Vascular Endothelial Growth Factor and its Soluble Receptor-1 as Surrogate Markers for Subjects with High-risk of Cardiovascular Disease

Vipa Boonkitticharoen[1*], Chanika Sritara[1], Prin Vathesatogkit[2], Supakajee Saengruang-Orn[3], Wipa Ratanachaiwong[4], Piyamitr Sritara[2]

[1]Department of Diagnostic and Therapeutic Radiology, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand, [2]Department of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok 10400, Thailand, [3]Department of Radiology, Phramongkutklao Hospital, Bangkok 10400, Thailand, [4]Medical and Health Office, Electricity Generating Authority of Thailand, Nonthaburi 11130, Thailand

ABSTRACT

Background: Vascular endothelial growth factor (VEGF) and its soluble receptor-1 (sVEGFR-1) can either act beneficially or adversely on cardiovascular health depending on the context. An understanding of VEGF regulation in subclinical atherosclerosis would help to identify individuals who may need more intensive efforts in risk modification as preventive measures. **Subjects and Methods:** The population in this study consisted of subjects without any modifiable risks and hypercholesterolemic subjects with low or high cardiovascular disease (CVD) risks. A total of 227 subjects were recruited from employees of the Electricity Generating Authority of Thailand and their cardiovascular risk factor history as well as clinical and laboratory data were collected. Plasma VEGF and sVEGFR-1 were measured using enzyme-linked immunosorbent assay. **Results:** Biphasic association between the sVEGFR-1-to-VEGF (R/V) ratio and the VEGF concentration indicated VEGF upregulation at concentrations above 12.99 pg/mL and VEGF entrapment at a concentration below this level. Rates of VEGF upregulation in low- and high-risk groups were respectively found to be 2.6-fold ($P = 0.01$) and 4.6-fold ($P = 0.03$) above the zero risk group. The R/V ratio, which is positively correlated to cholesterol level ($P < 0.01$), blood pressure and waist circumference ($P < 0.1$), was found to be associated with CVD risk severity in high-risk subjects ($P = 0.028$). At VEGF ≤18.58 pg/mL and R/V ratio >3.4, high-risk subjects compared to low-risk subjects had greater odds of being observed with severe risk scores (odds ratio = 8.2, $P < 0.0001$). **Conclusions:** VEGF upregulation in subjects with CVD risks is correctively controlled within the physiologic boundary and represents an attempt at vascular repair. Low VEGF levels and high R/V ratios in high-risk subjects are warning signs with respect to cardiovascular health and may indicate the need for more aggressive risk factor treatment in order to prevent the occurrence of undesirable cardiovascular events.

Keywords: Cardiovascular disease risk factor, the Electricity Generating Authority of Thailand risk score, hypercholesterolemia, soluble vascular endothelial growth factor receptor-1-to-vascular endothelial growth factor ratio, vascular endothelial growth factor protective function

INTRODUCTION

Vascular endothelial growth factor (VEGF) is an angiogenic cytokine of interest in cardiovascular medicine. Besides the angiogenic action, VEGF stimulates virtually all aspects of endothelial function, including permeability, proliferation, migration, nitric oxide (NO) production, and endothelial cell survival.[1] Over suppression of VEGF action by its soluble receptor-1 (sVEGFR-1) can lead to endothelial dysfunction.[2] Likewise, endothelial dysfunction can be induced by cardiovascular disease (CVD) risk factors through the generation of oxidative stress to deplete the availability of NO.[3]

VEGF is expressed in every vascularized tissue to maintain quiescent vascular beds of multiple organs, mediates physiologic angiogenesis in skeletal muscle

***Corresponding address:**
Dr. Vipa Boonkitticharoen, Department of Diagnostic and Therapeutic Radiology, Ramathibodi Hospital, 270 Rama VI Road, Mahidol University, Bangkok 10400, Thailand.
E-mail: vipa.bon@mahidol.ac.th

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response to exercise,[4] energy metabolism in adipose tissue,[5] and possibly regulates lipid metabolism.[6] In health, low physiologic VEGF levels modulate vascular tone and blood pressure through stimulation of NO production.[1] In atherosclerosis, elevating plasma VEGF along with decreasing sVEGFR-1 levels in relative to healthy controls observed in subjects with CVD risk factors and in patients with established atherosclerotic diseases[7-9] is suggested as an attempt to compensate for tissue damage or hypoxia, or simply a reflection of endothelial cell damage.[10] In the light of recent evidences, these alterations in VEGF and sVEGFR-1 secretion profiles may be regarded as a reparative response to vascular damage or endothelial dysfunction. For example, VEGF gene transfer inhibits thickening of the media and promotes vascular remodeling,[11] high circulating VEGF is related to a better systolic function in diabetic hypertensive patients with cardiac remodeling.[12] Paradoxically, VEGF may promote atherosclerosis progression through its ability in enhancing plaque inflammatory infiltration and plaque neovascularization.[13] A prospective study on association of VEGF with coronary heart disease mortality reveals that subjects with large CVD risk burden and elevated VEGF levels at baseline are at greater risks of death than those with lower VEGF levels and also less CVD burden.[14]

Whether VEGF response is vasculoprotective or pathogenic may involve pattern of VEGF regulation at a certain time under a specific context. For instance, VEGF-mediated angiogenesis during the early stage of obesity is protective for involvement in energy expenditure, whereas an angiogenic switch in the late stage will lead to a number of disorders such as type 2 diabetes, CVD, etc.[5] Abnormal suppression of VEGF by sVEGFR-1 causes hypertension and proteinuria in patients with preeclampsia[15] while enhanced sVEGFR-1 expression in corneal epithelial cells safeguards the transparency of the eyes by suppression of angiogenesis near the lens.[16]

Taken altogether, VEGF/sVEGFR-1 system might play roles in modulating CVD risk factors to result either beneficial or deleterious outcome in a context dependent manner. An understanding of VEGF action and regulation in subjects with high CVD risk might be helpful in identifying individuals who might need more aggressive treatment of risk factors to prevent progression of atherosclerosis and cardiovascular events. Among many CVD risk factors, hypercholesterolemia is unique for being sufficient to drive the development of atherosclerosis even in the absence of other known risk factors.[17] CVD, however, is caused by multiple risk factors, which interact synergistically to promote the disease development.[18] We conducted a cross-sectional study to investigate the dependence of VEGF levels on sVEGFR-1-to-VEGF (R/V) ratio to understand how VEGF levels were regulated by sVEGFR-1 in subjects without any modifiable risk factors, low-risk hypercholesterolemic subjects (with or without additional CVD risk factors excluding diabetes) and high-risk hypercholesterolemic subjects (with diabetes and or medical history of peripheral and CVDs) and to evaluate the association of VEGF levels or R/V ratios with profiles of the Electricity Generating Authority of Thailand (EGAT) scores[19] signifying 10-year risks of CVD validated for Thais.

SUBJECTS AND METHODS

Subjects

Subjects were selected among 2584 participants of EGAT cohort study on cardiovascular risks.[20] This study had a cross-sectional design and consisted of 56 subjects without any modifiable CVD risk factors and 171 hypercholesterolemic subjects with or without additional risk factors. The inclusion criteria were subjects aged between 35 and 65 years with or without the following CVD risk factors: Hypercholesterolemia (total cholesterol ≥240 mg/dL), hypertension (blood pressure ≥140/90 mmHg), diabetes (according to the World Health Organization criteria), abdominal obesity (waist circumference ≥90 cm for male and ≥80 cm for female), current smoker and medical history of CVD, cerebrovascular events and peripheral vascular disease. The exclusion criteria were subjects who had recent surgery, coronary bypass graft, post-myocardial infarction, stroke (within 12 weeks), acute infections requiring antibiotic therapy (within 2 weeks), history of neoplastic disease, and connective tissue disease. This study was approved by Ramathibodi Hospital Ethics Committee and written informed consents were obtained. Subjects completed a self-administered CVD risk factor questionnaire, underwent an oral glucose tolerance test and a physical examination, and gave blood samples for chemistry analyses.

Classification of risk category

Subjects were classified into three risk categories. A: The zero risk category recruited subjects without any modifiable CVD risk factors. B: The low-risk category included hypercholesterolemic subjects with or without the following risk factors: Hypertension, abdominal

obesity and cigarette smoking. C: The high-risk category enrolled hypercholesterolemic subjects with additional risk factors plus diabetes and/or medical history of CVD, cerebrovascular event, and peripheral vascular disease. Ten-year risks of CVD for individual subjects were calculated based on the EGAT scoring system as shown in Table 1.[19] EGAT scores ≤5 corresponding to ≤1% risks were characterized as non-significant health risks, scores between 6-10 and 2-4% risks as non-serious health risks, which can be nullified by changes in lifestyle and scores ≥11 and ≥4% risks as serious health risks, which required medical advices and therapy for preventive measures.

VEGF and sVEGFR-1 determination

For VEGF and sVEGFR-1 assays, 6 ml of peripheral blood was collected into a tube with EDTA coagulant and kept in an ice bucket. Plasma was prepared by centrifugation under 4°C at a speed of 1000 g for 15 min. The plasma sample was aliquoted and stored at −25°C until assay. Time for a blood sample processing and freezing was within 2 h after collection. Levels of VEGF and sVEGFR-1 were measured using two commercially available enzyme-linked immunosorbent assay kits (Quantikine R&D Systems, Abingdon, UK). The VEGF assay kit (DVE 00) measured only unbound fraction of VEGF while the sVEGFR-1 assay (DVR 100) measured total sVEGFR-1.[15] According to the manufacturer, the minimal detectable concentrations of VEGF and sVEGFR-1 were <9 and 5.01 pg/mL, respectively.

Statistical analysis

Parametric data are expressed as mean (standard deviation or standard error [SE]), nonparametric data as median (interquartile range or range) or actual numbers (percent). Differences among means were analyzed by one-way analysis of variance, between or among medians by χ^2 analyses of contingency tables. Frequency data between groups were analyzed by χ^2 – test or Fisher exact test as appropriate. Correlations for R/V ratio or sVEGFR-1 level with VEGF level were assessed by Spearman rank test. Analyses of linear regression lines were performed to assess relationships between R/V ratio versus VEGF level, and R/V ratio versus the odds of serious health risk. Pearson's correlation and multiple regression analysis were performed to identify CVD risk factors that were related to VEGF levels or R/V ratios. Unpaired *t*-test was used to evaluate the association of VEGF levels or R/V ratios with EGAT scores ≥11 versus scores <11. Data were analyzed using SPSS for windows version 18. All statistical tests were two-tailed and $P < 0.05$ was considered as statistical significance.

RESULTS

Association between VEGF level and sVEGFR-1-to-VEGF (R/V) ratio

Demographic characteristics of the study population are presented in Table 2. Despite the worsening of EGAT risk scores from the zero risk to the high-risk categories ($P < 0.001$), no significant differences in median levels of VEGF and sVEGFR-1 could be observed among risk categories. It remained to be investigated whether VEGF regulations in CVD risk groups were altered or compensated so as to normalize VEGF levels.

As a negative regulator of VEGF bioavailability, sVEGFR-1 had been demonstrated to trap VEGF thereby reducing free VEGF levels.[15] In this study, significant negative correlations shown by Spearman rank test could be observed between VEGF levels and R/V ratios (a mean correlation coefficient for all risk categories was −0.928, $P < 0.001$) but not with absolute sVEGFR-1 concentrations ($P \geq 0.2$). A representative scatter plot shown in Figure 1 displays a biphasic association which could be separated at a median VEGF level of 12.99 pg/mL. Regression line analyses demonstrated a greater slope for the association above the median (Figure 1). Slopes for subjects with low (B) and high-risk (C) categories were 2.59 ($P = 0.008$) and 4.58 ($P = 0.033$) folds greater than that of the zero risk (A). No

Table 1 EGAT scoring system[19]

Score	–2	0	2	3	4	5	6	8
Age (years)	35-39	40-44	45-99		50-54		55-59	60-65
Gender		Female		Male				
Cholesterol (mg/dL)		<280				>280		
Smoking		No	Yes					
Diabetes		No				Yes		
Hypertension		No		Yes				
Waist circumference*		Below		Above				

*Men≥90 cm, women≥80 cm

Table 2 Characteristics of the study population

Parameter	Risk category			*P* value
	A. Zero (*n*=56)	B. Low (*n*=117)	C. High (*n*=54)	
Gender (male/female)	28/28	84/33	48/6	<0.001*
Age (years)	49.0 (4.4)	50.8 (4.6)	51.9 (4.4)	<0.001*
Medical history (*n*)				
CVD	-	-	20	-
CVA	-	-	9	-
PVD	-	-	14	-
Diabetes mellitus	-	-	33	-
Hypertension	-	38	32	-
Current smoker	-	14	17	-
Total cholesterol (mg/dL)	203.6 (27.0)	265.3.2 (28.8)	227.0 (55.2)	<0.001*
Glucose (mg/dL)	93.5 (9.5)	97.5 8.6)	146.7 (83.7)	<0.001*
Systolic BP (mmHg)	114.7 (10.0)	122.6 (15.4)	132.3 (16.1)	<0.001*
Diastolic BP (mmHg)	74.0 (8.8)	79.4 (10.9)	85.0 (10.8)	<0.001*
Waist circumference (cm)	81.5 (8.1)	86.8 (9.3)	93.0 (8.3)	<0.001*
EGAT score	5 (4-8)	9 (6-12)	13 (11-17)	<0.001*
VEGF (pg/mL)	12.7 (9.0-18.1)	12.5 (7.3-19.6)	13.7 (6.8-18.3)	0.96
sVEGFR-1 (pg/mL)	54.1 (45.4-70.0)	56.6 (50-65.2)	53.4 (51.3-70.0)	0.39

Data are presented as means (SD), medians (interquartile range), or actual numbers; *Significant differences among risk categories: $P < 0.05$; CVD: Cardiovascular disease, CVA: Cerebrovascular accidents, PVD: Perivascular disease, BP: Blood pressure

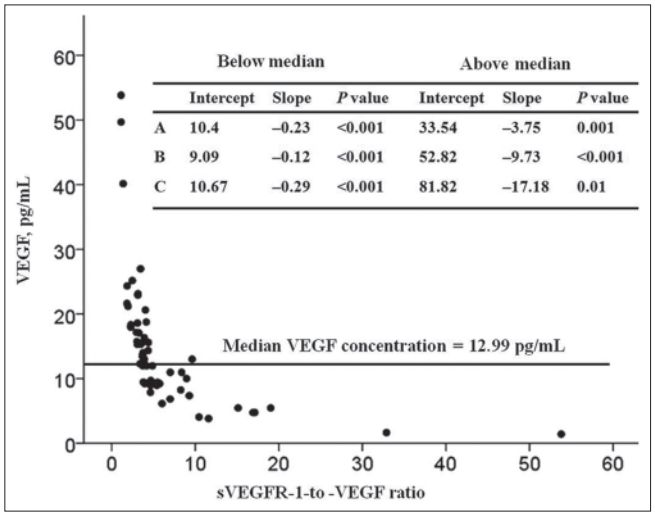

Figure 1. A representative scatter plot to demonstrate the correlation between soluble vascular endothelial growth factor (VEGF) receptor-1-to-VEGF (R/V) ratio and VEGF concentration. The insert: A = zero risk group, B = low-risk group and C = high-risk group.

significant differences in slopes among risk categories could be confirmed at VEGF concentration region below median.

Modulation of VEGF levels

To understand how VEGF concentrations were modulated, profiles of R/V ratios were analyzed across three VEGF regions defined as low, cut-off <12.99 pg/mL (50th percentile), intermediate, between 12.99 and 18.58 pg/mL (50-75th percentiles) and high, >18.58 pg/mL. R/V ratio was also defined as high at a cut-off >9 (75th percentile), intermediate, between 3-9 (25-75th percentiles) and low at <3 (Table 3). At low VEGF region, levels of VEGF were inversely modulated by sVEGFR-1. While at intermediate to high concentration regions, VEGF levels seemed to be increased by the mechanism of VEGF upregulation not by sVEGFR-1 down-regulation. Since VEGF concentrations did not change with changes in R/V ratios in contrast to what observed at the low VEGF region (Table 3).

Comparison of VEGF modulation between groups, R/V ratio profiles at low VEGF region in high risk category were significant different from those of zero risk category ($P = 0.013$). More subjects, 69.2% in compared to 32.1% of the zero risk, were associated with an R/V ratio >9, thereby the overall median VEGF concentration tended to be lower (i.e., 6.6 pg/mL in contrast to 9 pg/mL of the zero risk). At high concentration region, R/V ratio profiles for low-risk ($P = 0.005$) and high-risk category ($P = 0.015$) were significantly deviated from that of zero risk. Elevated VEGF levels could be observed in the high-risk category, but at a marginal significant level ($P = 0.097$).

R/V ratio in associating with CVD risk profile

Means (SE) for VEGF and R/V ratio in associating with EGAT risk scores ≥11 and <11 calculated for all risk categories are shown in Table 4. In overall, lower VEGF levels or higher R/V ratios tended to be associated with the high CVD risk scores rather than the low risk score. However, significant association could only be observed for R/V ratio in the high-risk group ($P = 0.028$). Multivariate analysis revealed the significant determinants for R/V ratio as cholesterol level (ß =5.283, $P = 0.009$), whereas

Table 3 Distributions of sVEGFR-1-to-VEGF (R/V) ratio at different VEGF levels

VEGF, pg/mL (Cutoff)	R/V ratio	A. Zero risk category		B. Low risk category		C. High risk category	
		n (%)	VEGF, pg/mL	*n* (%)	VEGF, pg/mL	*n* (%)	VEGF, pg/mL
Low	>9	9 (32.1)	4.8 (1.4-7.4)	26 (44.1)	3.1 (0-7.6)	18 (69.2)	5.6 (0-9.1)
(<12.99)	3-9	19 (67.9)	9.5 (6.1-12.5)	33 (55.9)	10.7 (5.8-12.5)	8 (30.8)	10.9 (9.5-12.9)
	Total	28 (100)	9 (1.4-12.5)	59 (100)	7.3 (0-12.5)	26 (100)	6.6 (0-12.9)
	P value	-	-	0.35	0.35	0.013*	0.28
Intermediate	3-9	12 (80)	15.3 (13-17)	22 (81.5)	15.3 (13-18.5)	12 (80)	15.1 (13.6-18.3)
(12.99-18.58)	<3	3 (20)	18 (17.1-18.3)	5 (18.5)	17.4 (13.4-18.6)	3 (20)	17.6 (15.7-18)
	Total	15 (100)	15.6 (13-18.3)	27 (100)	15.6 (13-18.6)	15 (100)	15.4 (13.6-18.3)
	P value	-	-	1	1	1	1
High	3-9	6 (46.2)	21.8 (18.6-27)	2 (6.5)	21.8 (19.5-24.1)	-	-
(>18.58)	<3	7 (53.8)	25.2 (21.2-53.8)	29 (93.5)	23.5 (19.6-137.1)	13 (100)	30.2 (18.8-292.4)
	Total	13 (100)	23.1 (18.6-53.8)	31 (100)	23.5 (19.5-137.1)	13 (100)	30.2 (18.8-292.4)
	P value	-	-	0.005*	1	0.015*	0.097†

Values are median (range) unless otherwise stated; *Significant difference from zero risk group (*P*<0.05); †Borderline significance (*P*<0.1)

Table 4 Association of VEGF or sVEGFR-1-to-VEGF (R/V) ratio with serious and non-serious risk scores

Category	Parameter	EGAT scores≥11	EGAT scores<11	*P* value
A. Zero risk	VEGF, pg/mL	8.49 (6.84)	14.96 (1.38)	0.38
	R/V ratio	18.2 (14.69)	6.37 (1.05)	0.57
	n	2	54	-
B. Low risk	VEGF, pg/mL	12.48 (1.45)	16.59 (1.92)	0.172
	R/V ratio	7.92 (1.24)	9.09 (1.97)	0.705
	n	37	50	-
C. High risk	VEGF, pg/mL	20.16 (7.68)	19.99 (3.46)	0.99
	R/V ratio	8.31 (1.41)	4.46 (0.95)	0.028*
	n	37	17	-

Values are means (SE) unless otherwise stated; *Significant difference (*P*<0.05).

Table 5 Correlation of sVEGFR-1-to-VEGF (R/V) ratio with cardiovascular disease risk factors

Risk factors	Beta coefficients	*P* value
Age	−0.215	0.197
Sex	0.015	0.929
Smoking	−0.017	0.921
Systolic blood pressure	0.385	0.099†
Diastolic blood pressure	−0.273	0.23
Waist circumference	0.283	0.08†
Glucose	0.002	0.993
Total cholesterol	5.283	0.009*
HDL cholesterol	−0.698	0.056†
LDL cholesterol	−4.568	0.009*
Triglyceride	−1.965	0.015*

*Significant difference (*P*<0.05); †Borderline significance (*P*<0.1).

the systolic blood pressure (ß =0.385, $P = 0.099$) and waist circumference (ß =0.283, $P = 0.08$) only at the marginal significant level ($P < 0.1$). There were negative associations for HDL cholesterol (ß = −0.698, $P = 0.056$), low-density lipoprotein cholesterol (ß = −4.568, $P = 0.009$) and triglyceride (ß = −1.965, $P = 0.015$) (Table 5).

To evaluate how the odds favoring serious risk score changed with R/V ratios, different cut-offs for R/V ratio were applied to generate different series of data sets for curve fitting. Each data set composed of odds favoring EGAT scores ≥11 versus the median of a specified R/V ratio interval. Figure 2 depicts significant dependence of the odds favoring EGAT scores ≥ 11 on median R/V ratios for the high-risk group (slope = 0.429, $P = 0.008$), marginal significance for the low-risk (slope = 0.011, $P = 0.086$), and non-significance for the zero risk (slope = 0.007, $P = 0.16$).

As there was a trend that low VEGF and high R/V ratio to be associated with a serious risk score, this posed a question whether R/V alone or R/V in combination with VEGF level might represent a good predictor for subjects with greater odds of serious risk. Odds ratio (OR) comparing the odds favoring EGAT scores ≥11 between

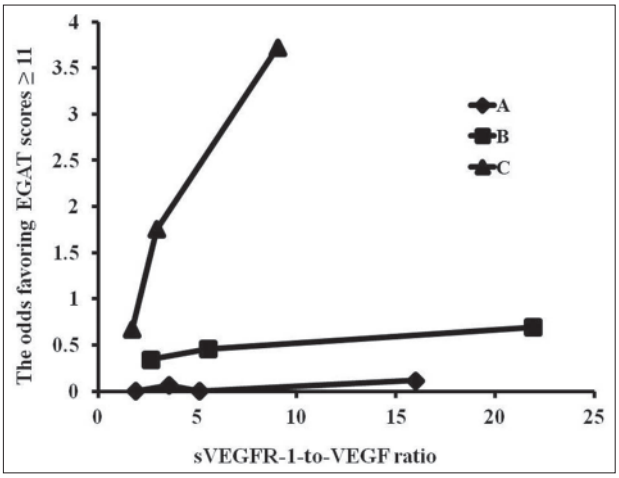

Figure 2. Relation between soluble vascular endothelial growth factor (VEGF) receptor-1-to-VEGF ratio and the odds favoring Electricity Generating Authority of Thailand scores ≥ 11 for the zero risk (A), the low-risk (B) and the high-risk category (C).

high- and low-risk subjects were calculated for different R/V cut-offs, VEGF cut-offs and combined VEGF and R/V ratio (Table 6). It was found that largest OR for R/V ratio was 7.6 at cut-off >3.4 ($P < 0.0001$), VEGF levels was 5.6 ($P < 0.001$) at cut-off <12.99 pg/mL, while

Table 6 VEGF level and R/V ratio in prediction of serious CVD risk scores

Marker	C. High-risk group		B. Low risk group		Odds ratio (95% CI)	*P* value
	≥11	<11	≥11	<11		
R/V ratio						
≤2	4 (40)	6 (60)	3 (25)	9 (75)	2.00 (0.32-12.33)	0.65
2.1-3.4	7 (63.6)	4 (36.4)	10 (31.3)	22 (68.7)	3.85 (0.91-16.22)	0.08
>3.4	26 (78.8)	7 (21.2)	24 (32.9)	49 (67.1)	7.58 (2.88-19.94)	<0.0001*
VEGF, pg/mL						
High (>18.58)	6 (46.2)	7 (53.8)	8 (25.8)	23 (74.2)	2.46 (0.63-9.55)	0.29
Intermediate (12.99-18.58)	11 (73.3)	4 (26.7)	7 (25.9)	20 (74.1)	4.49 (1.25-16.15)	0.004*
Low (<12.99)	20 (76.9)	6 (23.1)	22 (37.3)	37 (62.7)	5.61 (1.95-16.08)	<0.001*
Combined						
VEGF≥12.99 pg/mL, R/V ratio≤3.4	11 (52.4)	10 (47.6)	13 (32.5)	27 (67.5)	2.28 (0.77-6.74)	0.22
VEGF≤18.58 pg/mL, R/V ratio>3.4	26 (78.8)	7 (21.2)	24 (31.2)	53 (68.8)	8.20 (3.13-21.51)	<0.0001*

Data are *n* (%) unless otherwise stated; R/V ratio: sVEGFR-1-to-VEGF ratio; 95% CI: 95% Confidence interval; *Significant difference (*P*<0.05)

combining VEGF level with R/V ratio, i.e., VEGF levels ≤18.58 pg/mL and R/V ratio >3.4 yielded an OR of 8.2 (*P* << 0.0001) (Table 6).

DISCUSSION

Our study was in line with others for the observation of a broad range of VEGF concentrations varying from undetectable (<9 pg/mL) to 292.4 pg/mL. Different regulatory mechanisms might be responsible for this large variation. At low VEGF concentration region, i.e., below 12.99 pg/ml, sVEGFR-1 appeared to play a key role. Since levels of VEGF were modulated by a sVEGFR-1 dominant state suggested by large R/V ratios (i.e., varied from 3 to 100) far above the theoretical mass ratio of 2.22 required for a dimeric VEGF (45 kDa) trapping per sVEGFR-1 molecule (100 kDa).[21,22] At high VEGF region, VEGF upregulation rather than sVEGFR-1 down-regulation was likely to occur since sVEGFR-1 levels remained relatively unchanged over the entire range of VEGF concentrations.

Unlike previous studies mostly on Caucasian,[7-9] we did not obtain any significance elevated VEGF levels neither in low-risk nor high-risk subjects in relative to zero risk subjects, despite the observation of enhanced VEGF upregulation in subjects with CVD risk factors. It was likely that the amounts of VEGF produced might compensate for the amounts used for vascular repair leading to no net increase in absolute VEGF levels. In fact, elevated VEGF levels were observed in the high-risk group, but only at a borderline significant level. By these accounts, the enhanced VEGF upregulations in CVD risk groups might be correctively controlled within the physiologic boundary, not to permit aberrant angiogenesis. This was in contrast to our previous study in patients with head and neck cancer.[23] sVEGFR-1 was elevated along with the increase in VEGF levels. The elevated sVEGFR-1 was capable of normalizing the VEGF levels in low-grade tumor, but not in high-grade tumor. The dysregulated high VEGF levels were linked to tumor aggressiveness defined by a high-grade and advanced tumor stage.[23] In this study, neither sVEGFR-1 levels were increased in responding to VEGF elevation nor greatly decreased to allow VEGF elevation. By contrast, high VEGF levels appear to confer a protective effect to cardiovascular health and were in line with observations reported previously.[7-10]

Low physiologic VEGF levels for maintenance of vascular homeostasis are a well-established concept.[1] Too low the VEGF levels are adverse to vascular health as demonstrated by the side-effects such as hypertension and proteinuria caused by anti-VEGF antibody bevacizumab used for cancer treatment.[24] This is similar to the effects of systemic VEGF inhibition by sVEGFR-1 in preeclampsia.[15] sVEGFR-1 in modulation of VEGF levels in low concentration region could be described by a counter-balance mechanism between VEGF release and VEGF entrapment. For zero risk and low-risk categories, low VEGF levels were more likely to be dictated by the state of VEGF release, whereas the high-risk group by VEGF entrapment.

Our study highlighted a significance association of R/V ratio with CVD risk severity in subjects of high-risk, i.e., high R/V ratio with serious risk scores ≥11 and low R/V ratio with non-serious risk scores <11. Multivariate regression analysis revealed that total cholesterol levels were positively correlated to R/V ratios. This positive association implied the observation of high cholesterol levels at low VEGF levels or vice versa. Such association was reported elsewhere.[25,26] The possible role of VEGF in lipid metabolism has been recently addressed. VEGF was demonstrated to inhibit endothelial lipase leading to increased plasma HDL cholesterol in atherosclerotic prone mice.[6] Indeed, negative association between R/V ratios versus HDL cholesterol levels was also observed in this

series. Borderline positive associations were recognized for systolic blood pressure and waist circumference. These are factors that also contribute to EGAT scores. VEGF is known to regulate vascular tone and blood pressure through stimulating endothelial production of NO and prostacyclin.[1] Anti-VEGF in cancer treatment induces hypertension[24] in contrast to VEGF stimulation treatment of myocardial ischemia which causes hypotension.[27] Besides, microvascular rarefaction, a hallmark of hypertension, induced by anti-VEGF treatment is the consequence of deficient angiogenesis.[28] Altogether, this might partly explain the positive association of blood pressure with R/V ratio. The association of waist circumference, a measure of obesity, with R/V ratio was less clear. However, in an animal model at least, VEGF over-expression at early stage of obesity confers beneficial effect by browning the white adipose tissue leading to increase in energy expenditure and resistance to high fat diet-mediated metabolic insults. While inhibition of VEGF action results in weight gain and causes systemic insulin resistance. Paradoxically, anti-angiogenic action is protective in the context of obesity with preexisting adipose tissue dysfunction.[29]

In this study, high VEGF levels and low R/V ratio did not seem to be atherogenic, but rather protective to cardiovascular health based on the odds favoring EGAT scores ≥11 which was lower (odds = 1.1) than that (odds = 3.7) for low VEGF levels and high R/V ratio. In spite of the low VEGF levels, subjects with low CVD risks appeared to have a lower tendency to be observed with high-risk scores (32.1% chance) than those with high-risks (78.8% chance). This indicated that low physiologic VEGF concentrations were sufficient for reparative action in the low-risk group, but not in the high-risk group. VEGF repair insufficiency might point to the susceptibility in progression of atherosclerosis and development of the cardiovascular events. In fact, lower plasma VEGF and higher sVEGFR-1 levels were observed in patients with coronary artery disease (CAD) compared to control patients. Positive correlations between sVEGFR-1 level with both the risk of CAD and its angiographic severity were also reported.[30]

CONCLUSION

VEGF upregulations observed in hypercholesterolemic subjects with low and high CVD risks are correctively controlled within the physiologic boundary and regarded as reparative responses to the presence of CVD risk factors. R/V ratio, in correlating to cholesterol level ($P < 0.01$), systolic blood pressure and waist circumference ($P < 0.1$), was associated with CVD risk severity in subjects of high-risk category. Relatively low VEGF levels with high R/V are warning signs with respect to poor cardiovascular health in these subjects possibly due to VEGF insufficiency for the repair of vascular damages incurred by large CVD risk burden. Combined VEGF levels ≤18.58 pg/mL and R/V ratio >3.4 allows identification of subjects in high-risk category for aggressive treatment to prevent the progression of atherosclerosis and the occurrence of undesirable cardiovascular events.

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