
Haemoglobin (g/dL), mean±SD	14.36±0.21	14.53±0.20	0.57
Leukocytes (x10^3/mm^3), mean±SD	14.30±0.54	13.41±0.55	0.25
Platelets (x10^3/mm^3), mean±SD	259.84±84.45	281.04±75.24	0.18
Random blood glucose (mg/dL), mean±SD	173.17±79.02	157.80±73.81	0.32
Fasting blood glucose (mg/dL), mean±SD	130.29±51.72	133.11±52.10	0.78
Received initial treatment			
Primary PCI, n (%)	30 (60)	30 (67)	0.89
Fibrinolysis, n (%)	10 (20)	6 (14)	0.89
Unfractionated heparin, n (%)	30 (60)	28 (62)	0.78
Low-molecular weight heparin, n (%)	20 (40)	10 (22)	0.78
ACE-inhibitor, n (%)	50 (100)	35 (78)	0.89
Angiotensin-receptor blocker, n (%)	3 (6)	5 (11)	0.89
Beta blocker, n (%)	48 (96)	32 (71)	0.76
Statin, n (%)	50 (100)	45 (100)	N.A
Aspirin, n (%)	50 (100)	45 (100)	N.A
Clopidogrel, n (%)	50 (100)	45 (100)	N.A

*p<0.05 is considered as significant. N.A: not applicable.

An interesting innovative study supports this result. When apheresis was conducted to remove atherogenic lipoproteins, including LDL cholesterol, the concentration of circulating microparticles, which are platelet positive in patients, is reduced.(19) Moreover, the expression of antibodies CD41a and CD61 as the markers for platelet antigens was discovered to be significantly higher to induce PDMPs in plasma containing LDL fractions. The production of PDMPs and LDL cholesterol complexes was enhanced during platelet activation *ex vivo*.(20) Hence, patients with elevated LDL cholesterol level should be given more aggressive anti-platelets and anti-lipids to hinder further vascular complications.

Platelet-derived microparticles are defined as the small extracellular fragments shed from the plasma membrane of platelets with size ranging between 0.1 and 1 μm.(21) The markers used to detect PDMPs can vary from CD31, CD41, CD61 and CDG2P.(8) There were remarkable elevation of PDMPs level measured in patients presenting with acute coronary syndrome, diabetes mellitus type 2, hypertension and stroke. As the microparticles reflect the functional state of platelet activation, it is mandatory to monitor PDMPs counts in the plasma and evaluate the risk of thrombosis in ACS patients.(21) Acute coronary events leading to STEMI can yield sequence of consequences, such as releasing the inflammatory cytokines and promoting neutrophil-

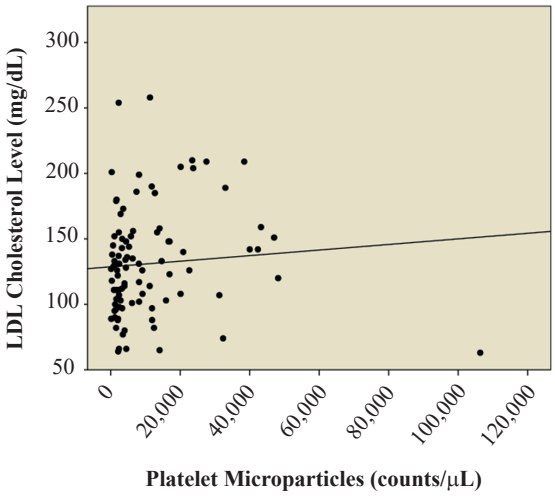

Figure 2. Scatter plot diagram of PDMPs and LDL cholesterol level showed a significant positive correlation.

mediated oxidative burst, hypoxia and necrosis of cardiac muscles. These events subsequently affect the process of PDMPs shedding and produce a direct consequence of atherothrombosis.(22,23) The higher level of PDMPs that are activated in STEMI patients, the greater risk of platelet activation and thrombus synthesis.(22) Although PDMPs increase due to platelet activation, there is a report stating that platelet counts and PDMPS were non-correlated.(24) However in this study, we found that both parameters were positively correlated.

Our finding showed that the amounts of PDMPs was significantly higher in patients with LDL cholesterol level ≥130 mg/dL. This finding is in support with the pathogenesis of AMI which starts with coronary atherosclerosis as the primary underlying process.(25) In the progression of AMI, the microparticles will be released to the circulation which demonstrate pro-atherogenic, pro-inflammatory, and immunomodulatory effects.(18) The rich component of phospholipid-expressing Annexin V in PDMPs has a pro-atherogenic and pro-coagulant characteristics.(26)

Platelets trigger the process of vascular inflammation in atherosclerosis and later, foster thrombus development in ruptured atherosclerotic plaque.(27.28) LDL cholesterol, in turn, is involved in the early phase of atherosclerosis through a recruitment of inflammatory cells to the sub-endothelium eliciting prothrombotic changes on the endothelial surface. Similarly, the presence of ox-LDL elevated the level of PDMPs as the microparticles posses the CD36 receptor that are specific for LDL, causing in severe damage of endothelial cells and further contributing to the progression of atherosclerosis. Moreover, one clinical study found that ACS subjects with high ox-LDL level had a higher concentration of PDMPs in their plasma as opposed to those with lower oxided LDL cholesterol level. (29,30)

Both parameters, platelets and LDL, contribute to the atherogenesis in acute coronary syndrome patients and influence one another. The relationships between activated platelets and ox-LDL affect several mechanisms, including (i) activation of inflammatory cells (*e.g.*, neutrophils, macrophages, smooth muscle cells, platelets and endothelial cells), (ii) regeneration of endothelial cells and (iii) the generation of foam cells. All these three processes eventually yield atherosclerosis. The presence of oxLDL-rich platelets further promote vascular inflammation and induce $CD34^{+}$ progenitor cells transformed into macrophages or foam cells.(29) The accumulated inflammatory cells then play a role in plaque destabilization through altering anti-adhesive and anti-coagulant into pro-coagulant tissue factor and ultimately progress to plaque rupture (ACS).(31)

The plasma concentration of PDMPs is influenced both by antiplatelet and lipid-lowering drugs.(30,32) The detected circulating microparticles were remarkably lower in the lipid-lowering treatment group regardless of cholesterol levels.(32) Statin therapy diminishes the level of PDMPs together with other biomarkers of activated platelet and inflammatory cells.(33) Besides LDL cholesterol, *in vivo* platetet activation was also increased in patients with hypertriglyceridemia, making elevated triglyceride level another risk factor for cardiovascular disease.(34) This study found a significant positive correlation between triglyceride level and PDMPs. It has been the mainstay lipid-lowering agent to effectively decrease PMPDs in

Table 3. The correlations between PDMPs count and other continous variables.

Continuous Variables	Spearman's Coefficient Correlation	*p*-value
Body weight	-0.077	0.44
Body height	-0.021	0.83
Body mass index	-0.193	0.06
Total cholesterol	0.238	0.01*
HDL cholesterol	-0.119	0.23
Triglyceride	0.198	0.04*
Haemoglobin	-0.042	0.67
Leukocytes	0.044	0.66
Platelets	0.195	0.04*
Fasting blood glucose	-0.18	0.07

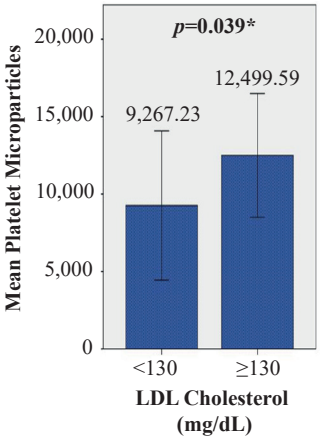

Figure 3. Histogram of mean PDMPs count between LDL cholesterol <130 mg/dL and LDL cholesterol ≥130 mg/dL. *Significant if $p<0.05$, tested with Mann-Whitney test.

patients with various cardiovascular risk factors, including the use simvastatin in hypertension and type II diabetes mellitus and atorvastatin in peripheral vascular diseases. (33) In our study, all subjects received high-intensity statins and loading-dose of antiplatelets therefore their impacts on PDMPs were negligible.

Several limitations of our study must be addressed. Firstly, the period of sample taken was considered as a large time span since during the 24 hours, there were many incidences that could possibly alter the sample characteristics and analysis. Secondly, PDMPs counts and LDL cholesterol levels measurement was not always conducted within the same time due to different procedures and operators causing them to not reflect the same time frame and condition of the patients. Lastly, the sample size used for this study was relatively small. Subsequently, this led to the persistent confounding factors affecting the LDL cholesterol level despite the effort of excluding some of those factors.

Conclusion

The levels of LDL cholesterol were significantly correlated with the amounts of PDMPs in subjects with AMI. A significantly higher PDMPs counts were found in subjects with LDL cholesterol level ≥130 mg/dL as compared to LDL cholesterol <130 mg/dL. Therefore, the higher the LDL cholesterol level, the more PDMPs released into the blood circulation during AMI episode. It is recommended to measure PDMPs in patients with high LDL cholesterol levels as both might be significant AMI biomarkers.

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Authors Contribution

KS, IP, DSM, and ABH were involved in concepting and planning the research. IP, DSM and ABH performed the data acquisition/collection. KS calculated the experimental data, performed the analysis, and drafted the manuscript. DSM and ABH aided in interpreting the results. ABH provided grants for research and publication. All authors discussed and gave critical revision and approved final manuscript.

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