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RESEARCH ARTICLE

Circulating Factors and Ultrasono-findings are Linked to Previous Atherosclerotic Burden and Recurrent Risk

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Abstract: ***Background*:** In the progression of atherosclerosis, platelet activation and the interaction of platelets with leukocytes play a crucial role in arterial thrombus formation and are associated with the pathophysiology of carotid and cerebrovascular disease (CVD), including ischemic stroke. With aged participants, we evaluated and followed up the change in circulating factor and platelet-leukocyte aggregate levels in participants with or without CVD history. This study investigated whether circulating factor changes and ultrasonographic characteristics link to CVD risk and other relating long-term outcomes.

***Materials and Methods*:** Two hundred fifteen participants who enrolled in the study were divided into two groups with CVD and without CVD history. We evaluated and analyzed the correlation between ultrasonography-based morphological characteristics and circulating factor-based functional changes in both groups.

***Results*:** There was no difference in p-selectin level between both groups. However, activated monocyte and platelet-monocyte aggregate levels were higher in patients with previous CVD than without previous CVD. Circulating factor and ultrasonographical characteristics were correlated in the group with CVD, whereas these factors were not correlated in the group without CVD.

***Conclusion*:** We found that circulating blood factor levels showed a different tendency in participants with and without CVD history. The results depict that atherosclerotic severity might depend on the history of CVD and progression of atherosclerosis. We suggest that the circulating factor levels, atherosclerotic severity, and history of CVD are considered in the observation of pathologic progression to manage the development of CVD risks and CVD relating outcomes.

Keywords: Circulating factors, atherosclerosis, platelet monocyte-aggregation, p-selectin, Mac-1, ultrasonography, cerebrovascular disease.

1. INTRODUCTION

Carotid artery atherosclerosis is associated with subsequent risk of new or recurrent cerebrovascular diseases, such as transient ischemic attack, stroke, and post-stroke vascular dementia, particularly in the modern day society [1-5]. With this association, several noninvasive measures for subclinical atherosclerosis have been undertaken in the predictive diagnosis of carotid and cerebrovascular diseases (CVDs). The size and number of plaques; intima-media thickness (IMT) of carotid vessel on ultrasonographic measurement of the carotid artery; and circulating factor activation, such as platelet, leukocyte, and platelet-leukocyte aggregate (PLA) activation, according to flow cytometry of peripheral blood, have been shown to be closely correlated with atherosclerotic ischemic stroke [5-8]. However, previous studies investigating the relationship between circulating levels of the above mentioned hematologic factors and carotid atherosclerosis had different population sizes and indicators to estimate the severity of carotid atherosclerosis, and thus yielded inconsistent findings [9-11].

In this context, some prospective clinical studies have been performed to determine whether the ultrasonographic type of carotid artery plaques influences the rate of restenosis as well as the incidence of future cardiovascular events [12, 13]. The initial assumption of these studies was that different plaque types reflect different disease processes. Moreover, it could progress to a specific atheroma at carotid bifurcation, where atheroma is mostly detected after local resection [14]. However, our previous investigations showed that there were no significant differences among detailed categorized IMT or plaque size (diameter) and plaque numbers [2].

It has been reported that atherosclerotic plaques leading to acute coronary syndromes develop at sites wherein mild coronary-artery stenosis is angiographically detected [13, 15]. Several important questions remain unanswered, including the value of circulating factor activation markers and platelet-leukocyte interactions in asymptomatic subjects with high risk for stroke, correlation between circulating molecules and atherosclerotic vascular condition, and potentiality of this correlation analysis as a predictor of future CVD. Nevertheless, little is known about the potential participation of circulating molecules in the early stage of arterial disease before the development of complications.

The aim of this study was to compare the carotid vascular condition and circulating factor changes in asymptomatic patients with or without a CVD history. In this study, we measured circulating factor levels in participants with and without a known history of CVD, and explored their potential relationships with CVD risk factors and subclinical atherosclerosis assessed by ultrasonography for long-term follow-up care.

2. MATERIALS AND METHODS

2.1. Participant and clinical Assessment

The study enrolled 215 patients who had visited the neurology clinic of Severance Hospital between August 2007 and February 2008. The participants were divided into two groups: with previous CVD and without previous CVD. Participants with major trauma, surgery, severe liver disease, renal failure, cancer, and chronic in-

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flammatory diseases within three months were excluded to avoid external events or parallel infection that could also activate platelets or leukocytes. Two to three years later (from 2009 to 2010), 48 participants were followed up, PLA level was re-assessed, and ultrasonography was also performed. Furthermore, in 12 participants, PLA level was re-evaluated, and follow-up of over 10 years was performed. The two groups consisted of sex- and age-matched subjects with no inflammatory condition over the last three months before the study and with a comparable atherosclerotic risk profile such as hypertension, hypercholesterolemia, diabetes mellitus, and smoking. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg or the current use of antihypertensive medications. Hypercholesterolemia was defined as total cholesterol level ≥ 220 mg/dL or the current use of cholesterol-lowering agents. Diabetes mellitus was defined as a glycated hemoglobin (HbA1c) level ≥ 5.8% or the current use of oral hypoglycemic agents. Participants were considered smokers if they were current smokers or had stopped smoking less than one month before enrollment in the study. Participants were considered to have CVD if they had a history of ischemic heart disease, atherosclerosis, aortic aneurysm, peripheral vascular disease, or ischemic stroke. Detailed history is presented in Table **1**. The study protocol was approved by the Committee for the Protection of Human Subjects in Research of Yonsei University, and written informed consent was obtained from all patients.

2.2. Carotid Ultrasonography

High-resolution B-mode ultrasonography was performed with a duplex-type scanner (Accuvix V10, Seoul, Korea) to evaluate carotid atherosclerosis. Carotid artery intima-media thickness (C-IMT) was measured offline on the far wall of the common carotid artery in a longitudinal view in a region free from plaques, using a computerized system. The upper normal limit for IMT was 1.0 mm, and lesions with IMT ≥ 1.1 mm were defined as atheromatous plaques. The measurements of plaques at the site of carotid artery were computed according to the distance from flow divider: above flow divider up 1.5 cm, internal carotid artery; below flow divider down to 1.5 cm, bifurcation; below flow divider down to 3 cm, common carotid artery [5]. According to Mannheim Consensus, carotid plaques were defined as focal protruding structures into the lumen with a size of at least 0.5 mm, 50% of IMT, or a distance of 1.5 mm from the media-adventitia interface to intima-lumen [16].

2.3. Blood Sampling and Flow Cytometry

Blood samples were taken from each participant and collected into Vacutainer tubes containing 0.5 mL of 3.2% buffered sodium citrate. Immediately thereafter, citrated blood sample was added to a fixation solution (4% formaldehyde) to minimize *ex vivo* platelet activation. In flow cytometry analysis, platelet activity was assessed in the whole blood. The collected whole blood was resuspended in Tyrode's solution, and then incubated with phycoerythrin (PE)-conjugated anti-CD41a to immunologically identify platelets. The samples were simultaneously incubated with fluorescein isothiocyanate (FITC)-conjugated anti-CD62P at saturating concentrations for 15 min at room temperature in the dark. Platelet-bound anti-CD62P was determined by analyzing 5,000 platelets for FITC fluorescence. Results were expressed as a percentage of antibody-positive platelets.

Red-blood-cell-lysed blood samples were used to determine Mac-1 expression and evaluate platelet-leukocyte interaction in the leukocyte population. Each subpopulation of leukocytes was identified on the basis of forward and sideward scatter properties of PE-CD45-positive leukocytes. Monocytes and lymphocytes were identified on the basis of strong expression of PE-CD14 and PE-CD154, respectively. Mac-1 expression was identified with CD11b-FITC signal as the mean fluorescence intensity (MFI). Subset PLAs (platelet-granulocyte, platelet-monocyte, and platelet-lymphocyte aggregates) were recorded with simultaneous detection of CD42b-FITC-labeled platelets. The percentages of three complex types were calculated. FITC-conjugated immunoglobulin G (IgG) and PE-conjugated IgG antibodies were used for isotype control experiments. A minimum of 50,000 cell events were analyzed in each assay. Blood samples were analyzed on the flow cytometer within 3 h of venipuncture. All antibodies used were purchased from BD Biosciences (New Jersey, USA). Blood samples were analyzed using LSR II (Becton Dickinson, San Jose, California, USA).

2.4. Statistical Analysis

Laboratory and clinical data were expressed as mean ± SD. Fluorescence-activated cell sorting (FACS) values were presented as medians. Clinical characteristics of the two groups were compared using t-test. Statistical significance among groups was determined *via* one-way analysis of variance (ANOVA). Probability values were two-tailed, and p-values < 0.05 were considered statistically significant (GraphPad Prism 6.0, La Jolla, CA, USA). Pearson correlation coefficients were used to assess the association between the parameters of carotid atherosclerosis based on ultrasonographic findings (mean IMT and plaque numbers) and changes in circulating factors in the group without or with previous CVD. Values of the coefficient constant r and R^2 were provided. A p-value ≤ 0.05 was considered statistically significant (SPSS, Windows version 17.0, Chicago, Illinois, USA).

3. RESULTS

Two hundred fifteen subjects were included in this study. All participants were over 60 years of age in average, which may have been a risk factors for carotid and cerebrovascular degenerative events. Participants characteristics are shown in Table **1**. There were no significant differences between the two groups in total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and fasting glucose levels (Table **1**). In all participants with and without CVD history, there was no difference in p-selectin levels (Fig. **1A**). Platelet activation was not affected by existing CVD history. Unlike platelet activation, monocyte activation by Mac-1 expression had an apparently higher MFI value in the group with previous CVD than in the group without previous CVD (Fig. **1B**, $p < 0.00001$). According to the pattern of Mac-1 expression, platelet-monocyte aggregation (PMA) had apparently higher percentage in the group with previous CVD than in the group without previous CVD (Fig. **1C**, $p < 0.00001$). Similarly, the levels of platelet-lymphocyte aggregate (PLA) and platelet-granulocyte aggregate (PGA) were significantly higher in the group with previous CVD group than in the group without previous CVD (data not shown). In this study, we assumed that PMA had a more significant role in the difference between the two groups, and focused on monocyte activation and interaction with platelets.

We assessed ultrasonography-based morphological characteristics as mean values of IMT and plaque numbers *versus* circulating factor-based functional changes in the two groups. Our previous investigations showed that there were no significant differences among detailed categorized IMT or plaque size (diameter) or plaque numbers [2]. Therefore, in this study, we used simple categories such as mean IMT and plaque numbers. The mean IMT and carotid atherosclerotic severity were significantly greater in the group with previous CVD than in the group without previous CVD (Fig. **2A**, $p = 0.0254$). The number of carotid plaques also showed significant difference between the two groups (Fig. **2B**, $p = 0.0280$).

Among participants enrolled in this study, there was a high prevalence of hypertension (without:with = 56.9%:69%), hyperlipidemia (without:with = 31.0%:22.0%), diabetes (without:with = 24.4%:24%), and smoking (without:with = 10.9%:22%). Although the participants were similar in age, the correlation between carotid atherosclerotic severity and vascular risk or circulating factors showed significant difference between the two groups (Table **2**). In

Table 1. Characteristics of the participants.

Variables	Without Previous CVD (n=113)	With Previous CVD (n=102)	*p*
Age (y)	63.2±6.8	65.1±10.2	0.9524
Male % (n)	46.0 (52)	64 (65)	0.8477
female % (n)	54.0 (61)	37 (37)	0.8194
Hypertension % (n)	56.6 (64)	69 (70)	0.9.22
Hyperlipidemia % (n)	31.0 (35)	22 (22)	0.8349
Diabetes mellitus % (n)	20.4 (23)	24 (24)	0.9194
Current smoking % (n)	10.9 (14)	22 (22)	0.6955
History of CVD % (n)	0 (0)	100 (102)	
Triglyceride, mg/dL	148.87±90.15	122.99±62.91	0.5918
High density lipoprotein, mg/dL	49.23±13.03	46.16±10.3	0.9198
Low density lipoprotein, mg/dL	116.62±74.11	112.11±65.79	0.8572
Total cholesterol, mg/dL	173.58±35.50	170.13±38.11	0.9969

Two-tailed t-test is done on each variable.

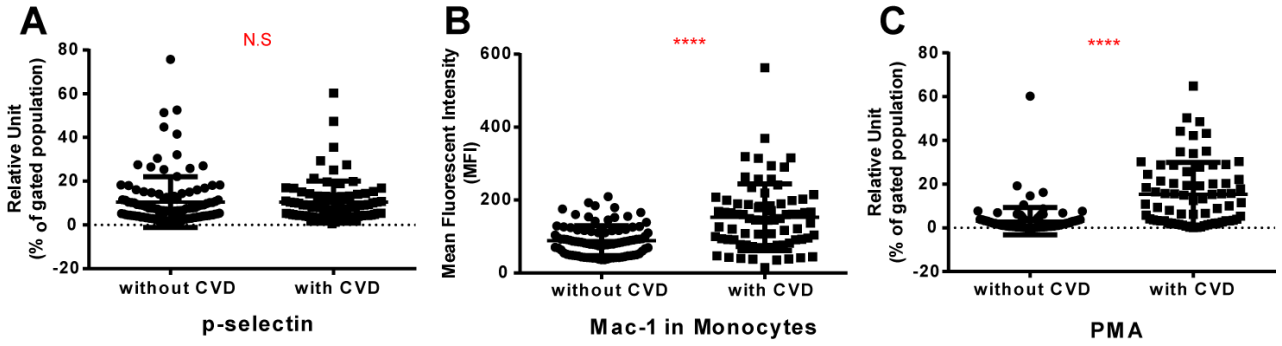

Fig. (1). Circulating blood factor changes by FACS analysis between the groups without and with previous CVD. **(A)** Between the two groups, p-selectin positive platelets were shown in percentage of event number per gated platelet population by FACS analysis. **(B)** Circulating levels of Mac-1-positive monocytes were presented with the mean fluorescent intensity (MFI) of CD14-positive monocytes. **(C)** Circulating levels of monocytes aggregated with platelets were shown in percentage of event number per monocyte subpopulation of whole leukocytes. NS, not significant, ****$p < 0.00001$ between the two groups.

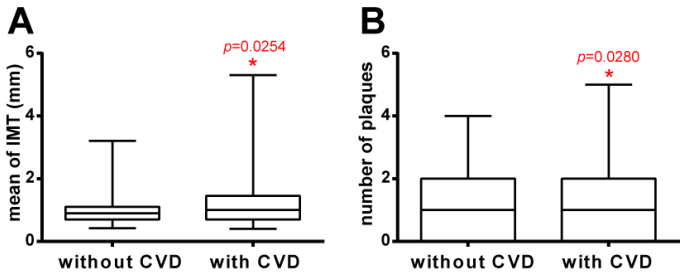

Fig. (2). Vessel atherosclerotic determinant differences in the two groups. **(A)** The mean intima-media thickness (IMT) and **(B)** plaque numbers are compared between the group without previous CVD and that with previous CVD. Bars represent median values, and the error bar, the standard deviation. *$p < 0.05$ between the two groups.

the group without CVD history, there was no correlation between circulating factors and atherosclerotic vessel factors, such as mean IMT or plaque number. In the group with CVD, the mean IMT was significantly correlated with PMA level ($p = 0.0321$). Furthermore, plaque numbers were deeply correlated with PMA level ($p = 0.0041$) and activated monocyte level (Mac-1, $p = 0.0098$).

Based on the results of the correlation analysis, we followed up with some patients to analyze circulating factor changes. Of all

initial participants, 48 patients were followed up two to three years later (2009 to 2010), and 12 patients were followed up 10 years later (2016). They all belonged to the group with CVD history, and have been regularly checked as outpatients and were cared for with appropriate medication and daily life workout. In all followed-up participants, p-selectin expression in circulating platelets did not show differences among the groups (Fig. **3A**). Meanwhile, Mac-1 expression in circulating monocytes showed a downward tendency according to the follow-up year. The difference between the two groups (with or without CVD history) showed a larger gap at the initial point ($p < 0.00001$), but in the longer-period follow-up, Mac-1 level decreased compared to that in the initial point but was still higher compared to the group without CVD history ($p < 0.001$). After the 10-year follow-up, Mac-1 level decreased more compared to that in the two-year follow-up (Fig. **3B**, $p < 0.05$). In case of PMA, the downward trend was similar to that for Mac-1 level which was even more reduced, indicating no difference in the group without CVD history at 10-year follow-up (Fig. **3C**).

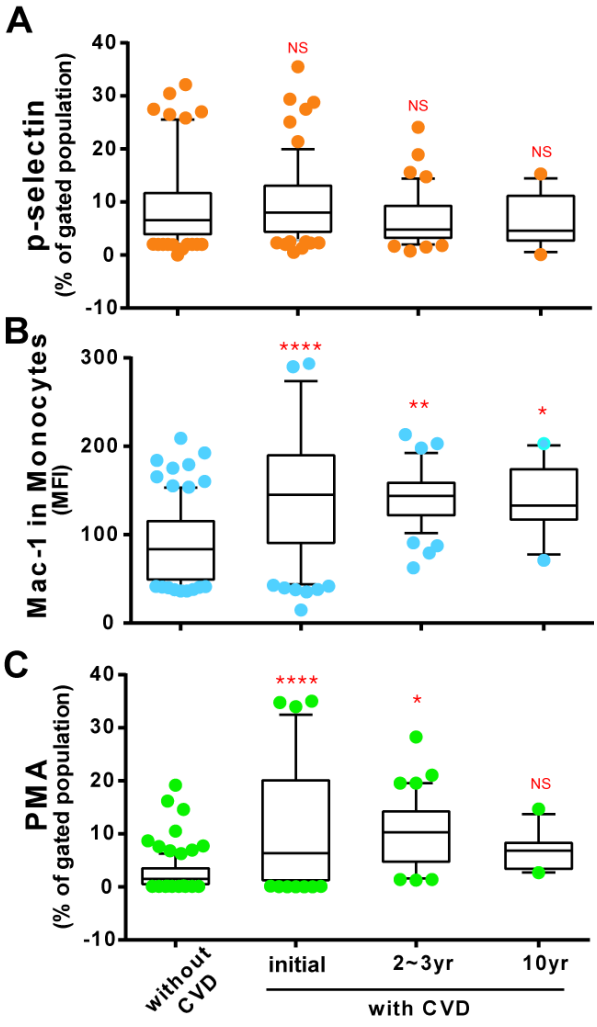

Fig. (3). Circulating blood factor changes according to follow-up. **(A)** p-selectin-positive platelets are shown in percentage of event number per gated platelet population. **(B)** Circulating levels of Mac-1-positive monocytes are presented with mean fluorescent intensity (MFI). **(C)** Circulating levels of monocyte aggregated with platelets (PMA) are shown in percentage of event number per monocyte subpopulation. Bars represent median values from 10th to 90th percentile, and the error bar, the standard deviation. NS, not significant, ****$p < 0.00001$, **$p < 0.001$, *$p < 0.05$ *vs* the group without CVD.

4. DISCUSSION

Based on the previous studies as well as our previous reports and data, we hypothesized that there is a correlation between atherosclerotic severity and circulating factors according to CVD history, particularly in aged patients. Our results showed no differences in the levels of p-selectin expression in circulating platelets between the two groups. However, Mac-1 level in monocytes and circulating PMA was significantly increased in the group with previous CVD (Fig. **1**). Likewise, the mean IMT and plaque numbers showed a more severe condition in the patient group with CVD history than in the group without CVD history (Fig. **2**).

Several studies have reported that the ultrasonographic characteristics of carotid artery plaques are related to the development of future cardiovascular events [13, 17, 18]. Moreover, atherosclerotic severity is closely associated with new or recurrent stroke or other vascular diseases, and carotid IMT and plaque number present this association well [1, 9]. Thus, we investigated whether carotid mean IMT and plaque numbers, as indicators of atherosclerotic severity, have significant correlation in aged participants. Corresponding to our hypothesis, carotid mean IMT and plaque number were significantly correlated with aged patient group with CVD history (Table **2**). In the present study, regression analysis did not show that CVD history was correlated with vascular risk factors such as mean IMT and plaque numbers, but revealed that circulating factors and PMA were independently correlated with CVD history. This might suggest a possibility for the prediction of CVD by analyzing the correlation between circulating factors and atherosclerotic severity in aged participants with previous CVD, as reflected in many previous studies [7, 8, 11, 14, 19].

We also confirmed that the levels of activated monocytes (Mac-1) and PMA increased as the plaque numbers or mean IMT increased in the group with previous CVD. However, in the group without previous CVD, circulating factors showed no significant changes, nor any tendency along with atherosclerotic severity, despite the participants' old age. This result indicated that, in contrast to the group with previous CVD, other risk factors did not affect the levels of circulating factors with changes in atherosclerotic severity in the group without previous CVD. This result indicates that all participants with previous CVD have a higher risk for recurrent CVD than those without CVD history. For participants with previous CVD, we interpreted that circulating factors increased during the primary cerebrovascular events, including stroke, attack may contribute to atherosclerotic severity and eventually to the second cerebrovascular event. The results in patients who were followed up for 2 and 10 years showed that the circulating factor level and atherosclerotic severity were maintained as low or stable (Fig. **3**). Among them, patients who developed recurrent CVD, including ischemic stroke, could not be observed, which might lead to sustained medical care.

Despite the long-term nature of our observation and follow-up, this study had several limitations. First, the sample sizes were small; 48 participants for 2-year and 12 participants for 10-year follow-up. During the follow-up period, many patients who failed to attend follow-up sessions died of non-vascular causes or developed another disease, such as severe inflammatory diseases. Considering the small size of our study subjects, further studies with a larger sample size during follow-up should be performed to confirm our findings and speculations on the incidence rate of CVD. Now, we have been recruiting patients for long-term follow-up for further study and concrete conclusion. Another limitation was that the circulating factor based on FACS analysis was also needed to determine a cutoff value for each blood cell population for standardization or generalization. Nonetheless, our findings may serve as a background for further evaluation of circulating factor changes, and might be applied to predict CVD in elderly people who need intensive care.

Table 2. Correlation analysis between without *vs* with previous CVD in each category.

		Without Previous CVD			With Previous CVD		
		p-selectin	MacI	PMA	p-selectin	MacI	PMA
Mean IMT	r	0.097	0.0608	0.0965	0.1752	0.2148	0.3148
	R^2	0.0094	0.0037	0.0093	0.0307	0.0585	0.0991
	p	0.3028	0.5649	0.3048	0.2141	0.0907	**0.0312***
Numbers of Plaques	r	0.3019	0.0036	0.0283	0.1562	0.3518	0.3565
	R^2	0.001	0.000	0.000	0.0244	0.1237	0.1271
	p	0.7350	0.9732	0.7641	0.2943	**0.0098****	**0.0041****

Ordinary oneway ANOVA test was performed.
Pearson's correlation coefficients, R squared, and associated two-tailed *p*-values are shown.

Collectively, we found that circulating factor levels showed a different tendency in aged people with and without previous CVD, and its correlation with atherosclerotic severity also depended on a history of CVD and progression of atherosclerosis. Ultrasonographic findings of carotid IMT and plaque number have not yet been used as reliable guidelines for the prediction of CVD or in the diagnosis of atherosclerotic ischemic stroke, since it remains unclear whether an increased carotid IMT or plaque number indicates the development of atherosclerotic ischemic stroke subsequently. Therefore, we suggest that pathologic progression in elderly patients with CVD history should be intensely observed while considering the circulating factor level and atherosclerotic severity. With further observational investigation, our results may be most effective in predicting the occurrence of atherosclerotic ischemic stroke.

CONCLUSION

Collectively, we found that the circulating factor levels showed a different tendency in aged people with and without previous CVD, and its correlation with atherosclerotic severity also depended on a history of CVD and progression of atherosclerosis. Ultrasonography findings of carotid IMT and plaque number have not yet been used as reliable guidelines for the prediction of CVD or in the diagnosis of atherosclerotic ischemic stroke because it is unclear whether an increased carotid IMT or plaque number indicates the development of atherosclerotic ischemic stroke subsequently. Therefore, we suggest that pathologic progression in elderly patients with CVD history should be intensely observed by considering the circulating factor level and atherosclerotic severity. With further observational investigation, our results also might be most effective in predicting the occurrence of atherosclerotic ischemic stroke.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol was approved by the Committee for the Protection of Human Subjects in Research of Yonsei University, Seoul, Republic of Korea,

HUMAN AND ANIMAL RIGHTS

No animals were used for studies that are the basis of this research. All the experiments on humans were in accordance with the Helsinki Declaration of 1975 as revised in 2013.

CONSENT FOR PUBLICATION

The written informed consent was obtained from all patients.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

REFERENCES

[1] Kokubo Y, Watanabe M, Higashiyama A, Nakao YM, Nakamura F, Miyamoto Y. Impact of intima-media thickness progression in the common carotid arteries on the risk of incident cardiovascular disease in the suita study. J Am Heart Assoc 2018; 7(11): e007720. [http://dx.doi.org/10.1161/JAHA.117.007720] [PMID: 29858361]

[2] Cho K, Kim J, Kim GW. Changes in blood factors and ultrasound findings in mild cognitive impairment and dementia. Front Aging Neurosci 2017; 9: 427. [http://dx.doi.org/10.3389/fnagi.2017.00427] [PMID: 29311909]

[3] Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997; 96(5): 1432-7. [http://dx.doi.org/10.1161/01.CIR.96.5.1432] [PMID: 9315528]

[4] Tsivgoulis G, Vemmos K, Papamichael C, *et al.* Common carotid artery intima-media thickness and the risk of stroke recurrence. Stroke 2006; 37(7): 1913-6. [http://dx.doi.org/10.1161/01.STR.0000226399.13528.0a] [PMID: 16728693]

[5] Prati P, Tosetto A, Vanuzzo D, *et al.* Carotid intima media thickness and plaques can predict the occurrence of ischemic cerebrovascular events. Stroke 2008; 39(9): 2470-6. [http://dx.doi.org/10.1161/STROKEAHA.107.511584] [PMID: 18617662]

[6] Cha JK, Jeong MH, Jang JY, *et al.* Serial measurement of surface expressions of CD63, P-selectin and CD40 ligand on platelets in atherosclerotic ischemic stroke. A possible role of CD40 ligand on platelets in atherosclerotic ischemic stroke. Cerebrovasc Dis 2003; 16(4): 376-82. [http://dx.doi.org/10.1159/000072560] [PMID: 13130179]

[7] Marquardt L, Anders C, Buggle F, Palm F, Hellstern P, Grau AJ. Leukocyte-platelet aggregates in acute and subacute ischemic stroke. Cerebrovasc Dis 2009; 28(3): 276-82. [http://dx.doi.org/10.1159/000228710] [PMID: 19609079]

[8] Tsai NW, Chang WN, Shaw CF, *et al.* The value of leukocyte adhesion molecules in patients after ischemic stroke. J Neurol 2009; 256(8): 1296-302.
[http://dx.doi.org/10.1007/s00415-009-5117-3] [PMID: 19353220]

[9] Hashimoto H, Kitagawa K, Kuwabara K, *et al.* Circulating adhesion molecules are correlated with ultrasonic assessment of carotid plaques. Clin Sci (Lond) 2003; 104(5): 521-7.
[http://dx.doi.org/10.1042/CS20020290] [PMID: 12570870]

[10] Michelson AD, Barnard MR, Krueger LA, Valeri CR, Furman MI. Circulating monocyte-platelet aggregates are a more sensitive marker of *in vivo* platelet activation than platelet surface P-selectin: studies in baboons, human coronary intervention, and human acute myocardial infarction. Circulation 2001; 104(13): 1533-7.
[http://dx.doi.org/10.1161/hc3801.095588] [PMID: 11571248]

[11] Ritter MA, Jurk K, Schriek C, *et al.* Microembolic signals on transcranial Doppler ultrasound are correlated with platelet activation markers, but not with platelet-leukocyte associates: a study in patients with acute stroke and in patients with asymptomatic carotid stenosis. Neurol Res 2009; 31(1): 11-6.
[http://dx.doi.org/10.1179/174313208X331590] [PMID: 18768115]

[12] Park HK, Chung JW, Hong JH, *et al.* Preceding intravenous thrombolysis in patients receiving endovascular therapy. Cerebrovasc Dis 2017; 44(1-2): 51-8.
[http://dx.doi.org/10.1159/000471492] [PMID: 28427054]

[13] Liapis CD, Kakisis JD, Dimitroulis DA, Kostakis AG. The impact of the carotid plaque type on restenosis and future cardiovascular events: a 12-year prospective study. Eur J Vasc Endovasc Surg 2002; 24(3): 239-44.
[http://dx.doi.org/10.1053/ejvs.2002.1714] [PMID: 12217286]

[14] Schmalbach B, Stepanow O, Jochens A, Riedel C, Deuschl G, Kuhlenbäumer G. Determinants of platelet-leukocyte aggregation and platelet activation in stroke. Cerebrovasc Dis 2015; 39(3-4): 176-80.
[http://dx.doi.org/10.1159/000375396] [PMID: 25720421]

[15] Stone GW, Maehara A, Lansky AJ, *et al.* A prospective natural-history study of coronary atherosclerosis. N Engl J Med 2011; 364(3): 226-35.
[http://dx.doi.org/10.1056/NEJMoa1002358] [PMID: 21247313]

[16] Mathiesen EB, Johnsen SH. Ultrasonographic measurements of subclinical carotid atherosclerosis in prediction of ischemic stroke. Acta Neurol Scand Suppl 2009; (189): 68-72.
[http://dx.doi.org/10.1111/j.1600-0404.2009.01210.x] [PMID: 19566503]

[17] Htun P, Fateh-Moghadam S, Tomandl B, *et al.* Course of platelet activation and platelet-leukocyte interaction in cerebrovascular ischemia. Stroke 2006; 37(9): 2283-7.
[http://dx.doi.org/10.1161/01.STR.0000236638.75591.61] [PMID: 16888273]

[18] Ogata T, Yasaka M, Yamagishi M, Seguchi O, Nagatsuka K, Minematsu K. Atherosclerosis found on carotid ultrasonography is associated with atherosclerosis on coronary intravascular ultrasonography. J Ultrasound Med 2005; 24(4): 469-74.
[http://dx.doi.org/10.7863/jum.2005.24.4.469] [PMID: 15784765]

[19] Dotsenko O, Chaturvedi N, Thom SA, *et al.* Platelet and leukocyte activation, atherosclerosis and inflammation in European and South Asian men. J Thromb Haemost 2007; 5(10): 2036-42.
[http://dx.doi.org/10.1111/j.1538-7836.2007.02711.x] [PMID: 17883700]