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Balance between angiogenic and anti-angiogenic isoforms of VEGF-A is associated with the complexity and severity of coronary artery disease

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ABSTRACT

Introduction: Assessing the complexity of coronary artery disease (CAD) is clinically important. Vascular endothelial growth factor A (VEGF-A) is a powerful and the most important regulator of angiogenesis. It has been reported that the anti-angiogenic isoform of VEGF-A (VEGF-A_{165}b) inhibits angiogenesis. The purpose of this study was to evaluate the relationship between the complexities of CAD using the Syntax score (SS) and the levels of circulating total VEGF-A or VEGF-A_{165}b.

Methods: A total of 268 patients who underwent percutaneous coronary intervention (PCI) were enrolled. Of these, 73 patients without acute coronary syndrome or previous revascularization were included in this study. These subjects were divided into two groups according to the SS. Circulating total VEGF-A and VEGF-A_{165}b levels were measured using an enzyme-linked immunosorbent assay.

Results: Circulating levels of total VEGF-A were significantly higher in the SS $>$ 22 (high SS) group than in the SS $\leq$ 22 (low SS) group ($p = 0.018$). Moreover, the ratio of VEGF-A_{165}b to total VEGF-A was significantly lower for the high SS group ($p = 0.004$). The levels of total VEGF-A independently predicted high SS after adjusting for other significant variables among patients who underwent PCI (odds ratio, 1.004; 95% CI, 1.001 to 1.006; $p = 0.01$).

Conclusions: These data indicated that high SS was associated with circulating levels of total VEGF-A and the ratio of VEGF-A_{165}b to total VEGF-A in patients with complex CAD.

1. Introduction

Coronary artery disease (CAD) is still one of the leading causes of mortality [1]. The synergy between percutaneous coronary intervention (PCI) with TAXUS and cardiac surgery score (Syntax Score [SS]) is useful for determining the optimal revascularization strategy for patients with CAD and for predicting clinical outcomes of patients who underwent PCI [2,3].

Angiogenesis is related to collateral development in CAD [4]. Neoangiogesis, which arises from the vasa vasorum, is likely the primary source of intraplaque hemorrhage. It is closely associated with plaque progression [5]. Vascular endothelial growth factor-A (VEGF-A) is considered a key regulator of physiological angiogenesis [6,7] and is associated with CAD [8,9]. Previous reports showed that neoangiogenesis lesions in coronary plaque are favored by local accumulation of VEGF-A [10,11]. However, it is now recognized that alternative VEGF-A gene splicing generates VEGF-A isoforms that differ in their biological actions. Proximal splicing that includes an exon 8a sequence results in pro-angiogenic VEGF-A_{165}a, whereas distal splicing inclusive of exon 8b yields the anti-angiogenic isoform of VEGF-A (VEGF-A_{165}b) [12,13]. However, the association between VEGF-A variants and CAD is not yet clear. Therefore, we hypothesized that the levels and balance of angiogenic and anti-angiogenic isoforms of VEGF-A would be associated with the complexity of CAD. The aim of this study was to evaluate the association between circulating total VEGF-A or VEGF-A_{165}b and the SS of stable CAD patients undergoing PCI.

Abbreviations: CAD, coronary artery disease; SS, Syntax Score; VEGF-A, vascular endothelial growth factor-A; PCI, percutaneous coronary intervention; AP, angina pectoris; ACS, acute coronary syndrome; HD, hemodialysis; HbA1c, glycosylated hemoglobin; eGFR, estimated glomerular filtration rate; PAD, peripheral artery disease; ABI, ankle brachial index; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; IVSd, inter ventricular septum diameter; PWd, posterior wall diameter; LVEF, left ventricular ejection fraction

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2. Methods

2.1. Patient selection

A total of 268 patients who underwent percutaneous coronary intervention (PCI) for stable angina pectoris (AP) or acute coronary syndrome (ACS) were enrolled between February 2015 and June 2016 at Nagoya University Hospital. Of these, 73 patients who underwent PCI for stable angina pectoris were included in this observational study. Exclusion criteria included the following: (i) patients undergoing hemodialysis (HD) treatment; (ii) those who had active malignancies or had undergone surgery or chemotherapy for malignancy < 1 year previously; (iii) those who had undergone PCI or coronary artery bypass graft for ischemic heart disease; and (iv) those who had collagen diseases. Interventionists and cardiac surgeons discussed which procedures were appropriate for all patients and which were most consistent with the patient's wishes. As controls, we enrolled 32 patients without malignancy who underwent coronary angiography due to clinical symptoms or exercise electrocardiogram abnormalities but had an SS of 0. They had normal left ventricular function and did not undergo cardiac surgery or HD. Written informed consent was obtained from all patients before their procedures.

2.2. Blood sample examinations

On the day of PCI after a 12-hour overnight fast and before heparin injection, blood samples were obtained from all patients. Serum samples were stored at − 80 °C until total VEGF-A and VEGF-A_{165}b measurements were performed. Studies of these human samples were performed after approval from the Ethical Committees of Nagoya University Graduate School of Medicine.

2.3. Measurement of total-VEGF-A and VEGF-A_{165}b

Serum total VEGF-A levels were determined by an enzyme-linked immunosorbent assay (ELISA) kit (Human VEGF Quantikine ELISA Kit, DVE00, R&D) according to the manufacturer's instructions. The limit of detection was 9 pg/ml (intra-assay and inter-assay coefficients of variation were 4.5% and 7.0%, respectively). Serum VEGF-A_{165}b levels were also determined using an ELISA kit (Human Vascular Endothelial Growth Factor-165b ELISA Kit, MBS720132, MyBiosource) according to the manufacturer's instructions. The limit of detection was 1 pg/ml (intra-assay and inter-assay coefficients of variation were < 10% and < 10%, respectively).

2.4. Angiographic analysis

The complexity of coronary artery lesions was quantified using the SS. Precise methods are described elsewhere [2]. Coronary lesions producing stenosis with ≥ 50% diameter in vessels ≥ 1.5 mm according to the baseline diagnostic angiogram were scored individually and then added together to provide the overall SS, which was calculated using the SS algorithm. Coronary angiograms were analyzed by two experienced observers who were blinded to the identities and clinical information of the patients. Consistent with the SYNTAX trial, the subjects who underwent PCI were divided into two groups: the low SS group (SS ≤ 22) and the high SS group (SS > 22) [3].

2.5. Study outcomes and definitions

Hypertension was defined as systolic pressure ≥ 140 mm Hg or diastolic pressure ≥ 90 mm Hg and/or under anti-hypertensive medication. Blood pressure was measured with an appropriate arm cuff and a mercury column sphygmomanometer after at least 10 min of rest in a sitting position. Diabetes mellitus was defined as a current diagnosis of diabetes, a fasting plasma glucose concentration ≥ 126 mg/dl or glycosylated hemoglobin (HbA1c) concentration ≥ 6.5% (National Glycohemoglobin Standardization Program), and/or use of any anti-hyperglycemic medication. The estimated glomerular filtration rate (eGFR) was calculated according to the following Japanese equation: eGFR (ml/min/1.73 m^2) = 194 × serum creatinine$^{-1.094}$ × age$^{-0.287}$ × 0.739 (for women) [14]. Peripheral artery disease (PAD) was defined as ankle brachial index (ABI) < 0.9, claudication, and/or previous revascularization. All echocardiographic parameters were measured according to the recommendations of the American Society of Echocardiography [15]. Images were obtained in the parasternal (long axis and short axis) and apical views. The following parameters were evaluated: left ventricular end-diastolic diameter (LVEDD; mm); left ventricular end-systolic diameter (LVESD; mm); interventricular septum diameter (IVSd; mm) at end-diastole; left ventricular posterior wall diameter (PWd; mm) at end-diastole; and LV mass (g). The LV mass was indexed for body surface area (LV mass index; g/m^2). The left ventricular ejection fraction (LVEF; %) was calculated according to the biplane Simpson's method and apical four-chamber and two-chamber views.

2.6. Statistical analysis

Continuous variables were generally presented as mean ± standard deviation or median (interquartile range) if they were non-normally distributed. Categorical variables were presented as a percentage. A comparison between two groups was performed using Student's test for normally distributed variables and the Mann-Whitney *U* test for non-normally distributed variables. Comparison between more than two groups was performed using the Kruskal-Wallis test. Categorical data were assessed using the chi-squared test. Pearson and Spearman rank tests were performed to define the correlation of the levels of VEGF-A with SS. To identify independent predictors of high SS, multivariate logistic regression analysis was performed for each parameter used as the dependent variable.

Probability < 5% was considered a statistically significant difference. Analysis was performed using SPSS version 24.0 (IBM, Armonk, NY, USA).

3. Results

3.1. Clinical characteristics

Table 1 shows the patient's clinical characteristics. After comparing patients without significant stenosis in the coronary artery and those who needed revascularization, the percentages of females and those with hypertension were higher for patients with an SS of 0. Patients with an SS of 0 had better LVEF; however, differences in these values were not statistically significant. When revascularization was necessary, patients with a high SS were significantly older than those with a low SS. In addition, they tended to have worse renal function. Fasting blood glucose and HbA1c were significantly higher for those with a high SS compared to those with a low SS. However, there were no significant differences in those parameters between the two groups. In the high SS group, LV contraction was decreased and LV mass index was increased. The presence of PAD was not significantly associated with SS.

3.2. Correlation between the levels of circulating total VEGF-A, VEGF-A_{165}b and SS

We next assessed the relationship between the levels of VEGF-A and SS. The levels of total VEGF-A were positively correlated with SS (Fig. 1A). In addition, the ratio of VEGF-A_{165}b to total VEGF-A were negatively correlated with SS (Fig. 1C). However, the level of VEGF-A_{165}b did not correlate with SS (Fig. 1B).