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Relationship of β2-Microglobulin to Arterial Stiffness in Japanese Subjects
(日本人対象者における血中β2ミクログロブリンと動脈硬度との関係)

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Short running head: β_2-microglobulin and arterial stiffness

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Abstract

β_2-microglobulin (β2m) is related to inflammatory diseases, but there have been few reports of a relationship between β2m and atherosclerosis. We have examined the influence of β2m on brachial-ankle pulse wave velocity (baPWV) to clarify whether it is related to arterial stiffness. baPWV, β2m, C-reactive protein (CRP), and conventional risk factors were measured in 614 males and 158 females. The adjusted means of baPWV were compared with the quartiles of β2m, and significant differences in baPWV were observed across the quartiles of β2m (P=0.037). After being adjusted for potential confounders, quartile 4 of β2m, quartile 4 of CRP, and the combination of high β2m plus high CRP were significantly associated with a high value of PWV (quartile 4 of β2m: OR 2.53, 95%CI, 1.31-4.89; quartile 4 of CRP: OR 2.27, 95%CI, 1.18-4.34; high β2m plus high CRP: OR 5.60, 95%CI, 2.38-13.2). These results suggest that β2m is associated with an increase of arterial stiffness. Further studies are needed to clarify whether β2m is related to atherosclerotic diseases, and whether the combination of β2m and CRP measurement is a useful predictor for the development of atherosclerosis.

Key Words; β_2-microglobulin; C-reactive protein; glomerular filtration rate; pulse wave velocity; arterial stiffness

1. **Introduction**

Atherosclerosis is now generally accepted to be an inflammatory disorder in the arterial wall (*1*), and the C-reactive protein (CRP) level is a strong predictor of cardiovascular events (*2-5*). Meanwhile, it has been reported that β_2-microglobulin (β2m) is related to inflammatory diseases (*6*) and β2m is now widely used in evaluation of many clinical conditions, such as dialysis-related amyloidosis (*7*), HIV disease (*8*), myeloma (*9*), leukemia (*10*), and collagen disease (*11*), for the estimation of the glomerular filtration rate (GFR) (*12*), and so on. However, there have been few reports of a relationship between β2m and atherosclerosis.

Pulse wave velocity (PWV) in known to be an indicator of arterial stiffness (*13, 14*), and there have been many reports on PWV and the development of atherosclerotic diseases (*15-17*). A simple noninvasive method for automatic measurement of brachial-ankle PWV (baPWV) has recently been developed. The technical simplicity and short sampling time of the new method make it more feasible for screening a large population than previous methods such as carotid-femoral PWV.

In this study, we have investigated the influences of β2m on arterial stiffness to clarify whether β2m is related to early stage atherosclerosis.

2. Methods

2.1 Subjects

The subjects were local government employees (8229 men and 2194 women) aged 35 years or more who had their annual health checkup during the period from April 2003 through March 2004. We used a self-administered questionnaire including items on clinical history, family history, smoking, alcohol consumption, educational status, frequency of exercise, menopausal status, and hormone-replacement therapy. The questionnaire was distributed to the subjects in advance of their annual health checkup, and was collected at the checkup. Answers to the questionnaire and written informed consent to view health checkup data were obtained from 3907 men and 1044 women (response rate: men 47.5%, women 47.6%). A total of 685 subjects (495 men, 190 women) were excluded for the following reasons: past history of coronary disease or stroke (n=136; 124 men, 12 women), low ankle/brachial pressure index (<0.9, n= 12; 11 men, 1 woman), PWV not measured (n= 600; 416 men, 184 women), or blood samples not measured (n=3; 3 women). Among this original study group consisting of 3412 male and 854 female subjects, we analyzed 614 male and 158 female subjects who requested optional examinations, including measurement of the serum β_2-microglobulin level.

This study was conducted with all the subjects' written informed consent and approved by the institutional ethical board for epidemiological studies of Hokkaido University Graduate

School of Medicine.

2.2 Data collection

Subjects were classified as either current smokers or nonsmokers, with the latter group including both never- and ex-smokers. Drinkers were defined as those who consumed alcohol once a week or more. With regard to leisure-time exercise (with perspiration), subjects were categorized as exercising "rarely or never", or "≥1 per week". Finally, two groups were used to categorize subjects according to their educational attainment: "high school education or less" and "more than high school education."

Anthropometric measures (height, body weight, and waist and hip circumferences) were recorded by a standardized protocol. The body mass index (BMI) was calculated as weight (kg)/height (m^2).

Blood samples were drawn from the antecubital vein of the seated subject with minimal tourniquet use after a 12-h fast. Specimens were collected in siliconized glass vacuum tubes containing sodium fluoride for blood glucose, and no additives for serum.

Total cholesterol (TC) levels were measured by an enzymatic method (Wako, Osaka, Japan). The triglyceride (TG) levels were measured by an enzymatic method (Daiichi Pure Chemicals, Tokyo, Japan), high density lipoprotein cholesterol (HDL-C) level by a direct method (Daiichi

Pure Chemicals), uric acid (UA) by an enzymatic method (Daiichi Pure Chemicals), creatinine by an enzymatic method (KANTO KAGAKU, Tokyo, Japan), blood glucose levels by an amperometric method (ARKRAY, Kyoto, Japan), and β2m by a latex immunoassay (Eiken Chemical, Tokyo, Japan).

The CRP levels were measured by nephelometry, with a latex particle-enhanced immunoassay (N Latex CRP II; Dade Behring, Tokyo, Japan). The assay could detect 0.004 mg/dL of CRP. Undetectable CRP values were recorded as 0.002 mg/dL.

All blood variables except for CRP were measured at Daiichi Clinical Laboratories, Inc. (Sapporo, Japan), a commercial hematology laboratory, where the measurements of TC and HDL cholesterol were all standardized by the Lipid Standardized Program of the Centers for the Disease Control and Prevention (Atlanta, GA). CRP was measured at Mitsubishi Kagaku Bio-Clinical Laboratories, Inc. (Tokyo, Japan), a commercial hematology laboratory.

The estimated GFR was calculated using the Cockcroft-Gault formula (*18*) adjusted for body surface area (BSA) as follows:

Cockcroft-Gault = (140 – age)/Scr * weight/72 * 1.73/BSA,

where Scr is the serum creatinine concentration (mg/dL) and weight is measured in kilograms. In females, a correlation factor (0.85) was used. BSA was estimated using the DuBois formula (*19*).

baPWV was measured using a volume-plethysmographic apparatus (Form PWV/AVI; model

BP-203RPEII, Colin Co., Komaki, Japan). Details about this instrument and its use have been described elsewhere (*20-23*). The subjects were examined in the supine position. This device records the phonocardiogram, electrocardiogram, and volume pulse form and arterial blood pressure at both the left and right brachia and ankles.

Blood pressure, heart rate (HR), and the ankle brachial index (ABI) were measured using the pulse-wave velocimeter at the same time that PWV was measured. ABI was the ratio of ankle systolic blood pressure (SBP) to brachial SBP, and the right and left ABIs were measured simultaneously. In all the studies, baPWV was obtained after an at least 5-min rest..

2.3. Statistical analysis

The subjects were categorized according to quartiles of β2m values. The data are presented as the mean $\pm$ SD, the median (and interquartile range) for variables with a skewed distribution, or percentages, and analysis of variance (ANOVA), the Kruskal-Wallis test, or the χ^2-test was used to compare data for these groups. The adjusted mean of PWV was compared among the quartiles of β2m, with analysis of covariance (ANCOVA) with age, gender, BMI, SBP, HR, TC, HDL-C, blood glucose, log TG, UA, estimated GFR, log CRP, smoking status (smoker/nonsmoker), alcohol consumption (drinker/rarely or never), frequency of exercise ($\geq$1/week/rarely or never), educational attainment (high school education or less/more than high

school education), medication for hypertension, medication for hyperlipidemia, and medication for diabetes. Logistic regression analyses were used to evaluate whether quartiles of β2m and CRP were related to a high value of PWV (tertile three). As the next step, combined variables (low β2m (≤1.7 mg/dL) plus low CRP (≤0.080 mg/dL); low β2m (≤1.7 mg/dL) plus high CRP (≥0.081 mg/dL); high β2m (≥1.8 mg/dL) plus low CRP (≤0.080 mg/dL); and high β2m (≥1.8 mg/dL) plus high CRP (≥0.081 mg/dL)) were created, and their association with the high value of PWV was evaluated. Odds ratios (OR) and 95% confidence intervals (95%CI) were calculated before and after adjustment for potential confounders. All of the above-mentioned potential confounders except log CRP were included in the multivariate logistic regression models as independent variables. To avoid multicollinearity, DBP was not included in these models.

P-values <0.05 were considered to be statistically significant. All analyses were conducted using the SPSS software package Version 12 for Windows (SPSS Inc., Chicago, IL).

3. Results

Characteristics of the groups in the β2m category are shown in Table 1. Gender, age, SBP, DBP, HDL-C, UA, CRP, estimated GFR, medication for hypertension, and PWV were significantly different in the group in the β2m category. Also, in crude regression analysis, β2m was significantly associated with age (Pearson's coefficient: 0.15; $P<0.0001$).

Next, the adjusted means of baPWV were compared with the quartiles of β2m (Figure). Significant differences in baPWV were observed across the quartiles of β2m ($P=0.037$; P for trend=0.069).

In unadjusted logistic regression analysis (Table 2), quartile 4 of β2m (reference quartile 1 of β2m), quartiles 2, 3 and 4 of CRP (reference quartile 1 of CRP), and the combinations of "high β2m plus high CRP" and "high β2m plus high CRP" (reference: low β2m plus low CRP) were significantly associated with a high value of PWV. After being adjusted for age, BMI, SBP, heart rate, TC, HDL-C, log TG, UA, smoking status, alcohol consumption, frequency of exercise, educational attainment, medication for hypertension, medication for hyperlipidemia, and medication for diabetes, the associations with quartiles 2 and 3 of CRP disappeared, but quartile 4 of β2m, quartile 4 of CRP, and the combinations of "high β2m plus high CRP" and "high β2m plus high CRP" were significantly associated with a high value of PWV (quartile 4 of β2m: OR 2.53, 95%CI, 1.31-4.89; quartile 4 of CRP: OR 2.27, 95%CI, 1.18-4.34; high β2m plus high CRP:

OR 1.86, 95%CI, 1.18-2.95; high β2m plus high CRP: OR 5.60, 95%CI, 2.38-13.2). These results were not substantially affected even if we used DBP as an independent variable instead of SBP.

4. Discussion

A significant relationship between CRP and PWV has been reported (*24, 25*), but, to the best of our knowledge, this is the first study to clarify the significant association between β2m and PWV.

The end-stage renal disease (ESRD) population has increased arterial stiffness, and the PWV level is a strong independent predictor of all-cause and cardiovascular mortality (*26*). It has been reported that elevated PWV is significantly associated with reduced GFR (*27*), and that β2m is a marker of GFR (*12*). Thus, GFR is a strong confounder in analyses of the association between β2m and arterial stiffness, and our analyses were adjusted for estimated GFR. We speculate therefore that the inflammatory factor of β2m is related to arterial stiffness.

In addition, we showed that the combination of high β2m plus high CRP was significantly related to a high value of PWV with a higher OR (5.60). Since, in some inflammatory disorders, β2m is regarded as necessary for, or as a discriminative marker of, inflammation (*12-15, 28-30*), this might indicate the inflammation that can not fully be estimated using only CRP.

β2m has been identified as the light chain common to the HLA-A, -B, and -C major histocompatibility complex antigens, and is expressed on the surface of virtually all normal nucleated cells. The surfaces of lymphocytes and monocytes are particularly rich in β2m, and lymphocytic synthesis and expression are further augmented by stimulation with mitogens or with

interferons (*31*). Viral infections such as infectious mononucleosis, cytomegalovirus (CMV), and influenza A are associated with pronounced increases in the serum β2m concentration (*32*). CMV-seropositive individuals have endothelial dysfunction and impaired responses to nitric oxide (*33*). Thus, chronic persistent viral infections may be related to the β2m concentration and arterial stiffness.

Meanwhile, it has been reported that β2m inhibits the growth of, and induces apoptosis or necrosis in tumor cells such as leukemia and myeloma cells (*34, 35*). Xie et al. suggested that it would be of interest to examine whether β2m at high concentrations could also induce apoptosis or necrosis in normal cells, including endothelial cells and fibroblasts, because apoptotic or necrotic bodies and released enzymes and cytokines could act as chemoattractants for mononuclear cells, and they speculated that β2m may be a potential initiator of the inflammatory response (*36*).

Diets and exercise inducing weight loss lower the CRP level (*37, 38*), and smoking and alcohol consumption are related to the CRP level (*39, 40*). Exercise induces an increase in the rate of β2m excretion into the urine (*41*). But the relationships between β2m, diet, and lifestyle have not been fully investigated. It is therefore necessary to elucidate the influences of diet and lifestyle on β2m.

The present study has several limitations. First, this study could not identify a causal role for

β2m in the pathogenesis of arterial stiffness. Second, we measured only estimated GFR, using the Cockcroft-Gault formula. Since the direct assessment of GFR is rather complicated, we believe that estimated GFR is sufficient for a large population study. Third, only 4951 of the 10423 subjects that participated in the original study completed the questionnaire required for participation in this study. Since all of the present subjects requested optional examinations at their annual health checkup, they might have been more worried about their health than the general population. The age of subjects who requested the optional examinations was significantly higher than that of subjects who did not request the optional examinations (50 years vs 48 years). And the baPWV of subjects who requested the optional examinations was higher than that of subjects who did not request the optional examinations (1351 cm/s vs 1343 cm/s), but the difference was not significant. Thus, this study's subjects had slightly higher age and baPWV, but because the analyses were adjusted for many possible confounders, we believe that β2m was actually related to the high value of PWV. Fourth, since the subjects requested the optional examinations at their annual health checkup, they might have been more worried about their health than the general population. But the subjects who had past histories of coronary disease, stroke, or low ankle/brachial pressure were excluded, and the analyses were adjusted for many possible confounders. Fifth, conventional methods of measurement of PWV are carotid femoral and heart-ankle PWV, and the significance of baPWV for the prediction of cardiovascular events

has not been published. The carotid femoral and heart-ankle PWV mainly reflect a property of the aorta (elastic artery), but baPWV involves properties of both the aorta and lower limb arteries (muscular artery). However, the validity and reliability of baPWV have been reported (*42*). Yamashita et al. (*20*) reported that baPWV was significantly correlated with aortic PWV measured directly by a catheter pressure transducer ($n=41$, $r=0.87$, $P<0.01$); the coefficient of variation of interobserver reproducibility was 8.4% in their study, and that of intraobserver reproducibility was 10.0%. The path length was estimated from the height of each subject based on the superficial measurements in the Japanese population, suggesting possible errors. However, use of the equation should not have seriously biased the reliability of the PWV measurements, because the Pearson's correlation coefficient between the estimated length and the actual surface measurement was higher than 0.9 (*43*). And baPWV can be measured noninvasively and automatically. Therefore, we believe that baPWV is useful for population-based studies. Sixth, the sample size was relatively small. The lack of a significant relationship between quartile 3 of β2m and a high value of PWV would seem to have been due to the small sample size. In addition, when the logistic regression analyses were performed separately for men and women, the odds ratios of men were consistently significant. However, the odds ratios of women were not significant, even though the odds ratios of women were similar to those of men. Finally, we could not obtain data on the subjects' income, although all the subjects worked for one local government.

We therefore believe that the subjects were socioeconomically similar, and our data were adjusted for educational attainment, so it was considered that the influence of socioeconomic status on the adjusted analysis was practically nil.

In summary, our results suggest that β2m is associated with an increase of arterial stiffness. Because β2m is measured easily and is in widespread use, further studies are needed to clarify whether β2m is related to atherosclerotic diseases, and to elucidate whether the combination of β2m and CRP measurement is a useful predictive strategy for the development of atherosclerosis.

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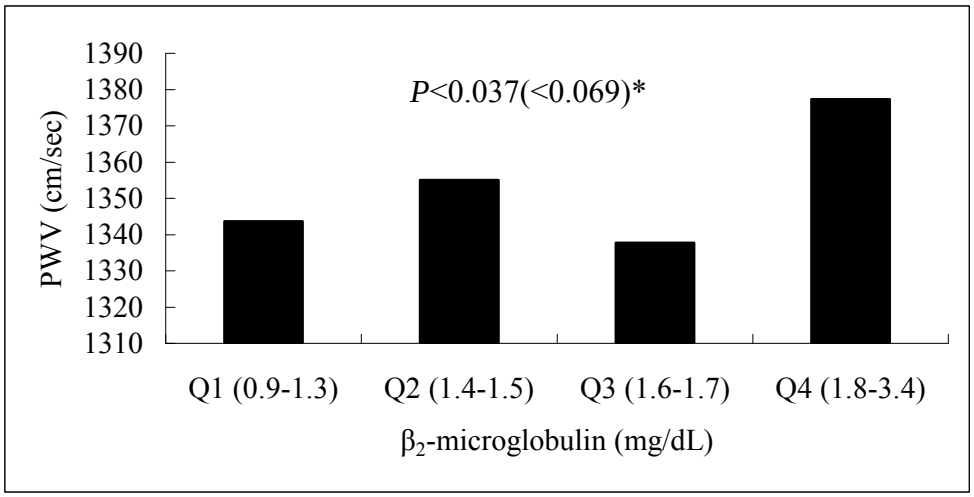
Figure

Adjusted Means of baPWV Compared among Quartiles of β_2-microglobulin.



Adjusted for age, gender, BMI, SBP, HR, TC, HDL-C, FBS, logTG, UA, logCRP, estimated GFR, smoking status, alcohol consumption, frequency of exercise, , educational attainment, medication for hypertension, medication for hyperlipidemia, and medication for diabetes.

* *P* value for difference (*P* for trend)

Table. 1 Characteristics in Quartiles of β_2-microglobulin.

	β_2-microglobulin category				*P*-value
	Quartile 1 (n=223)	Quartile 2 (n=226)	Quartile 3 (n=185)	Quartile 4 (n=128)	
β_2-microglobulin range (mg/dL)	0.9-1.3	1.4-1.5	1.6-1.7	1.8-3.4	
Gender (male, %)	67.4	82.3	85.4	88.3	<0.00001
Age (y)	48.6 ± 6.3	50.1 ± 6.0	50.4 ± 6.3	51.2 ± 5.5	<0.0001
BMI (kg/m^2)	23.3 ± 3.1	23.5 ± 2.9	24.0 ± 2.9	23.6 ± 2.9	0.14
SBP (mmHg)	119.2 ± 12.5	119.7 ± 15.4	124.2 ± 16.8	123.2 ± 16.3	<0.01
DBP (mmHg)	75.2 ± 11.0	75.6 ± 10.9	78.3 ± 11.6	77.5 ± 11.3	<0.01
Heart rate (bpm)	60.7 ± 9.5	61.1 ± 10.0	61.7 ± 9.7	61.3 ± 9.2	0.77
Total cholesterol (mg/dL)	209.4 ± 33.2	209.3 ± 31.6	205.9 ± 30.8	203.1 ± 32.6	0.23
Triglycerides (mg/dL)	93 (62-146)	102 (76-145)	101 (73-164)	105 (75-147)	0.22
HDL cholesterol (mg/dL)	59.9 ± 15.0	57.4 ± 14.1	55.6 ± 14.4	53.2 ± 13.2	<0.001
Fasting glucose (mg/dL)	97.3 ± 22.6	98.8 ± 25.0	95.9 ± 13.3	95.2 ± 15.1	0.33
Uric acid (mg/dL)	5.3 ± 1.3	5.6 ± 1.2	5.9 ± 1.2	6.0 ± 1.2	<0.00001
CRP (mg/dL)	0.035 (0.018-0.065)	0.034 (0.020-0.077)	0.046 (0.025-0.083)	0.055 (0.028-0.125)	<0.001
Estimated GFR (mL/min per 1.73m^2)	122.3 ± 19.1	105.2 ± 18.6	102.7 ± 19.4	97.8 ± 17.0	<0.00001

Current smoker (%)	39.9	36.7	38.9	39.6	0.46
Drinker (%)	69.1	72.1	73.0	65.6	0.48
Frequency of exercise (%)					
Rarely or never	59.2	48.2	56.8	60.9	0.05
≥1week	40.8	51.8	43.2	39.1	
Educational attainment (%)					
High school education or less	52.8	46.5	45.9	51.6	0.4
More than high school education	47.2	53.5	54.1	48.4	
Medication for					
Hypertension (%)	5.1	5.3	13.0	15.6	<0.0001
Hyperlipidemia (%)	6.9	5.7	5.9	2.3	0.34
Diabetes (%)	0.9	1.8	1.6	0.8	0.76
PWV (cm/s)	1314 ± 177	1347 ± 204	1365 ± 196	1407 ± 213	<0.001

Variables are presented as mean±SD, median (interguatile range) for skewed variables, or percentage

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; CRP, C-reactive protein; GFR, glomerular filtration rate; PWV, pulse wave velocity.

Table. 2 Association of β_2-microglobulin, CRP levels, and their Combination with High Values of PWV (tertile three category).

Parameter	Unadjusted OR (95%CI)		*P*-value	Adjusted OR[a] (95%CI)		*P*-value
β_2-microglobulin						
Quartile 1 (0.9-1.3 mg/dL)	reference			reference		
Quartile 2 (1.4-1.5 mg/dL)	1.50	0.99 to 2.26	0.06	1.45	0.82 to 2.57	0.20
Quartile 3 (1.6-1.7 mg/dL)	1.30	0.84 to 2.01	0.24	0.73	0.40 to 1.36	0.32
Quartile 4 (1.8-3.4 mg/dL)	2.40	1.51 to 3.83	<0.001	2.53	1.31 to 4.89	<0.01
C-reactive protein						
Quartile 1 (<0.004-0.021 mg/dL)	reference			reference		
Quartile 2 (0.022-0.040 mg/dL)	1.81	1.14 to 2.89	<0.05	1.39	0.74 to 2.61	0.30
Quartile 3 (0.041-0.080 mg/dL)	2.03	1.28 to 3.23	<0.01	1.24	0.65 to 2.39	0.52
Quartile 4 (0.081-8.36 mg/dL)	3.14	2.00 to 4.96	<0.00001	2.27	1.18 to 4.34	<0.05
Combination						
Low β_2m ($\leq$1.7 mg/dL) and low CRP ($\leq$0.080 mg/dL)	reference			reference		
High β_2m ($\geq$1.8 mg/dL) or High CRP ($\geq$0.081 mg/dL)	1.78	1.28 to 2.49	<0.001	1.86	1.18 to 2.95	<0.01
High β_2m ($\geq$1.8 mg/dL) and high CRP ($\geq$0.081 mg/dL)	4.86	2.54 to 8.91	<0.00001	5.60	2.38 to 13.2	<0.0001

[a]Adjusted for age, gender, BMI, SBP, HR, TC, HDL-C, FBS, logTG, UA, estimated GFR, smoking status , alcohol consumption, frequency of exercise, educational attainment, medication for hypertension, medication for hyperlipidemia, and medication for diabetes.

Abbreviations

β2m: β_2-microglobulin

CRP: C-reactive protein

GFR: glomerular filtration rate

PWV: pulse wave velocity

baPWV: brachial-ankle pulse wave velocity

BSA: body surface area

OR: odds ratio

CVD: cerebrovascular disease

BMI: body mass index

TC: total cholesterol

TG: triglyceride

HDL-C: high density lipoprotein cholesterol

UA: uric acid

HR: heart rate

ABI: ankle brachial index

ANOVA: analysis of variance

CI: confidence interval

SBP: systolic blood pressure

DPB: diastolic blood pressure

ESRD : end-stage renal disease