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Original Article

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Associations Between Plasma Kinin B1 Receptor Levels and the Presence and Severity of Coronary Artery Disease

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Aim: Kinin B1 receptor (KB1R) was shown to be up-regulated in human carotid atherosclerotic lesions. Serum KB1R levels were also reported to be high in patients with stroke. However, KB1R deficiency increased atherosclerotic lesions. Therefore, the role of KB1R in atherosclerosis remains unclear. Moreover, no study has reported blood KB1R levels in patients with coronary artery disease (CAD).

Methods: We measured plasma KB1R levels in 375 patients undergoing coronary angiography. The severity of CAD was represented as the numbers of >50% stenotic vessels and segments and the severity score.

Results: CAD was found in 197 patients, of whom 89 had 1-vessel disease (1-VD), 62 had 2-VD, and 46 had 3-VD. Plasma KB1R levels were higher in 197 patients with CAD than in 178 without CAD (median 83.3 vs. 73.7 pg/mL, $p<0.01$). A stepwise increase in KB1R levels was found depending on the number of stenotic vessels: 77.1 in 1-VD, 87.8 in 2-VD, and 88.5 pg/mL in 3-VD ($p<0.025$). A high KB1R level (>90.0 pg/mL) was present in 30% of patients with CAD(-), 39% of 1-VD, 50% of 2-VD, and 48% of 3-VD ($p<0.025$). KB1R levels correlated with the number of stenotic segments and the severity score (r=0.14 and r=0.17, $p<0.01$). In multivariate analysis, KB1R levels were an independent factor associated with CAD. Odds ratio for CAD was 1.62 (95%CI=1.02-2.58) for high KB1R level >90.0 pg/mL.

Conclusion: Plasma KB1R levels in patients with CAD were high and were associated with the presence and severity of CAD independent of atherosclerotic risk factors.

Key words: Atherosclerosis, Coronary artery disease, Kinin B1 receptor

Introduction

Kinins are proinflammatory and vasoactive peptides that act through the activation of two G protein-coupled receptors, denoted as B1 and B2 receptors (KB1R and KB2R). Kinins are known to be increased in inflammation and tissue injury, eliciting vasodilation, increasing vascular permeability and recruiting inflammatory cells to the injury sites by activating KB1R and KB2R[1, 2]. However, KB1R is weakly expressed under healthy conditions but markedly increased during inflammation or tissue injury, while KB2R is constitutively expressed[1, 2].

KB1R expression is induced by proinflammatory cytokines including IL-1β and TNFα, and KB1R activation stimulates NFκB activation, leading to increased IL-1β and TNFα synthesis[1]. IL-1β increased KB1R mRNA expression and induced leukocyte rolling, adherence and emigration in the mesenteric venules of mice, while such IL-1β-induced leukocyte rolling, adherence and emigration were shown to be attenuated in KB1R-deficient ($KB1R^{-/-}$)

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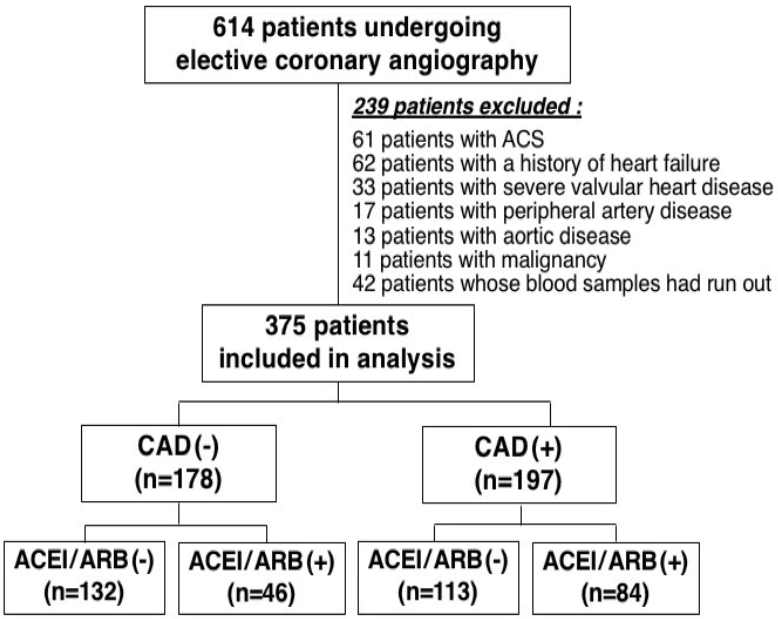

Fig. 1. Patients' selection flow chart

mice[3]. In KB1R$^{-/-}$ mice, the accumulation of leukocytes, especially neutrophils, into inflamed tissue was also shown to be markedly reduced, suggesting that KB1R plays an essential role in the initiation of inflammatory responses and that leukocyte recruitment to the inflamed tissue is mediated by kinins mainly via KB1R[4]. Because atherosclerosis is recognized to be a chronic inflammatory disease[5, 6] and because inflammatory responses, especially leukocyte recruitment, are implicated in the early stage of atherosclerosis[1], KB1R is expected to play an important role in the development of atherosclerosis.

In atherosclerotic tissues of the carotid, coronary and femoral arteries obtained at autopsy, intense immunolabelling for KB1R was demonstrated in endothelial cells, foamy macrophages, and inflammatory cells within plaques[7]. KB1R mRNA and protein expression were also shown to be upregulated in carotid atherosclerotic plaques obtained from patients who underwent carotid endarterectomy, thus suggesting that kinin-mediated inflammation contributes to the formation of atherosclerotic plaques[8, 9]. Using flow cytometry, plasma from patients with acute vasculitis was found to contain high levels of microvesicles that were positive for KB1R[10, 11]. Moreover, serum KB1R levels were reported to be high in patients with a history of stroke within 90 days[12]. However, blood KB1R levels in patients with coronary artery disease (CAD) have not been elucidated. To elucidate the associations between plasma KB1R levels and the presence and severity of CAD, we measured plasma KB1R levels in 375 patients undergoing coronary angiography.

Methods

Study Patients

In July 2008, we started our study to prospectively collect blood samples and clinical data from patients undergoing coronary angiography. Our study was approved by the institutional ethics committee of our hospital (R07-054/R15-056). After written informed consent was obtained, overnight-fasting blood samples were taken on the morning of the day when angiography was performed. A total of 614 consecutive patients who underwent elective coronary angiography for suspected CAD at Tokyo Medical Center from July 2008 to September 2016 participated in this study. Patients with a history of percutaneous coronary intervention or cardiac surgery were not included in this study. Of the 614 patients, 61 with acute coronary syndromes (ACS), such as acute myocardial infarction (MI) and unstable angina, 62 with a history of heart failure, and 33 with severe valvular heart disease were excluded from this study **(Fig. 1)**. Moreover, 17 patients with known peripheral artery disease, 13 with known aortic disease, 11 with malignancy, and 42 whose blood samples had run out were excluded. As a result, we measured plasma KB1R levels in 375 patients. Hypertension was defined as blood pressures of ≥ 140/90 mmHg or on drugs. Of the 375 study patients, 212 (57%) were taking anti-

hypertensive drugs, of whom 130 (35%) were taking angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs). Hyperlipidemia was defined as an LDL-cholesterol level of >140 mg/dL or on drugs, and 134 (36%) patients were taking statins. Diabetes mellitus (DM) (a fasting plasma glucose [FPG] level of ≥ 126 mg/dL or on treatment) was present in 95 (25%) patients, and 129 (34%) were smokers (≥ 10 pack-years).

Measurements of Plasma KB1R and C-Reactive Protein (CRP) Levels

Blood samples were collected in EDTA-containing tubes, and plasma was stored at −80℃. Plasma KB1R levels were measured using an enzyme-linked immunosorbent assay (ELISA) with a commercially available kit (Human B1 bradykinin receptor [BDKRB1] ELISA Kit; Cusabio, Houston, TX, USA) according to the manufacturer's instructions. According to the data supplied by the manufacturer, the detection range was from 25 to 1,600 pg/mL. The intra- and inter-assay coefficients of variation were <8% and <10%, respectively. Plasma high-sensitivity CRP (hsCRP) levels were also measured using a BNII nephelometer (Dade Behring, Tokyo, Japan).

Coronary Angiography

Angiograms were recorded on a cineangiogram system (Philips Electronics Japan, Tokyo, Japan). CAD was defined as at least one coronary artery having >50% luminal diameter stenosis on angiograms. The severity of CAD was represented as the numbers of >50% stenotic coronary vessels and >50% and >25% stenotic segments and the severity score of stenosis. The degree of coronary stenosis in each segment was scored from 0 to 4 points (0, ≤ 25%; 1, 26%-50%; 2, 51%-75%; 3, 76%-90%; 4, > 90% stenosis), and then the severity score was defined as the sum of scores of all segments. Coronary artery segments were defined as 29 segments according to the Coronary Artery Surgery Study (CASS) classification. All angiograms were evaluated by a single cardiologist (Y.M.) who had been blinded to the clinical and laboratory data.

Statistical Analysis

Differences between 2 groups were evaluated by unpaired *t*-test for parametric variables, by Mann-Whitney *U* test for nonparametric variables, and by chi-squared test for categorical variables. Differences among ≥ 3 groups were evaluated by an analysis of variance with Scheffe's test for parametric variables, by Kruskal-Wallis test for nonparametric variables, and by chi-squared test for categorical variables. Correlations between plasma KB1R levels and hsCRP levels or the severity of CAD were evaluated by Spearman's rank correlation test. To determine the cut-off point of KB1R levels for CAD, a receiver–operating characteristic (ROC) curve was created, and the optimal cut-off point was determined as the point where the Youden index was highest. Regarding the cut-off point of hsCRP levels, the previously reported cut-off point of 1.0 mg/L for CAD was used[13, 14]. A forward stepwise multiple logistic regression analysis was performed to determine the independent association between KB1R levels and CAD. All statistical analyses were carried out using the SPSS software package, ver. 25 (IBM, Tokyo, Japan). A p value of <0.05 was considered to be statistically significant. The results are presented as the mean ± SD or the median value.

Results

Among the 375 study patients, CAD was present in 197 patients (53%) (1-vessel disease [1-VD], n=89; 2-VD, n=62; 3-VD, n=46). Compared with 178 patients without CAD, 197 with CAD were older and had a male predominance; higher prevalence of hypertension, hyperlipidemia, and DM; and lower HDL-cholesterol and higher hsCRP (median 0.58 vs. 0.46 mg/L, p<0.005) levels **(Table 1)**. Notably, plasma KB1R levels were significantly higher in patients with CAD than in those without CAD (median 83.3 vs. 73.7 pg/mL, p<0.01) **(Fig. 2)**. A stepwise increase in KB1R levels was found depending on the number of >50% stenotic vessels: 73.7 in CAD(-), 77.1 in 1-VD, 87.8 in 2-VD, and 88.5 pg/mL in 3-VD and were highest in 3-VD (p<0.025) **(Fig. 2)**. The optimal cut-off point of KB1R levels for CAD was determined to be 90.0 pg/mL by the ROC curve and the Youden index. The area under ROC curve (AUC) for KB1R levels was 0.58 (95%CI=0.52–0.64) **(Fig. 3)**. A high KB1R level (>90.0 pg/mL) was present in 30% of patients with CAD(-), 39% of 1-VD, 50% of 2-VD, and 48% of 3-VD, respectively (p<0.025) **(Table 1)**. Furthermore, KB1R levels significantly correlated with the number of >50% stenotic segments and the severity score (rs=0.14 and rs=0.17, respectively; p<0.01) **(Fig. 4)** and correlated with age (rs=0.14), HbA1c (rs=0.19) and hsCRP levels (rs=0.12) (p<0.01) **(Table 2)**.

Since ACEI or ARB treatment may affect plasma KB1R levels, the 375 study patients were divided into 2 groups by whether or not they were taking ACEI or ARB. Of the 375 patients, 130 (35%) were taking ACEI or ARB, including 11 and 119 taking ACEI

Table 1. Clinical characteristics and plasma KB1R levels of patients with and without CAD

	ALL (n = 375)	CAD(-) (n = 178)	p value CAD(-) vs. CAD	CAD (n = 197)	1-VD (n = 89)	2-VD (n = 62)	3-VD (n = 46)	p value among 4 groups
Age (years)	67 ± 11	64 ± 12	< 0.001	69 ± 10	68 ± 10	69 ± 10	72 ± 8	< 0.001
Gender (male)	254 (68%)	108 (61%)	0.008	146 (74%)	65 (73%)	43 (69%)	38 (83%)	0.019
BMI (kg/m^2)	24.0 ± 4.0	24.1 ± 4.5	0.263	24.0 ± 3.4	24.4 ± 3.7	23.9 ± 3.0	23.3 ± 3.3	0.649
Hypertension	258 (69%)	106 (60%)	< 0.001	152 (77%)	65 (73%)	47 (76%)	40 (87%)	< 0.001
SBP (mmHg)	132 ± 19	128 ± 21	0.073	133 ± 18	132 ± 17	136 ± 21	131 ± 17	0.082
Anti-hypertensive drugs	212 (57%)	81 (46%)	< 0.001	131 (66%)	55 (62%)	40 (65%)	36 (78%)	< 0.001
ACEI/ARB	130 (35%)	46 (25%)	< 0.001	84 (43%)	34 (38%)	28 (45%)	22 (48%)	< 0.001
DM	95 (25%)	28 (16%)	< 0.001	67 (34%)	25 (28%)	24 (39%)	18 (39%)	< 0.001
HbA1c (%)	6.1 ± 0.8	6.0 ± 0.7	0.001	6.3 ± 0.9	6.1 ± 0.8	6.4 ± 0.9	6.3 ± 1.0	0.001
Smoking	129 (34%)	52 (29%)	0.057	77 (39%)	38 (43%)	24 (39%)	15 (33%)	0.143
Hyperlipidemia	186 (50%)	73 (41%)	< 0.005	113 (57%)	50 (56%)	36 (58%)	27 (59%)	0.017
Statin	134 (36%)	48 (27%)	< 0.005	86 (44%)	38 (43%)	27 (44%)	21 (46%)	0.009
LDL-C (mg/dL)	113 ± 30	113 ± 27	0.613	114 ± 32	111 ± 34	116 ± 32	118 ± 29	0.478
HDL-C (mg/dL)	55 ± 14	59 ± 15	< 0.001	52 ± 13	55 ± 14	51 ± 11	48 ± 13	< 0.001
hsCRP levels (mg/L)	0.54 [0.28, 1.29]	0.46 [0.22, 1.12]	0.016	0.58 [0.32, 1.32]	0.57 [0.30, 1.30]	0.57 [0.31, 1.11]	0.70 [0.42, 1.80]	0.040
> 1.0 mg/L	111 (30%)	49 (28%)	0.471	62 (31%)	25 (28%)	19 (31%)	18 (39%)	0.474
KB1R levels (pg/mL)	78.9 [59.1, 102.3]	73.7 [54.8, 94.8]	0.006	83.3 [62.1, 106.4]	77.1 [60.4, 102.7]	87.8 [65.3, 115.7]	88.5 [71.8, 107.9]	0.021
> 90 pg/mL	143 (38%)	54 (30%)	0.006	88 (45%)	35 (39%)	31 (50%)	22 (48%)	0.017

Data represent the mean ± SD or the number (%) of patients, with the exception of hsCRP and KB1R levels which are presented as the median value and interquartile range.
BMI = body mass index; SBP = systolic blood pressure; DM = diabetes mellitus;
LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol.

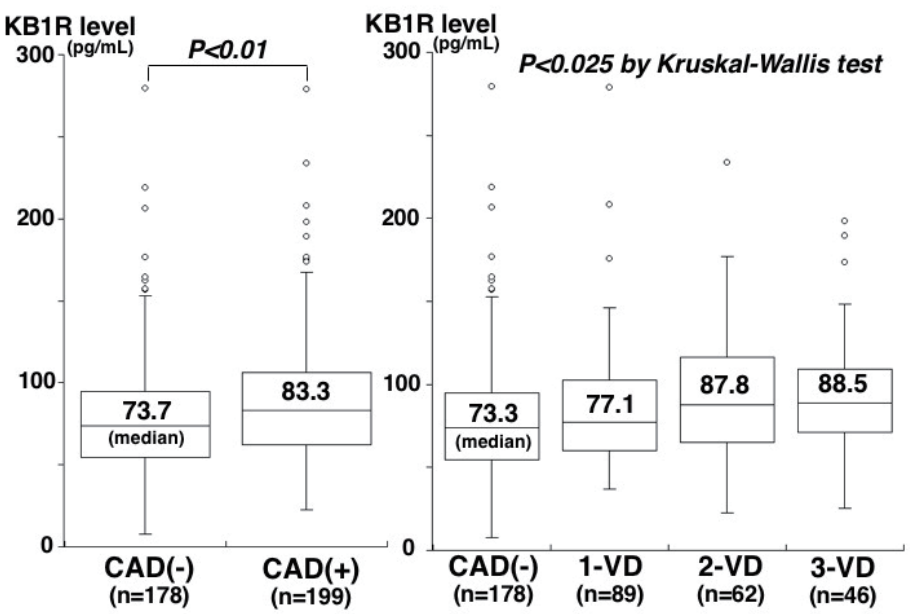

Fig. 2. Plasma KB1R levels and the presence of CAD or the number of > 50% stenotic coronary vessels

Plasma KB1R levels were significantly higher in CAD than in CAD(-) (p < 0.01) (left). Moreover, KB1R levels in 4 groups of CAD(-), 1-VD, 2-VD, and 3-VD were 73.3, 77.1, 87.8, and 88.5 pg/mL, respectively, and were highest in 3-VD (p < 0.025 by Kruskal-Wallis test) (right). The central line represents the median, and the box represents the 25th to 75th percentiles. The whiskers represent the lowest and highest value in the 25th percentile minus 1.5 IQR and 75th percentile plus 1.5 IQR, respectively.

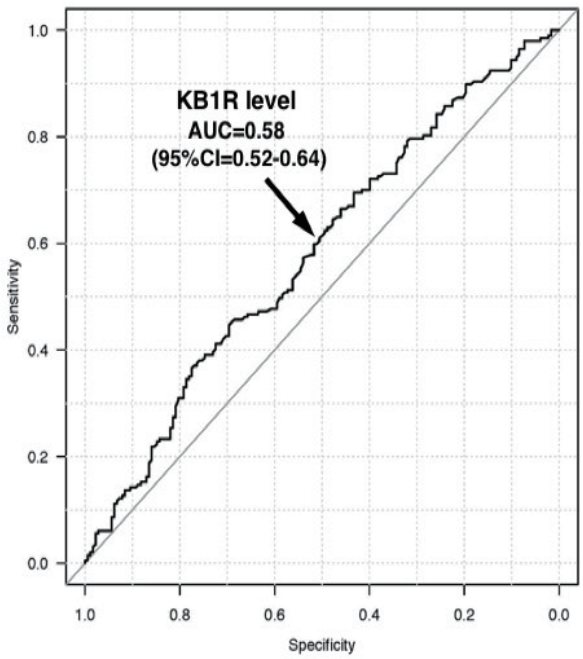

Fig. 3. ROC curve of KB1R levels for the diagnostic ability of CAD

Regarding the diagnostic ability of KB1R levels to predict CAD, the area under ROC curve (AUC) for KB1R levels was 0.58 (95%CI = 0.52–0.64).

and ARB, respectively. Although patients taking ACEI/ARB were older (70 ± 9 vs. 65 ± 12 yrs) and more often had CAD (65% vs. 46%) than those not taking ACEI/ARB ($p < 0.001$), no significant difference was found in KB1R levels between patients taking and not taking ACEI/ARB (80.1 vs. 77.0 pg/mL) **(Table 3)**. Notably, among the 245 patients not taking ACEI/ARB, KB1R levels were much higher in patients with CAD than in those without CAD (86.1 vs. 73.6 pg/mL, $p < 0.01$) **(Table 3)** and correlated better with the number of stenotic segments and the severity score (rs = 0.18 and rs = 0.22, $p < 0.005$). However, among the 130 patients taking ACEI/ARB, KB1R levels did not differ between patients with and without CAD (80.1 vs. 79.0 pg/mL) **(Table 3)** and did not correlate with the number of stenotic segments or the severity score (p = NS).

To elucidate the independent association between KB1R levels and CAD, variables (age, gender, hypertension, ACEI/ARB use, hyperlipidemia, statin use, DM, smoking, and hsCRP and KB1R levels) were entered into a multiple logistic regression model. KB1R levels were a significant factor associated with CAD. The odds ratio for CAD was 1.62 (95%CI = 1.02-2.58) for the high KB1R level of > 90.0 pg/mL ($p < 0.05$) **(Table 4)**. Moreover, KB1R levels were a

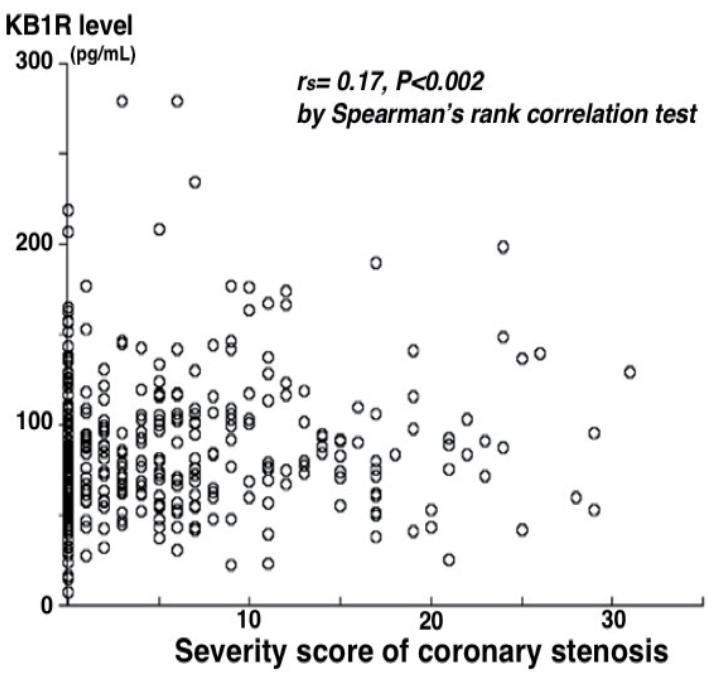

Fig. 4. Correlation between plasma KB1R level and the severity score of stenosis

Plasma KB1R levels significantly correlated with the severity score of coronary stenosis (rs = 0.17, $p < 0.002$).

Table 2. Correlations between plasma KB1R levels and the severity of CAD as well as atherosclerotic risk factors

	rs*	*p* value
The severity of CAD		
The number of >50% stenotic segments	0.14	0.007
The number of >25% stenotic segments	0.16	0.002
The severity score of stenosis	0.17	0.001
Atherosclerotic risk factors		
Age (years)	0.14	0.006
BMI (kg/m^2)	0.02	0.647
Systolic blood pressure (mmHg)	0.02	0.660
Diastolic blood pressure (mmHg)	-0.01	0.790
LDL-cholesterol level (mg/dL)	-0.01	0.811
HDL-cholesterol level (mg/dL)	-0.08	0.136
FPG (mg/dL)	0.09	0.083
HbA1c (%)	0.19	0.001
hsCRP level (mg/L)	0.12	0.017

*By Spearman`s rank correlation test.

Table 3. Clinical characteristics of patients taking ACEI/ARB and those not taking ACEI/ARB

	ACEI/ARB(-) (*n* = 245)	CAD(-) (*n* = 132)	CAD(-) vs. CAD	CAD (*n* = 113)	ACEI/ARB (-) vs. (+)	ACEI/ARB(+) (*n* = 130)	CAD(-) (*n* = 46)	CAD(-) vs. CAD	CAD (*n* = 84)
Age (years)	65 ± 12	63 ± 12	< 0.001	68 ± 10	< 0.001	70 ± 9	70 ± 9	0.415	71 ± 9
Gender (male)	162 (66%)	77 (58%)	0.008	85 (75%)	0.424	92 (71%)	31 (67%)	0.671	61 (73%)
Hypertension	128 (52%)	60 (45%)	0.030	68 (60%)	< 0.001	130 (100%)	46 (100%)		84 (100%)
SBP (mmHg)	131 ± 21	128 ± 22	0.014	135 ± 19	0.545	133 ± 17	134 ± 16	0.401	132 ± 17
On drugs	82 (33%)	35 (27%)	0.018	47 (42%)	< 0.001	130 (100%)	46 (100%)		84 (100%)
ACEI/ARB	0 (0%)	0 (0%)		0 (0%)		130 (100%)	46 (100%)		84 (100%)
DM	53 (22%)	17 (13%)	< 0.001	36 (32%)	0.033	42 (32%)	11 (24%)	0.187	31 (37%)
HbA1c (%)	6.1 ± 0.8	5.9 ± 0.7	0.002	6.3 ± 0.9	0.120	6.2 ± 0.9	6.1 ± 0.9	0.239	6.3 ± 0.9
Smoking	83 (34%)	38 (29%)	0.092	45 (40%)	0.858	46 (35%)	14 (30%)	0.495	32 (38%)
Hyperlipidemia	114 (47%)	48 (36%)	< 0.001	66 (58%)	0.128	72 (55%)	25 (54%)	0.975	47 (56%)
Statin	73 (30%)	28 (21%)	0.002	45 (40%)	0.001	61 (47%)	20 (43%)	0.690	41 (49%)
LDL-C (mg/dL)	116 ± 31	113 ± 27	0.118	119 ± 34	0.030	109 ± 29	111 ± 29	0.467	107 ± 28
HDL-C (mg/dL)	56 ± 15	60 ± 15	< 0.001	52 ± 14	0.042	53 ± 12	54 ± 12	0.578	53 ± 12
hsCRP levels (mg/L)	0.52 [0.27, 1.29]	0.43 [0.21, 1.21]	0.013	0.60 [0.35, 1.31]	0.607	0.55 [0.30, 1.23]	0.51 [0.30, 1.35]	0.525	0.56 [0.30, 1.35]
KB1R levels (pg/mL)	77.0 [57.0, 101.5]	73.6 [53.3, 91.8]	0.008	86.1 [59.8, 108.9]	0.297	80.1 [62.7, 103.1]	79.0 [58.6, 106.0]	0.483	80.1 [65.7, 102.4]
>90 pg/mL	90 (37%)	36 (27%)	0.001	54 (48%)	0.611	52 (40%)	18 (39%)	0.975	34 (40%)
CAD	113 (46%)	0 (0%)		113 (100%)	< 0.001	84 (65%)	0 (0%)		84 (100%)

Data represent the mean ± SD or the number (%) of patients, with the exception of hsCRP and KB1R levels which are presented as the median value and interquartile range.
SBP = systolic blood pressure; DM = diabetes mellitus; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol.

significant factor associated with multi-vessel disease (2-VD or 3-VD). The odds ratio for multi-vessel disease was 1.73 (95%CI = 1.07-2.73) for the high KB1R level of >90.0 pg/mL ($p < 0.05$) (Table 4).

Discussion

In the present study, plasma KB1R levels were significantly higher in patients with CAD than in those without CAD, and they positively correlated

Table 4. Factors associated with CAD and multi-vessel disease (Multiple logistic regression analysis of the 375 study patients)

	Odds ratio (95% CI)	*p* value
CAD		
Age (per 10 years increase)	1.58 (1.28-1.95)	<0.001
Male	2.76 (1.65-4.60)	<0.001
Hyperlipidemia	2.15 (1.34-3.42)	0.001
Diabetes mellitus	1.88 (1.10-3.21)	0.021
KB1R level (>90.0 pg/mL)	1.62 (1.02-2.58)	0.042
Multi-vessel disease (2-VD or 3-VD)		
Age (per 10 years increase)	1.51 (1.18-1.93)	0.001
Male	1.80 (1.05-3.09)	0.033
Hypertension	1.82 (1.03-3.20)	0.039
Diabetes mellitus	1.98 (1.18-3.32)	0.010
KB1R level (>90.0 pg/mL)	1.73 (1.07-2.83)	0.027

The dependent variable was the presence of CAD and multi-vessel disease.
The analysis included age, gender, hypertension, ACEI/ARB use, hyperlipidemia, statin use, diabetes mellitus, smoking, and hsCRP (>1.0mg/L) and KB1R (>90.0 pg/mL) levels.

with the severity of CAD, defined by the numbers of stenotic vessels and segments and the severity score. However, the correlation between KB1R levels and the severity of CAD was blunted by taking ACEI/ARB. High plasma KB1R levels were found to be a significant factor for CAD, especially multi-vessel disease, independent of atherosclerotic risk factors, ACEI/ARB use and hsCRP levels.

Kinins are proinflammatory peptides that increase in inflammation, thereby eliciting vasodilation, increasing vascular permeability and recruiting inflammatory cells mainly by activating KB1R[1, 2]. KB1R activation increases IL-1β and TNFα synthesis and plays a pivotal role in the process of inflammation[1, 4]. Atherosclerotic disease, such as CAD, is recognized as an inflammatory diasease[5, 6]. In atherosclerotic plaques obtained at autopsy, intense immunolabelling for KB1R was demonstrated in endothelial cells, macrophages and inflammatory cells within plaques[7]. KB1R mRNA expression was also reported to be upregulated in carotid atherosclerotic plaques obtained from endarterectomy[8, 9]. In ApoE$^{-/-}$ mice fed a high-fat diet, KB1R mRNA expression was shown to be markedly upregulated in atherosclerotic plaques, especially in the region with low shear stress[8]. These findings thus suggest that KB1R may play an important role in the progression of atherosclerosis as well as inflammation.

Kinins are also vasoactive and cause vasodilatation. KB1R-mediated vasodilation was reported to be endothelium-dependent in rat coronary and rabbit carotid arteries[2]. KB1R activation was shown to increase NO production in endothelial cells[2]. In cultured vascular smooth muscle cells (VSMCs), KB1R antagonist abolished the inhibitory effect of kallikrein on VSMC proliferation, and KB1R agonist inhibited platelet-derived growth factor-stimulated VSMC proliferation[15]. In rat carotid arteries after balloon angioplasty, KB1R mRNA expression was increased, and local delivery of adenovirus carrying kallikrein gene at the injured site reduced neointima formation, suggesting that KB1R may play a protective role against neointima formation after angioplasty[15]. In ApoE$^{-/-}$ mice, increased KB1R mRNA expression was found with a Western-type diet, and atherosclerotic lesions were shown to be increased in ApoE$^{-/-}$KB1$^{-/-}$ mice[16]. These findings thus suggest that KB1R may play a protective role against the progression of atherosclerosis. However, a further study is needed to elucidate the precise role of KB1R in atherosclerosis.

Using flow cytometry, patients with vasculitis were reported to have high levels of leukocyte-derived microvesicles bearing KB1R in plasma, suggesting that KB1R are secreted as microvesicles from cells into plasma[11]. The same group also showed that patients with acute vasculitis had high levels of endothelial KB1R-positive microvesicles in plasma, and KB1R-positive microvesicles from transfected embryonic cells induced neutrophil chemotaxis[10]. Regarding blood KB1R levels in patients with atherosclerotic disease, Liu *et al.*[12] measured KB1R levels in serum and carotid plaque specimens from 80 patients with stroke and 17 asymptomatic patients undergoing carotid endarterectomy. Serum KB1R levels were high in patients with stroke and correlated with histological

KB1R staining of carotid plaque specimens[12]. However, no study has reported blood KB1R levels in patients with CAD. Our study measured plasma KB1R levels in 197 patients with CAD and 178 without CAD. We reported that plasma KB1R levels were high in patients with CAD, and they correlated with the severity of CAD and hsCRP levels. KB1R levels were a significant factor associated with CAD, especially multi-vessel disease, independent of atherosclerotic risk factors and hsCRP levels. Our results thus suggest that KB1R may play a role in the progression of CAD and that plasma KB1R levels may reflect the severity of CAD and inflammation. However, the correlation between KB1R levels and the severity of CAD was significant but weak. Hence, plasma KB1R levels may reflect not only the severity of coronary atherosclerosis but also atherosclerosis in other vascular beds. Since we did not measure KB1R levels in coronary sinus, our study did not provide any information about the main source of plasma lKB1R in patients with CAD. Moreover, KB1R activation was shown to increase IL-1β and TNFα synthesis[1, 4], but we did not measure plasma IL-1β and TNFα levels. Therefore, further studies are needed to elucidate the major source of KB1R and the role of high plasma KB1R levels in the progression of CAD.

Regarding the effect of ACEI and ARB on vascular KB1R, ACEI treatment was shown to enhance vascular KB1R mRNA expression in rat[17], but others found that ACEI did not enhance KB1R in isolated aorta or umbilical veins[18]. In rat VSMCs, angiotensin II enhanced KB1R expression, and this enhanced KB1R expression was normalized by ARB[19]. The effects of ACEI and ARB on vascular KB1R remain unclear. In humans, KB1R expression on peripheral monocytes was shown to be upregulated in 17 patients with hypertension, and antihypertensive treatment, including ACEI and ARB, decreased KBR1 expression[20]. In our study, 35% of patients were taking ACEI or ARB. Between patients taking and not taking ACEI/ARB, no significant difference was found in KB1R levels. However, among patients not taking ACEI/ARB, KB1R levels were much higher in patients with CAD and correlated better with the severity of CAD. In contrast, among patients taking ACEI/ARB, KB1R levels did not differ between patients with and without CAD and did not correlate with the severity of CAD. These findings suggest that ACEI/ARB treatment may have some effect on plasma KB1R levels in patients with CAD.

In rat models, serum kinin levels were shown to increase with age, whereas the responsiveness of target cells to kinins decreased with age[21]. However, no study has reported the association between blood KB1R levels and age. As shown in **Table 2**, KB1R levels as well as age stepwisely increased depending on the number of stenotic vessels and were highest in 3-VD. Moreover, KB1R levels significantly correlated with age ($r=0.14$). However, both KB1R levels and age were found to be independent factors associated with CAD as shown in **Table 4**.

Our study has several limitations. First, in our study, angiography was used to evaluate coronary atherosclerosis. Angiography cannot visualize plaques and only shows lumen characteristics. However, intravascular ultrasound (IVUS) or optical coherence tomography (OCT) which can visualize plaques, were not always performed in our patients. Second, in a rat model of MI, myocardial KB1R expression was shown to be upregulated after MI[22]. However, no patients with ACS, such as acute MI, who usually need emergent coronary angiography, were included in our study. A further study in patients with ACS will be needed to clarify the potential role of KB1R in this syndrome. Finally, our study was cross-sectional in nature and could not establish causality, since it only depicted some associations and proposed some hypotheses.

Conclusions

Plasma KB1R levels in patients with CAD were found to be high and to be associated with the presence and severity of CAD independent of atherosclerotic risk factors and ACEI/ARB use. Our results suggest that KB1R may play a role in the progression of CAD and that plasma KB1R levels may reflect the severity of CAD.

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Conflict of Interest

Our study has no conflicts of interest to disclose.

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