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The Relationship between the Transcranial Doppler Pulsatility Changes and the CYP2C19 Genotype on Clopidogrel Treatment

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Background: We hypothesized that cytochrome P450 2C19 (CYP2C19) polymorphisms in clopidogrel-treated patients would influence cerebral vessel viscosity, which was related to transcranial Doppler (TCD) pulsatility index (PI). Hence, we explored the relationship between the CYP2C19 genotype status and the TCD PI changes in acute ischemic stroke patients on clopidogrel treatment using serial TCD examinations.

Methods: Patients (aged ≥40 years) who developed ischemic stroke within 7 days of onset of symptom from June 2018 to May 2019 were recruited for this study. Patients with high-risk cardiac sources of emboli, or stroke of other determined etiology as a stroke subtype, were excluded from the study. TCD was performed 5 (±2) days and followed up 180 (±30) days after onset of stroke.

Results: Eighty-four patients were enrolled for this study. Patients were categorized into decreased and increased PI groups. Good genotype for clopidogrel metabolism was found in 38% of the decreased-PI patients and 25% of the increased-PI patients (p=0.252). CYP2C19 genotype status was not associated with PI changes in both groups. Hematocrit (Hct) was significantly related to PI changes.

Conclusion: CYP2C19 genotype status was not related to TCD PI changes in acute ischemic stroke patients on clopidogrel treatment. Hct was the major determinant factor of PI. The negative association of Hct with cerebral blood flow velocity and its positive association with blood viscosity are plausible explanations for this phenomenon.

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INTRODUCTION

The transcranial Doppler (TCD) pulsatility index (PI) describes the shape of a spectral waveform and measures cerebral vascular resistance.[1] High PI indicates high-resistance vascular beds with low diastolic flow and peaked waveforms. Low PI indicates low-resistance beds with high diastolic flow and rounded waveforms. High PI suggests increased downstream vascular resistance in the cerebral circulation. PI increases with age and the presence of hypertension, diabetes, vascular dementia, and small artery disease. PI is also influenced by physiological factors, such as the partial pressures of oxygen and carbon dioxide and arterial pressure.[2] The major factors that affect PI are cerebral blood flow velocity (CBFV) and blood viscosity (BV).[3] CBFV is influenced by the length and cross sectional area of the vessel, age, sex, the partial pressures of oxygen and carbon dioxide, and arterial pressure.[4] BV is the measurement of blood resistance to flow, and is characterized by

blood thickness and stickiness.[5-7] Increased BV is related to elevated concentrations of blood cells and plasma components and low deformability and high aggregability of erythrocytes.[7,8]

We previously reported that the middle cerebral artery (MCA) PI was significantly associated with BV in acute ischemic stroke patients.[9] Increased mean MCA PI was associated with old age, presence of diabetes, and increased BV in our study. We also demonstrated that prior antithrombotic use was highly related to decreased BV in acute ischemic stroke patients.[10] Inhibition of platelet aggregation and enhancement of erythrocyte deformability may be associated with decreased BV when antithrombotic drugs are used. Evidence has begun to emerge concerning the relationship between the cytochrome P450 2C19 (CYP2C19) polymorphisms in clopidogrel-treated patients and stroke recurrence and mortality following ischemic stroke,[11,12] but there is no evidence that CYP2C19 polymorphisms in clopidogrel-treated patients influence cerebral vessel viscosity after ischemic stroke.

Considering that TCD PI is associated with BV in acute ischemic stroke patients, we hypothesized that CYP2C19 polymorphisms in clopidogrel-treated patients would influence cerebral vessel viscosity, which was related to TCD PI, and this effect could be demonstrated by serial TCD PI evaluations. Hence, this study was designed to evaluate the difference between the propensities of a good genotype group for clopidogrel metabolism and a poor genotype group to reduce the PI in acute ischemic stroke patients on clopidogrel treatment using serial TCD examinations.

SUBJECTS AND METHODS

1. Patients

Patients (aged ≥40 years) who developed ischemic stroke within 7 days of symptom onset from June 2018 to May 2019 were recruited for the study. The patient baseline demographics and medical histories were assessed at admission. Systemic investigations were executed for all patients. Each patient underwent brain MRI and at least one vascular imaging study, such as MR angiography (MRA) or CT angiography (CTA). Echocardiography and 24-hour Holter monitoring were performed in selected patients to detect the potential cardiac sources of embolism. Stroke subtype classification was performed according to the trial of ORG 10172 in the acute stroke treatment classification system.[13] Patients with high-risk cardiac sources of emboli, or stroke of other determined etiology as a stroke subtype, were excluded from the study. All patients received proper treatment, including anti-hypertensive drugs, statin, and rigorous control of other vascular risk factors, during the study.

The study was approved by the Institutional Review Board of the Sanggye Paik Hospital (IRB No. 2018-01-002-001). All patients provided written informed consent before study enrollment.

2. CYP2C19 genotyping assay

CYP2C19 genotype of the study population was measured using the Seeplex CYP2C19 ACE genotyping system (Seegene, Seoul, Korea) and Real-Q CYP2C19 genotyping kit (Biosewoom, Seoul, Korea). Each patient was classified as an ultrarapid metabolizer (UM; *1/*17, *17/*17), extensive metabolizer (EM; *1/*1), intermediate (IM)/unknown metabolizer (*1/*2, *1/*3 and *2/*17, *3/*17), or poor metabolizer (PM; *2/*2, *2/*3, *3/*3) based on the CYP2C19 genotype status. For this study, UM or EM status patients were allocated to the good genotype group for clopidogrel metabolism, while IM/unknown metabolizer or PM status patients were allocated to the poor genotype group.[1]

3. TCD

Methods of TCD examination used in our study have been published previously.[1,14] Briefly, TCD was performed with a Companion III (Nicolet Biomedical, Inc., Madison, Wisconsin, USA) according to the standard operating manual, 5 (±2) days after the onset of stroke. Doppler signals from the MCA were obtained at depths of 54 mm, 58 mm, 60 mm, and 62 mm. Doppler signals from the basilar artery (BA) were acquired at depths of 80 mm, 84 mm, 88 mm, and 92 mm. Gosling's PI was calculated as the difference between the peak systolic and end diastolic velocities (PSV and EDV, respectively) divided by the mean flow velocity (mFV) of each artery.

The depths that showed the highest mFV values were used as parameters for PI analysis and in the follow-up TCD studies. TCD was followed up and laboratory tests, including hemoglobin (Hb) and hematocrit (Hct), were conducted 180 (±30) days after onset of stroke. The PI changes were calculated using serial TCD examinations. Decreased PI suggested that the PI value in the baseline TCD study minus 180 days was positive, and vice-versa. For patients with either significant (>50%) stenosis or occlusion of relevant arteries in this study, non-relevant arterial PI was evaluated. An experienced sonographer executed all TCD examinations, and throughout the trial, the CYP2C19 genotype status of the patients was masked from the sonographer.

4. Statistical analysis

Variables were verified for normality using the Kolmogorov-Smirnov test. Descriptive data were expressed as number (percent) or mean±standard deviation. The patient characteristics among groups were analyzed using one-way analysis of variance with the Tukey's *post hoc* test for continuous variables, wher-

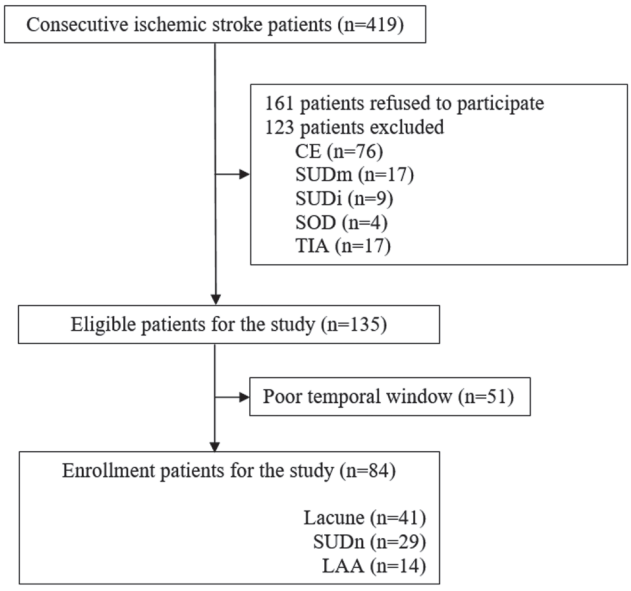

FIG. 1. Trial profile. CE; cardioembolism, SUDm; stroke of undetermined etiology (SUD), more than two causes identified, SUDi; SUD, incomplete evaluation, SOD; stroke of other determined etiology, TIA; transient ischemic attack, SUDn; SUD, negative work-up, LAA; large artery atherosclerosis.

TABLE 1. Baseline characteristics of the study population

	Total (n=84)	LAA (n=14)	Lacune (n=41)	SUDn (n=29)	*p*
Age (years)	64.5±12.4	61±10.71	67.4±10.9	62.1±14.46	0.185
Female	26 (31)	4 (29)	15 (58)	7 (24)	0.528
Hypertension	56 (67)	9 (64)	27 (66)	20 (69)	0.943
Diabetes mellitus	22 (26)	5 (36)	9 (22)	8 (28)	0.586
Dyslipidemia	34 (41)	8 (57)	13 (32)	13 (45)	0.207
Coronary artery disease	4 (5)	0 (0)	1 (2)	3 (10)	0.204
Current smoking	30 (36)	4 (29)	17 (41)	9 (31)	0.874
Good genotype for clopidogrel metabolism	28 (33)	3 (21)	16 (39)	9 (31)	0.459
Relevant artery velocity					
Peak systolic velocity	88±29.59	96±16.54	88±35.95	86±24.98	0.598
End diastolic velocity	39±14.54	44±10.19	38±16.86	38±12.72	0.336
Mean flow velocity	56±18.74	62±11.1	55±22.26	54±16.27	0.412
Relevant artery PI					
Left MCA	0.91±0.18	0.84±0.19	0.94±0.17	0.9±0.18	0.214
Right MCA	0.93±0.21	0.86±0.26	0.97±0.2	0.9±0.18	0.192
BA	0.95±0.18	0.88±0.21	0.99±0.14	0.93±0.19	0.066

Values are presented as numbers (%) or mean±standard deviation.
LAA; large artery atherosclerosis, SUDn; Stroke of undetermined etiology, negative work-up, PI; pulsatility index, MCA; middle cerebral artery, BA; basilar artery.

ever applicable. Categorical data were examined by chi-square statistics. Univariate analyses of patient characteristics were completed using an independent sample *t* test or the Mann-Whitney *U* test for continuous variables and the chi-square test for categorical variables. The Pearson's and the Spearman's correlation coefficients were calculated to evaluate the correlations between PI changes and patient characteristics. Statistical analyses were performed using SPSS version 25.0 for Windows (IBM Co., Armonk, NY, USA).

RESULTS

Fig. 1 shows the patient flow and the identified reasons for exclusion from the study. In total, 419 patients were screened during the trial. Of these, 161 patients refused to participate in the study, 123 patients were not eligible for the study, and 51 patients showed poor temporal window. Therefore, 84 patients (20%) were enrolled for this study. Brain diffusion-weighted imaging was performed in 95% of the patients. All patients

Table 2. Patients' characteristics and laboratory findings according to the changes of pulsatility index

	Decreased PI (n=56)	Increased PI (n=28)	*p*
Age (years)	64.3±10.67	69±10.47	0.62
Female	16 (29)	10 (36)	0.504
Hypertension	35 (63)	21 (75)	0.875
Diabetes mellitus	14 (25)	8 (29)	0.795
Dyslipidemia	23 (41)	11 (39)	0.912
Coronary artery disease	2 (4)	2 (7)	0.598
Current smoking	17 (30)	13 (46)	0.813
Good genotype for clopidogrel metabolism	21 (38)	7 (25)	0.252
Stroke in anterior circulation	30 (54)	14 (50)	0.861
Baseline study			
Hb (g/dL)	14.3±1.71	13.9±1.66	0.32
Hct	41.9±4.7	40.7±4.13	0.226
Left MCA PI	0.91±0.18	0.9±0.19	0.793
Right MCA PI	0.93±0.19	0.93±0.25	0.953
BA PI	0.95±0.19	0.94±0.16	0.816
180-day study			
Hb (g/dL)	13.9±1.73	13.9±1.58	0.963
Hct	41.2±4.65	40.9±4.17	0.788
Left MCA PI	0.84±0.16	0.95±0.2	0.013*
Right MCA PI	0.86±0.2	0.97±0.21	0.045*
BA PI	0.84±0.18	1.02±0.17	0.001*
Changes in Hb	0.5±1.05	0.1±1.36	0.114
Changes in Hct	1±3.11	-0.2±3.58	0.118
Changes in PI			
Left MCA PI	0.08±0.14	0.04±0.18	0.004*
Right MCA PI	0.09±0.17	0.04±0.18	0.006*
BA PI	0.11±0.1	0.08±0.11	0.001*
Relevant artery	0.13±0.13	0.1±0.08	0.001*

Values are presented as numbers (%) or mean±standard deviation.
PI; pulsatility index, Hemoglobin; Hb, Hematocrit; Hct, MCA; mean middle cerebral artery, BA; basilar artery.
*Significant *p* is marked with.

underwent at least one vascular imaging study (MRA in 96% and CTA in 14%). Anterior circulation stroke was seen in 52% of the patients. The most frequent stroke subtype was lacunar stroke (41, 49%), followed by stroke of undetermined etiology negative work-up (29, 35%) and large artery atherosclerosis (14, 16%). The patient baseline characteristics are shown in Table 1. The mean age was 64.5±12.4 years, and 31% of the patients were women. Of these, 67% had a history of hypertension, 26% had a history of diabetes, 41% had a history of dyslipidemia, 5% had a history of coronary artery disease, and 36% were current smokers. Additionally, 21% of the patients were on antiplatelets, 25% were on statins, and 45% were on antihypertensive drugs at admission. There were no differences in the baseline characteristics among the patients with different stroke subtypes. The relevant artery velocity and PI values were also not different among the groups. PSV, EDV, and mFV were highly associated with PI. PSV was positively correlated with PI (r=0.437, p=0.029). mFV and EDV was negatively related to PI (r<-0.439, p<0.023).

During the trial, aspirin and clopidogrel were given to 77 patients (92%), and clopidogrel alone was given to the remaining 8%. For this study, patients were categorized into decreased-PI and increased-PI groups. Table 2 shows the patient characteristics and laboratory findings according to the PI changes. No significant differences were observed in the medical histories and laboratory findings between the patients of the two groups. Good genotype for clopidogrel metabolism was found in 38% of the decreased-PI patients and 25% of the increased-PI patients (p=0.252). At the end of the 180-day study, we observed that the MCA and BA PI changed significantly with changes in Hct (p<0.013), suggesting that Hct was the major determinant factor of PI. Table 3 shows the correlation analysis of the relevant artery PI with patient characteristics. In the decreased-PI group, 180-day Hb and Hct were negatively associated with PI changes. In the increased-PI group, baseline Hb and Hct were significantly related to PI changes, also suggesting that Hct was the major determinant factor of PI. CYP2C19 genotype status for clopidogrel metabolism was not associated with PI changes in both groups.

DISCUSSION

TCD PI is the reflection of multifactorial, pleiotropic events that occur in the systemic and cerebral vascular systems. Instructive studies on TCD PI and acute

Table 3. Pearson's and Spearman's correlation of relevant artery TCD PI with patients' characteristics

	Decreased relevant artery PI (n=46)		Increased relevant artery PI (n=24)	
	r	p	r	p
Age	0.228	0.175	0.98	0.672
Female sex	-0.042	0.752	-0.105	0.652
Hypertension	0.315	0.051	0.286	0.208
Diabetes mellitus	0.103	0.533	0.301	0.185
Dyslipidemia	0.174	0.291	-0.235	0.305
Current smoking	-0.223	0.173	0.075	0.746
Good genotype for clopidogrel metabolism	-0.174	0.29	-0.25	0.273
Baseline Hb	-0.296	0.067	0.507	0.019*
Baseline Hct	-0.248	0.128	0.516	0.017*
180-day Hb	-0.364	0.025*	0.341	0.131
180 days Hct	-0.344	0.034*	0.305	0.179
Changes in Hb	0.096	0.568	0.17	0.461
Changes in Hct	0.125	0.455	0.196	0.394

TCD; transcranial Doppler, PI; pulsatility index, Patients with either significant (>50%) stenosis or occlusion of relevant artery are excluded for analysis.
*Significant p is marked with.

ischemic stroke are limited,[3,15] and no studies have addressed the serial PI changes associated with CYP2C19 polymorphisms in acute ischemic stroke patients on clopidogrel treatment. We speculated that CYP2C19 polymorphisms in clopidogrel-treated patients might influence cerebral vessel viscosity, which was related to TCD PI. However, CYP2C19 genotype status for clopidogrel metabolism was not associated with PI changes in this study. Our previous study demonstrated that in the clopidogrel treatment group, a good CYP2C19 genotype for clopidogrel metabolism was associated with a 31% decrease in the relative risk of recurrent stroke. However, a 41% decrease in the relative risk of recurrent ischemic stroke in the good CYP2C19 genotype-group has been observed.[16] Informative studies on pharmacological therapies for reducing BV, which may be related to decreased PI, are limited.[17,18] One study showed that in patients with subclinical carotid or femoral atherosclerosis, clopidogrel reduced the mean low-shear BV by 18% after 3 weeks of treatment.[18] Aspirin and dipyridamole are more effective in reducing low-shear BV than the efficacy exhibited by aspirin alone.[19] Since our study failed to demonstrate the relationship between the TCD PI changes and the CYP2C19 genotype in clopidogrel-treated patients, further studies that focus on the CYP2C19 genotype and BV are essential to evaluate the effects of antithrombotic therapy on PI changes.

Our study confirmed that Hct was clearly related to PI changes. These results were in concordance with those of previous studies that showed positive correlation between Hct and PI.[4,20,21]

The major factors that affect PI are CBFV and BV.[3] Decreases in Hct are associated with increased CBFV, which, in turn, is related to decreased PI.[20,21] BV reflects frictional interactions between blood components and erythrocytes within the systemic vascular system.[10] The major determinants of BV are erythrocyte aggregation and deformability, Hct, and plasma viscosity. Of these, Hct is the most essential factor, and alterations in Hct contribute to rheological discrepancies.[22] Increases in Hct are related to increased BV. The positive association of Hct to BV and arterial oxygen carrying capacity may be a plausible explanation for this phenomenon.

The strength of this study was that it incorporated a prospective longitudinal design using serial TCD examinations. Unlike traditional cross-sectional single studies, this study was used to evaluate the Hct and TCD PI changes in acute ischemic stroke patients. In the decreased-PI group, 180-day Hct was negatively associated with PI changes. Baseline Hct was positively related to PI changes in the increased PI group. However, there were several limitations, including small sample size, in this study. Of the 419 patients that were screened during the trial, only 84 (20%) were enrolled for this study. Though a previous report indicated that 41% of the Korean participants belonged to the good genotype-group for clopidogrel metabolism,[23] our study showed that only 33% belonged to the good genotype-group. These results might be due to the small sample size, which might have affected the study results. Second, clopidogrel response might have been affected by several clinical factors. Owing to the small sample size, we could not evaluate the clinical factors associated with clopidogrel response variability. Finally, hydration might be associated with Hct and BV changes. One study demonstrated that BV was significantly higher at admission, but was normalized after 2 weeks of hydration.[5] In our study, all blood samples and TCD studies were not performed on the same day at baseline. These limitations should be considered during the interpretation of our data.

In conclusion, the CYP2C19 genotype status was not associated with TCD PI changes in acute ischemic stroke patients on clopidogrel treatment. Hct was the major determinant factor of PI. Physicians should be aware that TCD PI may be affected by Hct in serial TCD examinations. The negative association of Hct with CBFV and its positive association with BV are plausible explanations for this phenomenon.

Conflicts of interest

No potential conflicts of interest relevant to this article was reported.

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