Angiology
2016, Vol. 67(2) 146-150

DOI: 10.1177/0003319715579781
ang.sagepub.com
SAGE

Increased Mean Platelet Volume in Familial Hypercholesterolemia

Atilla Icli, MD[1], Fatih Aksoy, MD[2], Gökay Nar, MD[1], Haci Kaymaz, MD[3], Mehmet Fatih Alpay, MD[4], Rukiye Nar, MD[5], Aydın Guclu, MD[6], Akif Arslan, MD[2], and Abdullah Dogan, MD[7]

Abstract

Familial hypercholesterolemia (FH) is a genetic disorder of lipoprotein metabolism and increases the risk of premature cardiovascular diseases. In patients with FH, platelet function may be activated; however, the extent of this activation and its etiology are unclear. We aimed to evaluate the mean platelet volume (MPV), a marker of platelet activation, in patients with FH. The study group consisted of 164 patients with FH and 160 control patients. Controls were matched for age, gender, hypertension, and smoking. The MPV was significantly higher in patients with FH than in controls (9.2 $\pm$ 0.4 vs 7.9 $\pm$ 0.6 fL, respectively; $P < .001$). Platelet count was significantly lower among patients with FH when compared to control patients (259 $\pm$ 51 vs 272 $\pm$ 56 $\times$ 10^3/L, respectively; $P = .03$). In linear regression analysis, MPV was independently associated only with total cholesterol ($\beta = .6$, 95% confidence interval: 0.004-0.008, $P < .001$). We have shown that MPV was increased in patients with FH and that it was independently associated with total cholesterol level.

Keywords

mean platelet volume, platelet activation, platelet count, familial hypercholesterolemia

Introduction

Familial hypercholesterolemia (FH) is a genetic disorder with autosomal dominant trait and characterized as severe elevations in plasma total and low-density lipoprotein cholesterol (LDL-C) levels. Familial hypercholesterolemia increases the risk of cardiovascular disease (CVD) in the early stages of life.[1,2] Accelerated atherosclerosis is a feature of FH.[3] Familial hypercholesterolemia can also be associated with impaired endothelial function, increased intima–media thickness, platelet hyperactivity, and prothrombotic state.[4-7] Evidence from several studies suggest increased platelet activity and shortened platelet survival in patients with FH.[7-9] Platelet activation has a crucial role in the pathogenesis of atherosclerotic lesion and cardiovascular events in patients with FH.[10]

There has been an increasing interest in the mean platelet volume (MPV) as a new cardiovascular risk factor.[11] An elevated MPV is considered as an indicator of larger and more reactive platelets.[12,13] There is evidence that larger platelets are metabolically and enzymatically more active and have a greater prothrombotic potential.[14] Some studies have demonstrated increased coagulation activity and platelet function in patients with hyperlipidemia.[7,8,15,16] Therefore, we aimed to investigate the platelet indices including MPV and platelet count in patients with FH.

Methods

Study Population

The study group consisted of 164 consecutive patients with FH (87 females, mean age = 39.6 $\pm$ 11.6 years) and 160 normolipidemic controls (89 females, mean age = 38.4 $\pm$ 9.9 years) matched for age, gender, smoking, and hypertension. Familial hypercholesterolemia was defined according to the Dutch Lipid Network Clinical Criteria outlined by the World Health Organization.[17] This cross-sectional study was conducted at 2 medical centers, namely, Ahi Evran University and Suleyman Demirel University in Turkey between 2010 and 2014. Male

[1] Department of Cardiology, Ahi Evran University, Kirsehir, Turkey
[2] Department of Cardiology, Suleyman Demirel University, Isparta, Turkey
[3] Department of Neurosurgery, Ahi Evran University Education and Research Hospital, Kirsehir, Turkey
[4] Department of Cardiovascular Surgery, Ahi Evran University, Kirsehir, Turkey
[5] Department of Biochemistry, Ahi Evran University, Kirsehir, Turkey
[6] Department of Nephrology, Ahi Evran University, Kirsehir, Turkey
[7] Department of Cardiology, Katip Celebi University, Izmir, Turkey

Corresponding Author:
Atilla Icli, Ahi Evran Üniversitesi Tıp Fakültesi, Kardiyoloji ABD, 40100 Kırşehir, Turkey.
Email: atillaicli@hotmail.com

and female patients aged 18 years or older with a first diagnosis of FH were eligible for inclusion in the present study. Fifty-two patients were not included in this study since they had exclusion criteria (see subsequently). Blood samples for platelet indices were taken before starting any antilipidemic agent. A complete medical history and physical examination were obtained from all participants. Data collected on the demographic and clinical characteristics of the patients included age, history of CVD, hypertension, type 2 diabetes mellitus, smoking status, and findings on physical examination. Body mass index was calculated as body weight (kg) divided by the square of height (m^2). Secondary causes of hypercholesterolemia were excluded, including nephrotic syndrome, liver disease, hypothyroidism, and diabetes mellitus. In addition, other exclusion criteria were as follows: left ventricular systolic dysfunction, known coronary artery disease, acute coronary syndrome, atrial fibrillation, history of renal or liver disease, malignancy, hematological disorders, acute or chronic infection, current use of lipid-lowering agents, current stroke, and consumption of >30 g alcohol/d. The study was approved by the local ethics committee, and all patients gave their informed consent.

Biochemical Measurements

Blood samples were drawn from the antecubital vein by careful vein puncture with a 21-G sterile syringe without stasis at 08:00 to 10:00 hours after a fasting period of 12 hours. Blood samples were collected as described earlier into EDTA and citrate vacutainers. Total cholesterol, high-density lipoprotein cholesterol, and triglycerides were measured by a commercially available enzymatic colorimetric assay (Roche, Basel, Switzerland). The LDL-C levels were calculated by the Friedewald formula.[18] The MPV was measured in a blood sample collected in dipotassium EDTA tubes (Vacuette) within 30 minutes to prevent EDTA-induced swelling. An automatic blood counter (Beckman-Coulter Co, Miami, Florida) was used for whole blood counts. Glucose, creatinine, and the other blood profiles were determined by standard methods.

Statistical Analysis

Data were analyzed using the SPSS software version 15.0 for Windows (SPSS Inc, Chicago, Illinois). Continuous variables were reported as mean $\pm$ standard deviation and categorical variables as percentage. The Student t test or Mann-Whitney U test was used to compare continuous variables when appropriate. Categorical variables were compared with the chi-square test. In addition, correlation analysis was performed to identify the relationship of MPV with other variables. Variables showing correlation with MPV at a level of significance of $P < .05$ were included into the linear regression analysis and were made to determine the independent association between MPV and confounders. These variables were hypertension, smoking, platelet count, total cholesterol, and LDL-C. Statistical significance was defined as a 2-tailed $P < .05$.

Table 1. Demographic and Clinical Characteristics of Patients With FH and Controls.[a]

	Patients With FH, n = 164	Controls, n = 160	*P* Value
Age, years	39.6 ± 11.6	38.4 ± 9.9	.32
Sex (M/F)	77/87	71/89	.64
BMI, kg/m^2	28.8 ± 3.3	28.1 ± 4	.08
SBP, mm Hg	126.6 ± 8.8	125.1 ± 11.4	.15
DBP, mm Hg	80.4 ± 5.5	79.5 ± 5.9	.16
Heart rate, beats/min	72 ± 7.4	70.9 ± 6.1	.14
Hypertension, n (%)	13 (7.9)	15 (9.4)	.64
Smoking, n (%)	17 (10.4)	20 (12.5)	.54
Aspirin, n (%)	22 (13.4)	13 (8.1)	.12

Abbreviations: FH, familial hypercholesterolemia; M/F, male to female; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[a]*P* value is for comparison between control and study population.

Table 2. Comparison of Laboratory Characteristics in Patients With FH and Controls.

Variables	Patients With FH, n = 164	Controls, n = 160	*P* Value
Glucose, mg/dL	99 ± 43	99 ± 11	.91
Creatinine, mg/dL	0.86 ± 0.2	0.87 ± 0.1	.85
Total cholesterol, mg/dL	362 ± 43	198 ± 33	<.001
Triglycerides, mg/dL	158 ± 58	148 ± 64	.11
LDL-cholesterol, mg/dL	282 ± 40	123 ± 43	<.001
HDL-cholesterol, mg/dL	48 ± 10	46 ± 9	.12
AST, IU/L	30 ± 10	29 ± 12	.33
ALT, IU/L	27 ± 13	28 ± 12	.45
WBC, $\times 10^3$ cells/μL	7.72 ± 1.90	7.45 ± 1.39	.14
Hemoglobin, g/dL	14.3 ± 1.1	14.1 ± 1.1	.13
Platelet count, $\times 10^3$ cells/μL	259 ± 51	272 ± 56	.03
MPV, fL	9.2 ± 0.4	7.9 ± 0.6	<.001

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; FH, familial hypercholesterolemia; WBC, white blood cell; LDL, low-density lipoprotein; HDL, high-density lipoprotein MPV, mean platelet volume.

Results

Clinical features and laboratory findings of the FH and control groups are summarized in Table 1. There were no statistically significant differences between the 2 groups in respect of age, gender, smoking, systolic and diastolic blood pressures, and levels of glucose, creatinine, hemoglobin level, and white blood cell count. The MPV was significantly higher among patients with FH when compared to controls (9.2 ± 0.4 vs 7.9 ± 0.6 fL, $P < .001$). In contrast, platelet count was significantly lower in patients with FH compared to controls (259 ± 51 vs 272 ± 56 × 10^3/L, $P = .03$, Table 2). In the correlation analysis, MPV was positively correlated with total cholesterol ($r = .73$, $P < .001$) and LDL-C ($r = .71$, $P < .001$) and negatively correlated with platelet count ($r = -.35$, $P < .001$; Table 3). Of these variables, total cholesterol ($\beta = .6$, 95% confidence interval: 0.004-0.008, $P < .001$) was independently associated with MPV in linear regression analysis.

Table 3. Variables Showing Significant Correlation With Mean Platelet Volume.

Variables	*r*	*P* Value
Total cholesterol, mg/dL	.73	<.001
LDL-cholesterol, mg/dL	.71	<.001
Platelet count	−.35	<.001

Abbreviation: LDL, low-density lipoprotein.

Discussion

We showed that MPV was significantly higher and independently associated with total cholesterol level in patients with FH compared to control participants. This may reflect platelet activation in these patients.

In FH, there are severe elevations in plasma total cholesterol and LDL-C levels, and FH leads to premature atherosclerosis and cardiovascular events at an early age.[1,3] High plasma cholesterol levels will also enhance the expression of cellular adhesion molecules, proinflammatory genes, and cytokines, leading to a low-grade systemic inflammatory status.[19] In addition, patients with FH have endothelial dysfunction.[4,5,19]

Some studies have demonstrated increased coagulation activity and platelet function in patients with hyperlipidemia.[6,7,20,21] Also, it has been suggested that FH alone may induce platelet activation and lead to prothrombotic state.[6,8,16] As a simple marker, MPV is considered to reflect platelet reactivity or activation.[13,22] Larger platelets are metabolically more reactive than smaller ones. Reactive platelets contain more prothrombotic material, with increased thromboxane A_2 and B_2 per unit volume and glycoprotein (Gp) IIb to IIIa receptor expression with greater aggregability in response to adenosine diphosphate and decreased inhibition of aggregation by prostacyclin in vitro.[11,23] Similarly, larger platelets create a tendency to the development of thrombosis. Accordingly, there has been increasing evidence that MPV may be a new cardiovascular risk factor and that the increased platelet activity may be associated with severity of atherosclerosis and greater risk of cardiovascular events.[11,14]

Previous studies have reported that elevated MPV can increase the risk of short- and long-term adverse cardiovascular outcome in patients with established CVD.[11,14] Furthermore, higher MPV is observed in thromboembolic events and coronary artery disease, hypertension, diabetes mellitus, hypercholesterolemia, and smoking, suggesting a common mechanism by which these factors may increase the risk of CVD.[14,24,25]

Platelet survival is one of the most reliable parameters of in vivo platelet activation reflecting the influence of various pro- and antiaggregatory effects.[26] Platelet survival and platelet counts have been reported to be significantly reduced in patients with FH.[27] This is an indicative of increased platelet turnover in FH and assuming that larger immature platelets predominate in this condition.[28] In the present study, we observed that platelet count was reduced in patients with FH. In the other hand, MPV was elevated in the same patients.

Previous studies have showed that FH can result in subclinical inflammation, impaired endothelial function, and increased intima–media thickness.[4,5] In addition, plasma high-sensitivity C-reactive protein (hsCRP) levels have been reported to be increased in children with FH.[29] Inflammation itself may influence megakaryocyte ploidy and lead to the production of more reactive and larger platelets.[30] The MPV has been shown to be significantly correlated with hsCRP levels in patients with hypertension.[31] Accordingly, it can be suggested that endothelial dysfunction and inflammation may contribute to elevation in MPV in patients with FH by stimulating the megakaryocyte ploidy.

P-selectin is known as platelet adhesion and activation molecule related to pathogenesis of atherothrombosis.[32] Guardamagna et al have demonstrated that there is a strong and significant association between P-selectin and plasma lipid levels in patients with FH.[33] Similarly, it is reported that elevated MPV can be associated with increased thromboxane A2 and B2 concentrations and higher expression of adhesion molecules, such as P-selectin and GpIIb/IIIa.[12,14]

Pirich et al have shown that dietary supplementation with fish oil containing small amounts of n-3 and n-6 fatty acids resulted in prolongation of platelet survival and the decrease in platelet activity in patients with hypercholesterolemia. In addition, LDL-C levels were reduced by the supplementation.[34] Similarly, lowering of the LDL-C levels by regular LDL-apheresis can decrease platelet aggregability and prolong platelet survival.[15,35] That study provides indirect support to our results.

Jagroop et al have reported that platelet cholesterol (PC) can be correlated with serum LDL-C cholesterol. They have suggested that the increase in PC content may affect platelet membrane fluidity, thereby resulting in platelet hyperactivity. Their study also supports our findings showing that high circulating cholesterol levels may be associated with the increased MPV reflecting platelet hyperactivity.[36]

Limitations of the Study

First, study data were collected from 2 centers. Second, we did not carry out a power analysis for inclusion of patients, since we did not have any data on which to base such a calculation. Third, we selected the patients with first diagnosis of FH and who did not receive any lipid-lowering agent. Thus, selection bias may have occurred.

In conclusion, our findings suggest that MPV was elevated in patients with FH compared to controls and that was associated with total cholesterol level. Because larger platelets are more active, and thereby prone to adhesion and aggregation, patients with FH having elevated MPV may have a higher risk of atherothrombotic cardiac events. Thus, such patients may need effective antiplatelet therapy in addition to intensive lipid-lowering treatment.

Authors' Note

AI, FA, GN, and AD conceived and designed the study. AI, FA, GN, HK, MFA, RN, AG, and AA performed the study and acquisition of

data. AI, AD, and AA analyzed and interpreted the data. AI and AD wrote the article and critical revision.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Hovingh GK, Davidson MH, Kastelein JJ, O'Connor AM. Diagnosis and treatment of familial hypercholesterolaemia. *Eur Heart J*. 2013;34(13):962-971.
2. Hopkins PN, Toth PP, Ballantyne CM, Rader DJ. Familial hypercholesterolemias: prevalence, genetics, diagnosis and screening recommendations from the National Lipid Association Expert Panel on Familial Hypercholesterolemia. *J Clin Lipidol*. 2011;5(3 suppl):S9-S17.
3. Neil HA, Huxley RR, Hawkins MM, Durrington PN, Betteridge DJ, Humphries SE. Comparison of the risk of fatal coronary heart disease in treated xanthomatous and non-xanthomatous heterozygous familial hypercholesterolaemia: a prospective registry study. *Atherosclerosis*. 2003;170(1):73-78.
4. Cheng HM, Ye ZX, Chiou KR, Lin SJ, Charng MJ. Vascular stiffness in familial hypercholesterolaemia is associated with C-reactive protein and cholesterol burden. *Eur J Clin Invest*. 2007; 37(3):197-206.
5. Kastelein JJ, Wiegman A, de Groot E. Surrogate markers of atherosclerosis: impact of statins. *Atheroscler Suppl*. 2003;4(1):31-36.
6. DiMinno G, Silver MJ, Cerbone AM, Rainone A, Postiglione A, Mancini M. Increased fibrinogen binding to platelets from patients with familial hypercholesterolemia. *Arteriosclerosis*. 1986;6(2):203-211.
7. Carvalho AC, Colman RW, Lees RS. Platelet function in hyperlipoproteinemia. *N Engl J Med*. 1974;290(8):434-438.
8. Corash L, Andersen J, Poindexter BJ, Schaefer EJ. Platelet function and survival in patients with severe hypercholesterolemia. *Arteriosclerosis*. 1981;1(6):443-448.
9. Blaha M, Pecka M, Urbankova J, et al. Activity of thrombocytes as a marker of sufficient intensity of LDL-apheresis in familial hypercholesterolaemia. *Transfus Apher Sci*. 2004;30(2):83-87.
10. Aviram M, Hussein O, Rosenblat M, Schlezinger S, Hayek T, Keidar S. Interactions of platelets, macrophages, and lipoproteins in hypercholesterolemia: antiatherogenic effects of HMG-CoA reductase inhibitor therapy. *J Cardiovasc Pharmacol*. 1998; 31(1):39-45.
11. Vizioli L, Muscari S, Muscari A. The relationship of mean platelet volume with the risk and prognosis of cardiovascular diseases. *Int J Clin Pract*. 2009;63(10):1509-1515.
12. Kamath S, Blann AD, Lip GY. Platelet activation: assessment and quantification. *Eur Heart J*. 2001;22(17):1561-1571.
13. Park Y, Schoene N, Harris W. Mean platelet volume as an indicator of platelet activation: methodological issues. *Platelets*. 2002; 13(5-6):301-306.
14. Chu SG, Becker RC, Berger PB, et al. Mean platelet volume as a predictor of cardiovascular risk: a systematic review and meta-analysis. *J Thromb Haemost*. 2010;8(1):148-156.
15. Sinzinger H, Pirich C, Bednar J, O'Grady J. Ex-vivo and in-vivo platelet function in patients with severe hypercholesterolemia undergoing LDL-apheresis. *Thromb Res*. 1996;82(4): 291-301.
16. Tremoli E, Maderna P, Colli S, Morazzoni G, Sirtori M, Sirtori CR. Increased platelet sensitivity and thromboxane B2 formation in type-II hyperlipoproteinaemic patients. *Eur J Clin Invest*. 1984; 14(5):329-333.
17. World Health Organization. *Familial Hypercholesterolemia—Report of a Second WHO Consultation*. Geneva, Switzerland: World Health Organization; 1999. WHO publication no. WHO/HGN/FH/CONS/99.2.
18. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18(6): 499-502.
19. Stokes KY, Cooper D, Tailor A, Granger DN. Hypercholesterolemia promotes inflammation and microvascular dysfunction: role of nitric oxide and superoxide. *Free Radic Biol Med*. 2002;33(8):1026-1036.
20. Croft KD, Beilin LJ, Vandongen R, Rouse I, Masarei J. Leukocyte and platelet function and eicosanoid production in subjects with hypercholesterolaemia. *Atherosclerosis*. 1990;83(2-3):101-109.
21. Mazeaud MM, Driss F, Le Quan Sang KH, et al. Biochemical and functional alterations associated with hypercholesterolemia in platelets from hypertensive patients. *Atherosclerosis*. 1992;94(2-3):201-211.
22. Boos CJ, Lip GY. Assessment of mean platelet volume in coronary artery disease—what does it mean? *Thromb Res*. 2007; 120(1):11-13.
23. Giles H, Smith RE, Martin JF. Platelet glycoprotein IIb-IIIa and size are increased in acute myocardial infarction. *Eur J Clin Invest*. 1994;24(1):69-72.
24. Pathansali R, Smith N, Bath P. Altered megakaryocyte-platelet haemostatic axis in hypercholesterolaemia. *Platelets*. 2001; 12(5):292-297.
25. Celebi OO, Canbay A, Celebi S, Sahin D, Aydogdu S, Diker E. The effect of admission mean platelet volume on TIMI frame count measured after fibrinolytic therapy in patients with acute ST-segment elevation myocardial infarction. *Turk Kardiyol Dern Ars*. 2009;37(5):307-311.
26. Thakur ML, Welch MJ, Joist JH, Coleman RE. Indium-LLL labeled platelets: studies on preparation and evaluation of in vitro and in vivo functions. *Thromb Res*. 1976;9(4):345-357.
27. Smith CC, Betteridge DJ. Reduced platelet serotonin content and release in familial hypercholesterolaemia. *Atherosclerosis*. 1997; 130(1-2):87-92.
28. Martin JF, Kristensen SD, Mathur A, Grove EL, Choudry FA. The causal role of megakaryocyte-platelet hyperactivity in acute coronary syndromes. *Nat Rev Cardiol*. 2012;9(11):658-670.
29. Ueland T, Vissers MN, Wiegman A, et al. Increased inflammatory markers in children with familial hypercholesterolaemia. *Eur J Clin Invest*. 2006;36(3):147-152.

30. Gasparyan AY, Ayvazyan L, Mikhailidis DP, Kitas GD. Mean platelet volume: a link between thrombosis and inflammation? *Curr Pharm Des*. 2011;17(1):47-58.
31. Yarlioglues M, Kaya MG, Ardic I, et al. Relationship between mean platelet volume levels and subclinical target organ damage in newly diagnosed hypertensive patients. *Blood Press*. 2011; 20(2):92-97.
32. Blann AD, Nadar SK, Lip GY. The adhesion molecule P-selectin and cardiovascular disease. *Eur Heart J*. 2003; 24(24):2166-2179.
33. Guardamagna O, Abello F, Saracco P, Baracco V, Rolfo E, Pirro M. Endothelial activation, inflammation and premature atherosclerosis in children with familial dyslipidemia. *Atherosclerosis*. 2009; 207(2):471-475.
34. Pirich C, Gaszo A, Granegger S, Sinzinger H. Effects of fish oil supplementation on platelet survival and ex vivo platelet function in hypercholesterolemic patients. *Thromb Res*. 1999;96(3): 219-227.
35. Blaha M, Kostal M, Lanska M, et al. The decrease of mean platelet volume after extracorporeal LDL-cholesterol elimination. *Atheroscler Suppl*. 2013;14(1):77-81.
36. Jagroop IA, Persaud JW, Mikhailidis DP. A new rapid method to measure human platelet cholesterol: a pilot study. *Clin Appl Thromb Hemost*. 2011;17(6):578-584.