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# Soluble Fms-Like Tyrosine Kinase-1 and the Progression of Carotid Intima-Media Thickness

## – 24-Month Follow-up Study –

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***Background:*** The relationship between fms-like tyrosine kinase-1 (sFlt-1), a soluble receptor for vascular endothelial growth factor (VEGF), and vascular disease has not been established, so this study aimed to elucidate the association between sFlt-1 and the progression of carotid intima–media thickness (IMT) in hypertensive patients.

***Methods and Results:*** The 120 hypertensive patients under medical control were enrolled and 112 completed the study (age 59±9 years, 57 females). Plasma VEGF and sFlt-1 levels were measured at enrollment. At baseline and 24-month visit, carotid IMT was measured and the association between sFlt-1 and IMT progression was assessed by linear regression. At baseline, age (r=0.186) and low level of high-density lipoprotein-cholesterol (HDL-C <40 mg/dl, r=0.214) were significantly related to carotid IMT. Over the 24 months, carotid IMT increased from 0.670±0.089 mm to 0.696±0.095 mm. There was a positive correlation between sFlt-1 tertiles and IMT change (P=0.05 by ANOVA). Upon multivariate analysis, log-transformed sFlt-1 level ($\beta$=0.137, P=0.003) and low HDL-C ($\beta$=0.048, P=0.04) were identified as predictors of IMT progression, independent of other confounding variables.

***Conclusions:*** High sFlt-1 level is predictive of carotid IMT progression in hypertensive patients. Low HDL-C level was also associated with IMT change. These observations support a high sFlt-1 level being indicative of progression of atherosclerosis. (*Circ J* 2010; **74:** 2211–2215)

**Key Words:** Atherosclerosis; Carotid arteries; Hypertension; Vascular endothelial growth factor; Vascular endothelial growth factor receptor 1

The role of angiogenesis in the early and advanced stages of atherosclerosis has long been investigated but remains an unresolved issue.[1] Many of the relevant studies have dealt with vascular endothelial growth factor (VEGF) and found that it is associated with angiogenesis under physiologic and pathologic conditions.[2] Fms-like tyrosine kinase-1 (Flt-1), a transmembranous tyrosine-kinase, is a receptor for VEGF. Flt-1 mRNA produces a soluble form of Flt-1 by alternative splicing, and soluble Flt-1 (sFlt-1) can be detected in the peripheral circulation.[2] Although sFlt-1 has been demonstrated to inhibit VEGF activity,[3] the association between sFlt-1 and human disease is unclear.[4] A few experimental and cross-sectional clinical studies have tried to evaluate the association between sFlt-1 and vascular diseases, but results have been inconsistent[4–7] and there is little consensus on the role of sFlt-1 in atherosclerosis so far.

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In human studies, surrogate markers of atherosclerosis have been widely used and carotid intima–media thickness (IMT) is 1 of the most popular markers.[8,9] Many epidemiological studies have accepted carotid IMT as a marker for atherosclerosis and cardiovascular risk.[10,11] Prior studies have shown several traditional risk factors, such as blood pressure (BP), diabetes mellitus, and dyslipidemia, to be predictive of IMT progression. In addition, there are a few studies addressing the possible influence of novel biomarkers on carotid IMT.[12,13] However, the relationship between sFlt-1 and progression of carotid IMT has not been systemically elucidated.

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**Table 1. Baseline Characteristics of the Subjects Who Completed the Study by Tertiles of sFlt-1 Level**

| | Low (<64.0 pg/ml, n=37) | Middle (64.0–72.0 pg/ml, n=37) | High (>72.0 pg/ml, n=38) | P value |
|---|---|---|---|---|
| **Age, years** | 60±10 | 60±8 | 58±9 | 0.65 |
| **Female sex, no (%)** | 20 (54) | 20 (54) | 17 (45) | 0.65 |
| **Medical history** | | | | |
| Diabetes mellitus, no (%) | 2 (5) | 4 (11) | 5 (13) | 0.51 |
| Hyperlipidemia, no (%) | 10 (27) | 10 (27) | 9 (24) | 0.91 |
| Current smoker, no (%) | 8 (22) | 10 (28) | 14 (39) | 0.29 |
| **Body mass index, kg/m²** | 25.4±3.0 | 24.3±2.5 | 25.0±2.4 | 0.24 |
| **Systolic BP, mmHg** | 129±14 | 131±14 | 126±14 | 0.31 |
| **Diastolic BP, mmHg** | 78±9 | 81±10 | 81±10 | 0.32 |
| **Laboratory examination** | | | | |
| Fasting glucose, mg/dl | 98±16 | 96±15 | 94±12 | 0.45 |
| Total cholesterol, mg/dl | 182±31 | 182±34 | 178±34 | 0.88 |
| Triglyceride, mg/dl* | 153 (57–383) | 129 (45–425) | 116 (45–425) | 0.41 |
| HDL-cholesterol, mg/dl | 53±13 | 54±15 | 50±12 | 0.36 |
| LDL-cholesterol, mg/dl | 97±28 | 100±31 | 103±29 | 0.71 |
| VEGF, $\mu$g/ml | 0.39±0.23 | 0.46±0.25 | 0.49±0.27 | 0.44 |
| sFlt-1, pg/ml* | 57 (38–64) | 67 (64–72) | 81 (72–155) | <0.001 |
| **Medications** | | | | |
| ≥3 antihypertensive agents | 11 (30) | 10 (27) | 4 (11) | 0.10 |
| Statin use, no (%) | 15 (41) | 12 (32) | 17 (45) | 0.54 |
| **Carotid IMT, mm** | | | | |
| Baseline | 0.672±0.077 | 0.688±0.097 | 0.671±0.089 | 0.19 |
| Follow-up | 0.672±0.107 | 0.704±0.082 | 0.711±0.093 | 0.17 |
| Change | 0.006±0.113 | 0.016±0.096 | 0.060± 0.116 | 0.05 |

*Log transformed before analysis.
sFlt-1, soluble fms-like tyrosine kinase-1; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VEGF, vascular endothelial growth factor; IMT, intima-media thickness.

Therefore, we planned to prospectively evaluate the association of sFlt-1 and carotid IMT progression. Hypertension is reported to be a major determinant of carotid IMT in the Korean population,[14] so we included only hypertensive patients who were stably treated with medications to minimize the potential influence of this risk factor in our analyses.

## Methods

### Study Subjects

Subjects were drawn from the patient database of the Cardiovascular Genome Center at Yonsei University Health System, Seoul, Republic of Korea. Between September 2004 and October 2006, treated hypertensive patients ranging from 20 to 80 years old were consecutively screened in the outpatient clinic. The inclusion criterion was hypertension under medical control for more than 3 months, and 120 patients were finally enrolled. The exclusion criteria were uncontrolled diabetes mellitus (fasting blood glucose ≥180 mg/dl), uncontrolled hypertension (systolic BP 180 mmHg or diastolic BP ≥110 mmHg), history of acute cerebrovascular accident or coronary artery disease, secondary hypertension, significant diseases of thyroid (thyroid stimulating hormone <lower limit of normal or >upper limit of normal (ULN)), liver (serum aminotransferase >2×ULN), or kidney (serum creatinine ≥1.5 mg/dl), history of inflammatory disease, or malignant neoplasm. The local ethics committee approved the study, and informed consent was given by all the participants.

### Study Protocol

Clinical data, including demographic variables, medical history, laboratory examination, and medication history, were obtained by a trained interviewer. Diabetes mellitus was defined as fasting blood glucose ≥126 mg/dl, postprandial blood glucose ≥200 mg/dl or current treatment with hypoglycemic agents. Hyperlipidemia was defined as low-density lipoprotein-cholesterol (LDL-C) ≥160 mg/dl. Follow-up visits occurred every 3–6 months over a period of 24 months. Patients were treated toward a goal of systolic BP (130 mmHg). Among the study subjects, 8 were excluded from the analysis, 3 because of withdrawal of consent and 5 because of failure to follow up.

### Laboratory Examination and Carotid IMT

At the time of enrollment, blood samples were collected from all subjects. Samples were analyzed within 4 h of collection or stored at –80°C until analysis. All analysis was performed by a local laboratory certified by the Korean Society of Laboratory Medicine. Lipid levels were measured using an auto-analyzer. Plasma levels of VEGF and sFlt-1 were measured by quantitative enzyme-linked immunoassay technique (ELISA: R&D System Inc, Minneapolis, MN, USA) according to the manufacturer's instructions. The coefficient of variation for both intra- and interassay precision was <7%.

At baseline and the 24-month visit, high-resolution carotid ultrasound was performed according to a standard protocol by 2 trained sonographers. Commercially available equipment (SSA 270A; Toshiba, Tokyo, Japan) with a 7.5-MHz linear transducer was used for the examination. B-mode images of

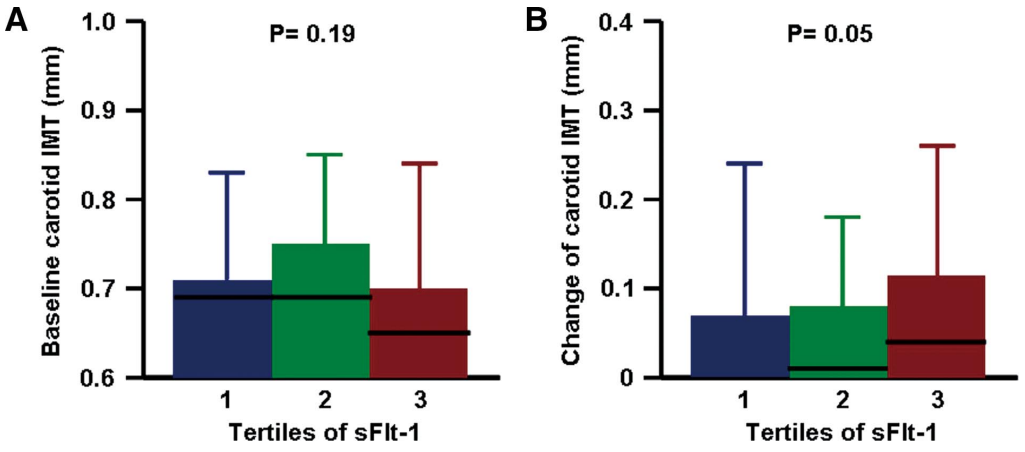


**Figure.** Baseline carotid IMT by tertile of sFlt-1 (**A**). IMT progression by tertile of sFlt-1 (**B**). IMT, intima-media thickness; sFlt-1, soluble fms-like tyrosine kinase-1.

the far wall of the distal right and left common carotid arteries were stored at the end-diastolic period. Carotid IMTs were measured by a single trained reader in a 1-cm segment from 1 cm proximal to the carotid bifurcation, using an automated system. IMT values for each side were averaged and used in all analyses. Intra-observer and inter-observer coefficients of variation for IMT were 5.4% and 6.4%, respectively.

### Statistical Analysis

Continuous variables with little skewness are listed as mean±SD, and continuous variables with a skewed distribution (triglyceride, sFlt-1) are listed as median (range). Categorical variables are presented as frequencies and group percentages. A log transformation was applied to triglyceride and sFlt-1 levels before analysis. Baseline parameters stratified by tertiles of sFlt-1 were compared using analysis of variance (ANOVA). The chi-square test was used to compare categorical variables in each group. Pearson correlation coefficients were calculated to evaluate the association between clinical risk factors and baseline carotid IMT.

To identify independent relationships between risk factors and carotid IMT progression, stepwise multivariate regression with forward selection was used. Only variables with $P<0.05$ in the univariate analysis were included in the multivariate analysis. As in previous studies, we found a significant inverse correlation between baseline carotid IMT and IMT progression ($r=-0.56$). Thus, we adjusted the baseline IMT in all analyses.[15] To determine potential differences in the determinants of carotid IMT progression between men and women, predictors of progression were analyzed separately by sex. All analyses used 2-tailed tests with a significance level of 0.05. The statistical software package SPSS version 12.0 (SPSS Inc, Chicago, IL, USA) was used for all analyses.

## Results

### Patients' Baseline Characteristics and Carotid IMT

The clinical characteristics of the patients who completed the study are stratified by tertile of sFlt-1 and listed in **Table 1**. Their mean age was 59±9 years, and 51% were female. Each group had similar clinical characteristics and baseline carotid IMT (**Figure A**). **Table 2** summarizes the correlation between clinical risk factors and carotid IMT at baseline. Carotid IMT was positively correlated with age (per 10 years, $r=0.186$) and low high-density lipoprotein-cholesterol (HDL-C <40 mg/dl, $r=0.214$), although there was no significant association between baseline IMT and sFlt-1 levels.

**Table 2. Correlation Between Clinical Variables and Baseline Carotid IMT**

| | r | P value |
|---|---|---|
| Age (per 10 years) | 0.186 | 0.049 |
| Female sex | 0.039 | 0.69 |
| Diabetes mellitus | –0.140 | 0.14 |
| Current smoker | 0.013 | 0.89 |
| Body mass index (per kg/m$^2$) | 0.079 | 0.41 |
| Systolic BP (mmHg) | 0.068 | 0.48 |
| Diastolic BP (mmHg) | 0.044 | 0.65 |
| Triglyceride ≥150 mg/dl | 0.012 | 0.90 |
| HDL-cholesterol <40 mg/dl | 0.214 | 0.02 |
| LDL-cholesterol ≥130 mg/dl | –0.092 | 0.35 |
| VEGF ($\mu$g/ml) | 0.028 | 0.82 |
| sFlt-1 (pg/ml)* | –0.149 | 0.12 |
| ≥3 antihypertensive agents | 0.003 | 0.98 |
| Statin use | –0.013 | 0.90 |

*Log transformed before analysis.
Abbreviations see in Table 1.

### sFlt-1 Levels and Carotid IMT Progression

During the 24 months, carotid IMT increased from 0.670±0.089 mm to 0.696±0.095 mm, and the mean IMT change was 0.026±0.111 mm. There was a significant relationship between baseline sFlt-1 and IMT progression ($P=0.05$ by ANOVA, **Figure B**). Univariate analysis showed that sFlt-1 ($P=0.002$) and low HDL-C ($P=0.02$) were positively correlated with IMT progression (**Table 3**). VEGF levels did not correlate with IMT change. There were no significant associations between other risk factors and IMT change. Upon multivariate analysis, sFlt-1 ($P=0.003$) and low HDL-C ($P=0.04$) were identified as independent predictors of IMT progression. This result was obtained after adjusting for the number of anti-

**Table 3. Determinants of Change in Carotid IMT in Univariate and Multivariate Analyses**

| | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | β | SE | P value | β | SE | P value |
| Age (per 10 years) | 0.014 | 0.011 | 0.20 | – | – | – |
| Female sex | –0.005 | 0.021 | 0.80 | – | – | – |
| Diabetes mellitus | 0.014 | 0.035 | 0.69 | – | – | – |
| Systolic BP (mmHg) | <0.001 | 0.001 | 0.97 | – | – | – |
| Diastolic BP (mmHg) | –0.001 | 0.001 | 0.28 | – | – | – |
| Current smoker | 0.007 | 0.023 | 0.77 | – | – | – |
| Body mass index (kg/m²) | –0.001 | 0.004 | 0.79 | – | – | – |
| Triglyceride ≥150 mg/dl | 0.003 | 0.022 | 0.90 | – | – | – |
| HDL-cholesterol <40 mg/dl | 0.054 | 0.024 | 0.02 | 0.048 | 0.023 | 0.04 |
| LDL-cholesterol ≥130 mg/dl | –0.025 | 0.027 | 0.35 | – | – | – |
| VEGF (μg/ml) | –0.006 | 0.050 | 0.91 | – | – | – |
| sFlt-1 (pg/ml)* | 0.146 | 0.045 | 0.002 | 0.137 | 0.045 | 0.003 |
| ≥3 antihypertensive agents | –0.050 | 0.025 | 0.054 | – | – | – |
| Statin use | –0.007 | 0.021 | 0.74 | – | – | – |

*Log transformed before analysis.
Abbreviations see in Table 1.

hypertensive agents and baseline IMT. Upon analysis by sex (Table S1), low HDL-C and sFlt-1 predicted IMT progression in men (n=55), whereas only diastolic BP was an independent predictor in women (n=57).

## Discussion

This study found that plasma sFlt-1 levels were positively associated with progression of carotid IMT. Our results also showed that low HDL-C was an independent predictor of IMT progression. Plasma VEGF levels were not related to IMT change. In our analyses by sex, the association between sFlt-1 and IMT progression was significant only in men. This study is the first to show, prospectively, a relationship between sFlt-1 level and progression of IMT.

Progression of IMT is affected by clinical risk factors. In longitudinal studies, traditional risk factors such as male sex, diabetes mellitus, smoking, systolic BP, and the levels of fasting glucose, triglycerides, HDL-C and LDL-C[10,11,16] correlate with IMT progression. In a recent study, emerging risk factors, including obesity and high insulin level, were reported as determinants of IMT change.[17] Biomarkers, such as asymmetric dimethylarginine[12] and soluble receptor for advanced glycation end-products, are also related to IMT progression.[13]

The relationship between angiogenesis markers and IMT progression has not been prospectively evaluated before. Instead, a few cross-sectional studies have investigated the correlations between angiogenesis markers and the risk of atherosclerosis. However, the results of those studies have been inconsistent. Some reported a significant correlation between VEGF and the presence or severity[18] of atherosclerotic vascular diseases, whereas others failed to show the same result.[19] Sanhofer et al recently reported a positive association between sFlt-1 and carotid IMT,[7] but in other reports sFlt-1 inversely correlated with atherosclerotic vascular diseases.[5,20]

In our study, higher sFlt-1 levels were related to increases in carotid IMT. The clinical relevance and possible mechanism of our findings are discussed below. The presence of higher plasma levels of VEGF and sFlt-1 in patients with hypertension, as compared with control subjects, was reported by Belgore et al, who suggested that altered angiogenesis in these patients may be related to endothelial change.[21] Furthermore, sFlt-1 has been demonstrated as inhibiting microvascular function, leading to endothelial dysfunction and proteinuria.[4,22] Accordingly, in our subjects sFlt-1 might have been involved in vascular pathogenesis in the change in IMT. On the other hand, recent studies have shown that sFlt-1 gene transfer reduces inflammation and cellular proliferation and inhibits post-injury neointimal formation.[6,23] Furthermore, Onoue et al reported that a low sFlt-1 level is associated with worsening of the atherosclerosis accompanying renal dysfunction.[24] The cause–effect relationship between sFlt-1 and atherosclerosis needs to be clarified by further studies. It is difficult to interpret the lack of association between sFlt-1 and baseline IMT in our data. Some have reported a positive relationship between sFlt-1 and carotid atherosclerosis in univariate analysis;[7] however, few data are available on this issue. Our results may partly depend on the number or the characteristics of the study subjects.

In our study, low HDL-C was an independent predictor of IMT progression, which is in agreement with previous reports that showed low HDL-C was a risk factor for IMT change in a variety of clinical situations, such as coronary artery disease,[16] familial hypercholesterolemia,[25] and metabolic syndrome.[17] However, unlike the results of other studies,[10,17] ours did not show predictive values of traditional risk factors and lipid parameters. In addition, several studies have reported the beneficial effects of medications, such as angiotensin-converting enzyme inhibitors[26] and statins,[27,28] on the progression of carotid IMT. However, neither the number of antihypertensive agents nor the use of statins was predictive of IMT change in our study. Although the reason for the lack of such relationships is unclear, the different risk profiles and ethnicity of our study subjects, their relatively advanced age (59±9 years) and the short follow-up (24 months) may have affected our results.

### Study Limitations

The major strength of our study is that it is the first to describe a prospective association between sFlt-1 and IMT progression. In addition, we conducted this study in a single center with accurate IMT measurement. However, several limitations of the study also need to be noted. First, although

we found a significant relationship between sFlt-1 and IMT progression, the size of the additive impact on other risk factors remains to be elucidated, because the associations shown in our study were not very strong. Second, we assessed the risk factors and biomarkers on a single occasion only. Inclusion of serial changes in risk factors and biomarkers in the analysis might have made our results clinically more useful. Third, many of our study subjects were on multiple medications, and we can not completely rule out their effect on the progression of atherosclerosis. However, we did try to minimize the medication effect in our analysis. Finally, the number of subjects is relatively small. If the sample size was larger and the follow-up longer, we may have obtained statistical significance for some important variables. However, the calculated power of our study was 0.89 and reasonable; it was estimated from the correlation coefficient of 0.295 between sFlt-1 and carotid IMT change, sample size of 112, and alpha of 0.05.

## Conclusion

We found that a high sFlt-1 level was associated with carotid IMT progression in hypertensive patients. Low HDL-C level was also predictive of IMT change. Our observations support that a high sFlt-1 level may be an indicator of the progression of atherosclerosis.

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### Supplementary files

**Table S1.** Determinants of Change of Carotid IMT in Men and Women

Please find supplementary file(s);
http://dx.doi.org/10.1253/circj.CJ-10-0432