Mean platelet volume is elevated in patients with patent foramen ovale

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Abstract

Introduction: Platelets play a major role in thromboembolic events. Increased mean platelet volume (MPV) indicates higher platelet reactivity and also a tendency to thrombosis. Patent foramen ovale (PFO), persistence of the fetal anatomic shunt between right and left atria, is strongly associated with cryptogenic stroke. The aim of this study is to determine the relationship between MPV and PFO and if such an association exists, whether higher MPV levels may require antiplatelet therapy before a thromboembolic event happens, together with a literature review.

Material and methods: Thirty patients (15 women, 15 men), free of any cerebrovascular events, were diagnosed with PFO by transesophageal echocardiography (TEE), enrolled as the study group. Thirty consecutive patients (16 women and 14 men), who were diagnosed as normal in TEE, were enrolled as the control group. These two groups were compared according to MPV and anatomical features of the right atrium.

Results: There was no significant difference between study and control groups in clinical features and also no difference was observed in platelet counts; however, MPV in the PFO group was significantly higher than the control group (8.38 ±0.93 fl and 7.45 ±0.68 fl respectively).

Conclusions: Our results indicate that elevated MPV may be detected in patients with PFO. This might be one of the explanations for the relationship between PFO and cryptogenic stroke; however, larger cohorts are warranted in order to define further mechanisms.

Key words: echocardiography, mean platelet volume, patent foramen ovale, stroke.

Introduction

Stroke is an important cause of mortality and morbidity [1]. The etiology of nearly 40% of strokes in patients could not be determined and these are called cryptogenic strokes [2]. Patent foramen ovale (PFO) is a known and important predisposing factor for cryptogenic stroke [3–5]. In the literature, PFO incidence among patients with cryptogenic stroke ranges between 40% and 55% whereas the incidence is 25% in the healthy population [6–9]. Paradoxical emboli, direct emboli, inappropriate coagulation and atrial arrhythmias are the major factors in the mechanism of stroke [10, 11].

Mean platelet volume (MPV) is a routine blood count parameter. The relations between PFO and atherosclerotic diseases and ischemic stroke have been identified [12]. Additionally, it was hypothesized that the changes in platelet size may also play a role in thrombotic events [13]. It has been shown that the ''giant platelets'' have a higher enzymatic activity and produce more thromboxane A2 [14]. Also MPV is associated with myocardial infarction, acute cerebral ischemia and transient ischemic attack (TIA) [4, 15]. Increased values of MPV were observed in patients with mitral stenosis [16].

To the best of our knowledge there are no data regarding the relationship between MPV and PFO. We hypothesized that, similar to mitral stenosis, the tendency for thrombocyte aggregation in patients with PFO may also be associated with stasis. The PFO, which has a tubular structure, leads to slow blood flow, which in turn stimulates platelet activation and *in situ* thrombus formation. As a result, MPV is an indicator of platelet activation. Furthermore, the deformation and the shear stress of the thrombocytes passing through the defect also lead to platelet activation, causing an increase in the MPV. This paper presents a comparison of MPV, between the normal population, as the control group, and the patients who were diagnosed with PFO during routine transesophageal echocardiography (TEE) and free of any stroke or TIA at the time of investigation, as the study group.

Material and methods

Study population

The study group was composed of patients who were incidentally diagnosed with PFO during TEE examination between December 2010 and August 2011. The indications of TEE were suspicion of PFO or insufficient views with transthoracic echocardiography. As the demographic features of the patients: hypertension was defined as systolic blood pressure greater than 140 mm Hg and diastolic greater than 90 mm Hg or normotensive patients with antihypertensive medication; diabetes mellitus was defined as blood glucose over 126 mg/dl after night fasting or 2 h post-prandial blood glucose over 190 mg/dl or normal levels of blood glucose with antidiabetic treatment; smoking was accepted as a factor if the patient smoked 10 or more cigarettes in a day; body mass index was calculated with the formula: weight in kilograms divided by the square of height in meters.

Patients with known history of stroke, TIA, thromboembolic events, congenital heart diseases (including mitral valve prolapse and bicuspid aorta), congestive heart failure, valvular heart disease, atrial fibrillation, any inflammatory diseases including pericarditis or myocarditis, hepatic or renal dysfunction, neoplastic diseases, hereditary causes of thromboembolism, documented coronary artery disease and those who received antiplatelet or anticoagulant therapy were excluded.

The study was approved by the institutional ethics committee. The details of the study were explained and informed consent was obtained from each patient prior to inclusion in the research.

Table I. Anatomic and procedural features

Parameter	Results
PFO radius, mean ± SD [mm]	4.81 ±3.93
Atrial septal aneurysm, *n* (%)	12 (40)
Chiari network, *n* (%)	8 (26)
Eustachian valve, *n* (%)	10 (33)
Spontaneous right left shunt, *n* (%)	11 (36)
Degree of shunt, *n* (%)	
Large (> 20 microbubbles)	23 (77)
Small (< 20 microbubbles)	7 (23)

Echocardiography

After undergoing routine transthoracic echocardiography, all patients underwent multiplane TEE (Vivid S-5, GE Systems, Norway) with saline contrast injection. Before the TEE study all patients were informed and educated about the Valsalva maneuver. The echocardiography studies were performed by two experienced echocardiographers who were blind about patients. Contrast material was prepared by mixing 9 ml of normal saline with 1.0 ml of patients' own blood and 1 ml of air through a three-way stopcock. The TEE was performed after administration of pharyngeal anesthesia with 10% lidocaine spray and 2 mg of midazolam intravenously for sedation. A 3.5–9 MHz multiplane TEE probe was passed to mid-above esophageal position, with longitudinal axis 90–100°, and then manipulated to provide adequate views of both atria, appendages, atrial septum, and mitral valve apparatus. All examinations were recorded for subsequent playback and analysis. Saline contrast injection was performed at rest and during the Valsalva maneuver. A PFO was judged to be present if at least one microbubble was seen in the left atrium within three cardiac cycles after maximum opacification of the right atrium [17]. If PFO was judged to be present, further imaging of the fossa ovalis area was undertaken in the vertical view to maximize the separation between the septum primum and secundum. The anatomic and procedural features of both groups are shown in Table I.

Biochemical studies

After 8 h of fasting the blood was drawn from an antecubital vein, stored in dipotassium-EDTA

tubes and studied within 1 h with an automatic hematology analyzer (Beckman Coulter, California, USA). The reference limits of MPV were accepted as 6–10 fl. The fasting blood glucose was measured with the hexokinase method and total cholesterol, high density lipoprotein (HDL), triglyceride (TG), and low density lipoprotein (LDL) were measured with the enzymatic method, accelerator selective detergent method, glycerol phosphate oxidase method and Friedewald formula, respectively, with an auto analyzer (Abbott Aeroset, Massachusetts, USA) [18].

Statistical analysis

Statistical analysis was performed with the computer program Statistical Package for the Social Sciences (SPSS) 19.0 (SPSS Inc. Chicago, Illinois, USA) for Windows by a professional statistician. Data are expressed as mean and standard deviation. The statistics of descriptive data are presented with frequency, ratio, mean ± standard deviation. The distribution of the data was tested with the Kolmogorov-Smirnov test. Non-parametric data were analyzed with the Mann-Whitney *U* test and parametric variables with the *t* test. Proportional variables were analyzed with the χ^2 test, if not suitable with Fisher's exact test. Descriptive variables were compared with Spearman correlation and classified variables with Pearson correlation analyses. A p value less than 0.05 was considered statistically significant.

Results

Mean age of the patients in the study group was 34.47 ±10.36 years and in the control group was 37.73 ±12.95 years. In both groups there were 3 diabetic patients. Hyperlipidemia was present in 10 patients and 6 patients in PFO and control groups, respectively. There were 3 hypertensive cases in the study group and 2 cases in the control group. Positive family history for PFO was identified in 8 patients in the study population whereas, although subjects did not have PFO, PFO was present in either first or second degree relatives among the control group patients. There were 8 smokers in the study group and 5 in the control group; however, 4 patients in each group had chronic obstructive pulmonary disease. Body mass index was 25.68 ±3.83 kg/m^2 in the PFO group and 24.21 ±3.89 kg/m^2 in the control group. Only one patient used oral contraceptives in the whole population. Ten patients in the study group had neurologically defined migraine and 6 in the control group. The aforementioned characteristics are shown in Table II. There were no statistically significant differences in demographic and clinical features – age, sex, hypertension, diabetes mellitus, hyperlipidemia, smoking, family history, migraine, oral contraceptive usage, chronic obstructive pulmonary disease, and body mass index – between the study and the control groups.

The blood parameters – blood glucose, creatine, total cholesterol, LDL, HDL, TG, hemoglobin, white blood cell count, and platelet count – are presented in Table III and they were not significantly different between the two groups. Interestingly, MPV was significantly higher in the PFO group than the control group (8.38 ±0.93 vs. 7.45 ±0.68 fl, $p < 0.001$) (Table III).

No statistically significant correlation was observed between the MPV and anatomical and procedural features such as PFO radius, spontaneous shunt, shunt degree, atrial septal aneurysm, or Eustachian valve (Table IV).

Discussion

Stroke currently ranks third among causes of death [19]. Strokes with no known causes comprise

Table II. Clinical and demographic features of patients

Parameter	PFO (n:30)	Control (n:30)	Value of *p*
Age, mean ± SD [years]	34.47 ±10.36	37.73 ±12.95	0.308
Sex (M/F), *n*	15/15	16/14	0.897
DM, *n*	3	3	1.000
HL, *n*	10	6	0.243
HT, *n*	3	2	0.640
Family history, *n*	8	7	0.766
Smoking, *n* (%)	8 (27)	5 (17)	0.347
Migraine, *n*	10	6	0.243
Oral contraceptive, *n*	1	1	1.000
COPD, *n*	4	4	1.000
BMI [kg/m^2]	25.68 ±3.83	24.21 ±3.89	0.145

M – male, F – female, DM – diabetes mellitus, HL – hyperlipidemia, HT – hypertension, COPD – chronic obstructive pulmonary disease, BMI – body mass index

Table III. Laboratory findings of PFO and control groups

Parameter	PFO (n:30)	Control (n:30)	Value of *p*
Glucose [mg/dl]	89.40 ±12.15	89.17 ±13.40	0.944
Urea [mg/dl]	27.30 ±5.77	26.40 ±8.02	0.620
Creatinine [mg/dl]	0.76 ±0.18	0.74 ±0.20	0.375
TChol [mg/dl]	169.60 ±33.77	177.07 ±34.35	0.399
LDL [mg/dl]	107.90 ±32.42	111.07 ±31.65	0.699
HDL [mg/dl]	43.93 ±11.58	47.17 ±10.57	0.263
TG [mg/dl]	88.90 ±47.16	95.80 ±42.82	0.555
Hemoglobin [g/dl]	13.32 ±1.61	13.46 ±1.28	0.710
WBC [× 10 m^3]	7.4 ±1.9	7.1 ±2.2	0.856
Platelet count [× 10^9]	244.5 ±58.7	256.8 ±54.9	0.302
MPV [fl]	7.45 ±0.68	8.38 ±0.93	< 0.001

PFO – patent foramen ovale, TChol – total cholesterol, LDL – low density lipoprotein, HDL – high density lipoprotein, TG – triglyceride, WBC – white blood cells, MPV – mean platelet volume

Table IV. Correlation of MPV with anatomical and clinical features

		PFO radius	Spontaneous shunt	Shunt degree	ASA	Eustachian valve
MPV	*r*	–0.139	0.047	–0.009	–0.044	0.101
	p	0.464	0.804	0.960	0.819	0.594

PFO – patent foramen ovale, ASA – atrial septal aneurysm, MPV – mean platelet volume

about half of all strokes [20]. Defined as the flap-like opening between the septum primum and secundum at the fossa ovalis section of the interatrial septum, PFO is demonstrated to be the crucial cause of cryptogenic strokes. Various mechanisms have been suggested as the cause of stroke in patients with PFO. The most valid is the paradoxical embolism [21]. In addition, a tendency to hypercoagulation and aggregation may be among the reasons for stroke in this particular patient group. Additionally, it is also believed that it causes in situ thrombus due to stasis resulting from sluggish flow through the PFO tunnel.

Aggregation is the clumping of the activated platelets. Platelet aggregation is the main step towards the formation of thrombus. Mean platelet volume is the determinant of platelet functions through an easy and inexpensive method and is a physiological variable with hemostatic significance [22]. Giant platelets are more reactive, produce more prothrombotic factors, and are clumped together more easily [13, 23, 24]. Giant platelets contain denser granules and they secrete more serotonin and β-thromboglobulin [25, 26]. Increased MPV is related to more *in vitro* aggregation in response to ADP and collagen [27].

Various studies demonstrate that shear stress formed by turbulent flow resulting from stenotic valves induces platelet activation [28, 29]. It has been shown that MPV increases in the presence of calcified aortic stenosis, one of the most significant valvular diseases that cause higher turbulent flow [30]. However, MPV is also elevated in mitral stenosis with low gradient sinus rhythm relative to aortic stenosis. Here, stasis developing in the left atrium may induce platelet activation [31]. Similarly, platelet activation can be stimulated with slow blood flow through tunnel-shaped PFO, and this may lead to in situ thrombus. Furthermore, the deformation and the shear stress of the thrombocytes passing through the defect also lead to platelet activation, causing an increase in MPV.

The fact that MPV was found higher in patients with PFO with no previous cerebrovascular event in our study might be a finding that supports the increase in the risk of stroke in those patients. As is known, the co-existence of PFO and atrial septal aneurysm increases the risk of stroke [31, 32]. In addition, the risk of stroke is also elevated in the presence of greater PFO diameter and spontaneous right-to-left shunt [33, 34]. In our study, there was no correlation between the increase in PFO diameter and presence of spontaneous right-to-left shunt and atrial septal aneurysm with MPV. In this study, our major hypothesis is that the tubular shaped PFO causes stasis leading to thrombocyte aggregation. If the correlation analysis were done by measuring the length of the PFO tunnel, the result might have been significant.

Anticoagulant and antiaggregant treatments are recommended for patients with PFO who experienced cerebrovascular events [35]. Additionally,

although there are few randomized controlled studies in this area, PFO is recommended to be closed with transcatheter technique or surgically, especially in high-risk patients [36]. However, in asymptomatic patients with PFO, with no previous cerebrovascular disease including transient ischemic attack, a consensus does not exist concerning commencing anticoagulant and antiaggregant treatment or closure [37, 38]. Although PFO gradually closes at early ages, sometimes the diameter is enlarged along with advanced age, and also it is known that the risk of stroke increases along with the increase in PFO diameter [39]. Patent foramen ovale is also associated in such cases predominantly with migraine, platypnea-orthodeoxia syndrome, and decompression sickness in divers [40]. When taken into consideration that it may lead to substantial labor loss in addition to the fact that stroke is a very significant cause of mortality and morbidity, there is a need for comprehensive randomized controlled studies in order to consider closure of the PFO in patients with right-to-left shunt that may be deemed risky for stroke accompanied by atrial septal aneurysm and Eustachian valve. When considering the mean platelet volume increase that was observed in our study, antiaggregant agents such as aspirin or clopidogrel could be considered for routine use for stroke prevention in this particular patient group. However, since enough evidence has not yet been obtained to be able to advocate this, it must also be taken into account that antiplatelet treatment also increases the risk of bleeding.

In our study, contrast TEE was used to investigate the PFO [41]. Even though TEE conducted with contrast is the gold standard in the diagnosis of PFO, pseudo negativities are also observed in such cases as insufficient contrast agent injection, insufficient or inappropriate Valsalva maneuver by the patients, increased left atrium pressure, and the use of upper extremity vein in the presence of Eustachian valve. In addition, pseudo negativity can also be found in cases as sinus venosus type or ASDs that cannot be imaged, when a giant Eustachian valve is mistakenly thought to be the atrial septum, with the effect of Valsalva maneuver, and in cases with pulmonary arteriovenous fistula. As is known, while inferior vena cava flow is directed to the fossa ovalis region, superior vena cava flow is through the tricuspid valve [5]. Thus, if a contrast study was conducted by utilizing the femoral artery, PFO might have been detected possibly in some members of the control group. However, femoral artery puncture is also associated with an increase in the risk of significant infection, arteriovenous fistula and thrombus. There is also a disadvantage as the contrast agent given through lower extremity arteries reaches the heart in a longer than expected period of time.

In our study, while a complete blood count was conducted by an automatic counter, EDTA was used as an anticoagulant. When EDTA is used as the anticoagulant, mean platelet volume increases due to platelet swelling. However, a change does not occur in time with a high concentration of sodium citrate (4/1 blood/citrate) [42]. And in another study, when an analysis was carried out 2 h after taking the blood sample, an increase in platelet size of < 0.5 fl was observed [43]. In our study, this negative characteristic of EDTA was eliminated by studying the blood samples no later than 1 h following blood withdrawal. When full blood sampling is conducted, due to different effects of anticoagulant agents and other preanalytical variables, it is recommended for each laboratory to give their own reference intervals for these platelet parameters [44].

The most significant restriction of our study is the low number of patients. In addition, use of right upper extremity veins for intravenous contrast analysis, and also not conducting transcranial Doppler USG in patients in whom we had doubts in diagnosing PFO, are the other limitations of our study. Use of the Valsalva maneuver in patients as a provocative maneuver can also be considered as a limitation since the number of microbubbles passing through right-to-left shunt differs based on the efficiency of the Valsalva maneuver and atrial pressure. Additionally, it is difficult for the flow from the superior vena cava in patients with a Eustachian valve to be directed to the fossa ovalis. Therefore, PFO diagnosis might be neglected in this patient group. Not scanning asymptomatic cerebral microinfarcts with cranial magnetic resonance imaging in the patient population with PFO can also be deemed another limitation. As the use of EDTA as an anticoagulant agent on full blood count samples might lead to swelling of platelets, it can be accepted as another limitation. Another limitation would be the absence of such indexes showing platelet activation markers in circulation as soluble p-selectin, soluble CD40 ligand and median platelet granularity, all analyzed together with MPV.

Patients with PFO with previous stroke and transient ischemic attack were determined as exclusion criteria in our study as most of these patients take such antiaggregant medicine as aspirin or clopidogrel. It was believed to be unsound to carry out an objective evaluation of MPV that is the indication of platelet activation in this patient group receiving antiaggregant treatment. However, MPV as an indication of platelet activation could have been compared in patients with PFO not having any antiaggregant treatment following any cerebrovascular events and asymptomatic PFO patients. Yet, there is quite a limited number of patients having those characteristics.

In conclusion, our study established increased MPV in patients with PFO who did not have any cerebrovascular event by utilizing contrast TEE. As far as the literature is concerned, our study is the first to demonstrate mean platelet volume in patients with PFO with no thromboembolic events. Among the various mechanisms leading to stroke in patients with PFO, an additional one could be the tendency of platelet aggregation due to increased MPV. Although our study fails to explain evidence that increased MPV cannot be an inborn feature of the subjects with PFO, the major finding of the research is the difference between groups in MPV. However, more comprehensive randomized studies are warranted to define underlying mechanisms of the observation and to establish its consequences. Only then may routine antiaggregant treatment for asymptomatic patients detected to have PFO be recommended.

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