Age Related Increase of Platelet Activation

Sang-Hwan Kim[1], Jee-Aee Im[2], Ji-Won Lee[3] and Duk-Chul Lee[3,†]

[1]Department of Family Medicine, School of Medicine, Eulji University, Daejeon, Korea.
[2]Department of Laboratory Medicine, MizMedi Hospital, Seoul, Korea.
[3]Departments of Family Medicine, Yonsei University, College of Medicine,
Yongdong Severance Hospital, Seoul, Korea

Platelets clearly play an important role in inflammatory responses. In this study, we aimed to evaluate the relationship between aging and platelet activation. A total number of 799 persons (383 males and 416 females), who were apparently healthy and aged more than 20 years were recruited by a health promotion center in a community-based hospital in Seoul, Korea. We collected material data about their medical history and health behavior. Platelet parameters including mean platelet component (MPC), mean platelet volume (MPV), and platelet component distribution width (PCDW) were determined within 1 hour after blood collection using the ADVIA 120 automated hematology analyzer. The MPC of the women (27.2±1.2) was significantly lower than that of the men (27.5±1.3). The MPC of all participants was found to decrease with increasing age ($P<0.01$). Study participants in their twenties had the highest MPC (27.7±1.1), followed by those in their thirties (27.6±1.1), forties (27.4±1.3), fifties (27.2±1.3), sixties (27.2±1.2) and seventies (27.1±1.2). Multiple regression analysis showed that aging and gender were related with MPC after adjusting for confounding factors, including age, gender, smoking habit, hypertension, diabetes, body mass index and total cholesterol level. The this study shows that aging is related to platelet activation. Future research will need to determine the implications of increased platelet activation with aging, especially regarding the increased incidence of cardiovascular diseases and related mortalities that occur in older age groups.

Key Words: Aging, Platelet activation, Mean platelet component

INTRODUCTION

Arterial thrombosis is the acute complication that develops on chronic lesions of atherosclerosis and causes heart attacks and strokes. Additionally, cardiovascular diseases are the major cause of morbidity and mortality in the elderly (Massarelli et al., 2000). Platelets, with fibrin, are prominent components of the thrombi (clots) that occlude arteries. Platelets may also participate in the development and progression of atherosclerotic plaque (Fuster et al., 1992; Ross, 1993).

Activated platelets have been associated with several

disorders, including coronary artery disease (Cahill, 1996a; Knight et al., 1997), Alzheimer's disease (Davies et al., 1997), myeloproliferative disorders (Cahill et al., 1996b), preeclampsia (Konijnenberg et al., 1997), and glomerular disease (Barnes, 1997). Furthermore, most risk factors of atherosclerosis including hypercholesterolemia (Broijersen, 1998), hypertension (Nityanand et al., 1993; Minuz et al., 1994), cigarette smoking (Nowak et al., 1987), and diabetes (Manduteanu et al., 1992) can increase the number of activated platelets in circulation.

Thanks to recent advances in automatic blood cell counting, a fast and accurate determination of platelet count and volume can be made. The density of platelets decreases upon activation, due to the release of alpha granules and dense granules (Corash et al., 1977). Mean platelet component (MPC) values have been shown to have a strong inverse correlation with the CD62P expression as a measure of platelet activation, and P-selectin secreted by platelet membrane (Chapman et al., 2003). Also related to platelet acti-

vation are the mean platelet volume (MPV) (Ahmed et al., 1993), which represents platelet size, and the platelet component distribution width (PCDW) (Mezzano et al., 1981; Lim et al., 2002).

As noted, there have been studies linking several diseases and platelet activation. To our knowledge, however, the relationship between aging and platelet activation has not been previously reported. In this study, we aimed to examine the variables related to MPC and to evaluate the relationship between aging and platelet activation.

MATERIALS AND METHODS

Study participants were recruited from a health promotion center in a community-based hospital in Seoul, Korea. The participants visited the hospital for a periodic health check-up. We recruited 383 males and 416 females that were apparently healthy and aged more than twenty years. We excluded patients with bleeding tendencies and thrombotic events, such as stroke and ischemic heart disease. All subjects signed an informed consent form approved by the Ethical Committee of the hospital.

We collected material data about the history and health behavior of the participants. Prior to testing, the participants independently completed a questionnaire regarding their medical history, cigarette smoking and exercise. In this study, hypertension was defined as having a history of taking associated medication or a rise in checked blood pressure above 140/90 mmHg. Diabetes was defined as having a history of taking associated medication or a rise in checked fasting serum glucose above 126 mg/dl.

Body weight was measured to the nearest 0.1 kg on an electronic scale. Subjects were weighed in light clothing and without shoes. Height was measured to the nearest 0.1 cm using a well mounted stadiometer. Body mass index (BMI) was defined as weight/height^2 in kg/m^2.

All samples were obtained in the morning after at least 8 hours of fasting. For the evaluation of platelet activation, we collected 5 ml of blood in a vacuum tube containing EDTA as an anticoagulant. A vacuum tube without an anticoagulant was used for examination of the others. After centrifugation, the serums were immediately stored at -80 ℃. Serum levels of fasting glucose, total cholesterol, HDL-cholesterol, and triglyceride were assayed using an ADVIA 1650 Chemistry system (Bayer, Tarrytown, NY, USA).

Platelet parameters, including MPC, MPV and PCDW were determined within 1 hour after blood collection using the ADVIA 120 automated hematology analyzer (Bayer, Tarrytown, NY, USA). Before being used for the examination, the instrument was calibrated using the Bayer SET-point calibrator and the Bayer OPTIpoint (Bayer, Tarrytown, NY, USA).

The effects of gender, age and diseases, including hypertension, diabetes, and others were assessed using a t-test for continuous variables and a χ^2-test for categorical variables. A Pearson correlation analysis was used to determine the relationship of platelet related variables with age, and analysis of variance (ANOVA) with a Tukey's multiple comparison analysis was used to determine the differences among MPC values of different age categories.

Multiple regression analysis, adjusted for age, gender, smoking habit, hypertension, diabetes, BMI and total cholesterol level, was used to determine the variables related to platelet activation. $P<0.05$ was considered statistically significant. All analyses were performed with the statistical package SAS for Windows V8.01 (SAS institute, Cary, NC, USA).

RESULTS

Between genders, there was no significant difference in the mean age and accompanied diseases, including hypertension and diabetes. Females had a significantly higher mean platelet count than males ($P<0.01$). In contrast, the MPC was significantly lower in females than in males ($P<0.01$) (Table 1).

Age was inversely correlated with MPC ($r=-0.18$, $P< 0.01$). However, there was no correlation between age and MPV (Table 2).

The MPC of all participants was found to decrease with increasing age ($P<0.01$). Study participants in their twenties had the highest MPC (27.7±1.1), followed by those in their thirties (27.6±1.1), forties (27.4±1.3), fifties (27.2± 1.3), sixties (27.2±1.2) and seventies (27.1±1.2). Tukey's multiple comparison analysis showed the MPC concentrations of participants in their fifties, sixties and seventies to be markedly lower than those of the participants in their twenties. Additionally, the MPC concentrations of the fifties and sixties groups were markedly lower than those of the thirties group (Fig. 1).

Table 1. Clinical characteristics and platelet parameters according to gender

Variables	Male (N=383)	Female (N=416)	P-value
Age (years)	48.4±13.9	47.4±14.5	0.33
Hypertension[a] N (%)	97 (25.3)	85 (20.4)	0.10
Diabetes[b] N (%)	8 (2.1)	6 (1.4)	0.45
Smoking habit N (%)			<0.01
Non-smoker	145 (37.9)	387 (93.0)	
Ex-smoker	74 (19.3)	17 (4.1)	
Current smoker	164 (42.8)	12 (2.9)	
BMI[c] (Kg/m²)	24.0±3.0	23.1±3.2	<0.01
Fasting glucose (mg/dl)	98.3±11.8	94.8±11.2	<0.01
Total cholesterol (mg/dl)	178.9±31.3	180.7±30.6	0.41
Platelet count (10³/µl)	247.6±54.9	260.0±61.4	<0.01
MPC[d] (g/dl)	27.5±1.3	27.2±1.2	<0.01
PCDW[e] (pg)	5.8±0.5	5.7±0.6	<0.05
MPV[f] (fl)	7.3±0.7	7.2±0.6	<0.05

P-values are calculated by t-test or χ^2-test.
[a]: hypertension was defined as having a history of taking associated medication or a rise in checked blood pressure above 140/90 mmHg
[b]: diabetes was defined as having a history of taking associated medication or a rise in checked fasting serum glucose above 126 mg/dl.
[c]: body mass index
[d]: mean platelet component
[e]: platelet component distribution width
[f]: mean platelet volume

Table 2. Correlation of platelet-related variables with age

Variables	Age	
	r	P-value
Platelet count (10³/µl)	-0.08	0.02
MPC[a] (g/dl)	-0.18	<0.01
PCDW[b] (pg)	0.12	<0.01
MPV[c] (fl)	0.04	0.30

Coefficients (r) and P-values are calculated by the Pearson correlation model.
[a]: mean platelet component
[b]: platelet component distribution width
[c]: mean platelet volume

Age was found to be an independent factor associated with MPC after adjustments were made for potential confounding factors such as subject age, gender, smoking habit, hypertension, diabetes, BMI and total cholesterol level (Table 3).

DISCUSSION

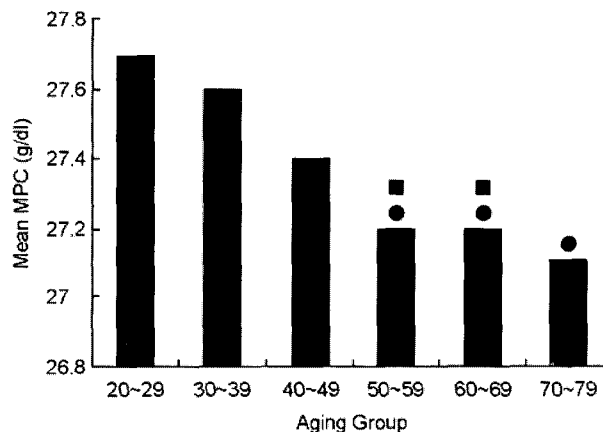This study shows that age is related to the increase of

Fig. 1. Mean platelet component (MPC) in the different age groups. The results of Tukey's multiple comparison analysis showed the MPC concentrations of the fifties, sixties and seventies groups were all markedly lower than those of the twenties and thirties groups. We compared the 20~29 year group (●) with the 30~39 year group (■) using Tukey's multiple comparison. P<0.01 by ANOVA analysis.

Table 3. Association between several factors and mean platelet component by multiple analysis[a]

Variables	β	S.E	P-value
Age	-0.02	0.01	<0.01
Gender (Female)	-0.22	0.11	<0.05
Hypertension[b]	-0.08	0.12	0.48
Diabetes[c]	0.60	0.33	0.07
Ex-smoker	0.09	0.15	0.57
Current smoker	0.08	0.13	0.56
BMI[d]	0.02	0.02	0.14
Total cholesterol	0.01	0.01	0.10

[a]: Calculated by multiple regression model using MPC as the dependent variable. The female group was compared to the male group, hypertension was compared to non-hypertension, diabetes was compared non-diabetes, and ex-smoker or current smoker was compared to non-smoker.
[b]: Hypertension was defined as having a history of taking associated medication or a rise in checked blood pressure above 140/90 mmHg.
[c]: Diabetes was defined as having a history of taking associated medication or a rise in checked fasting serum glucose above 126 mg/dl.
[d]: body mass index

the platelet activation. In this study, MPC was negatively correlated with age; the MPC concentration was highest in the twenties group (27.7±1.1) and lowest in the seventies group (27.1±1.2). In a study of 250 males and 250 females, Giacomini et al. (2001) found that the MPC concentration of the group aged above 65 years was lower than that of the group aged 45~65 years. However, they found no difference between the MPC concentration of the group aged 18~45

years and that of the group aged 45~65 years. The findings of Giacomini et al. (2001) are consistent with those of this study in that platelet activation increases with age. In this study, the MPC concentrations of those in their fifties, sixties or seventies were lower than those in their twenties or thirties. Furthermore, age was related to MPC after adjusting for confounding variables.

The mechanism of increased platelet activation with aging highlighted in this study can be explained as follows. A recent report demonstrated that the platelet membrane is deeply affected by oxidative stress in aged subjects (Martin-Valmaseda et al., 1999). Both the high content of peroxidation products and the altered physicochemical properties of the plasma membrane might be related to the increased platelet aggregability observed even in old but apparently healthy subjects (Hossain et al., 1999). Previous observations showed reduced plasma concentrations to be indicative of oxidative stress in centenarians compared with aged subjects (Paolisso et al., 1998). Rabinia et al. (Rabinia et al., 2003) indicated an age-associated trend in the platelet membrane concentrations of a biomarker of oxidative stress, as a malondialdehyde (MDA). A progressive increase in MDA occurred in subjects aged from 21~39 years to 60~79 years, but centenarians showed platelet membrane concentrations of MDA similar to the levels of the adult group and lower than those of elderly subjects. These results indicate numerous modifications of platelet behavior with aging. In addition, the results suggest that the plasma membranes of the centenarians are protected from oxidative damage, which is involved in the pathogenesis of several major age-associated diseases (Knight, 2000).

The limitations of this study are as follows. First, the history of the subject's intake of food and drugs that affect platelet parameters was insufficient. Second, despite our efforts to examine blood samples within 1 hour after sampling and at regular intervals, our results may have been influenced by the duration from blood sampling to assessment (Macey et al., 1999; Brummitt et al., 2000).

The new platelet parameter MPC may be used to detect resting or activated platelets. The measurement of MPC does not require specimen preparation, platelet activation-specific receptors, or activation-specific receptor labels. MPC values showed a strong inverse correlation with the CD62P expression as a measure of platelet activation (Chapman et al., 2003; Macey et al., 1999).

Because this study is of a cross-sectional design, we cannot elucidate whether the age-related increase of platelet activation is only a sign of thromboembolism, or if the increase is a sign of the pathogenesis of disease. Future research will need to determine the implications of increased platelet activation with aging, especially regarding the increased incidence of cardiovascular diseases and related mortalities that occur in older age groups.

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