quartile group were higher than those in each of the other three groups for the male subjects ($p < 0.05$, Mann–Whitney U test). On the other hand, there was no difference between mean crude CRP levels in the quartile groups for the female subjects. Higher intake of n-3PUFA was associated with lower percentage of smokers, lower percentage of drinkers, and higher percentage of subjects performing regular exercise.

Table 3 shows the results of multiple linear regression analyses using ln CRP as the dependent variable and smoking status patterns and other factors as independent variables. "Current smoking" and "ex-smoking" were significantly correlated with ln CRP levels in the male subjects but not in the female subjects. Age, BMI, systolic blood pressure, levels of HDLC, LDLC, and HbA1c, intake of SFA, and intake of n-3PUFA were related to ln CRP level in both sexes. Regular drinking was also correlated with ln CRP level, but regular exercise was not associated with ln CRP level. The high levels of correlation among the explanatory variables produce challenges for statistical modelling to ensure the results are not artifacts of collinearity. We also performed multiple regression analyses using the products of pairs of explanatory variables as independent variables to adjust for interactions between explanatory variables. The results were unchanged even after adjusting for interactions between explanatory variables (data not shown).

Non-adjusted and adjusted geometric mean levels of CRP in the quartile groups according to intake of n-3PUFA, according to intake of long-chain n-3PUFA, or according to intake of ALA are shown in Table 4. Multiple comparisons showed significant difference only between the Q1 category and Q4 category according to intake of n-3PUFA (0.54 vs. 0.46, $p < 0.05$) in male subjects. Linear trends across quartile groups according to intake of n-3PUFA or according to intake of long-chain n-3PUFA existed both in male and female subjects. The higher the intake of n-3PUFA was, the lower adjusted CRP level was. The higher the intake of long-chain PUFA was, the lower the adjusted CRP level was. A linear trend across quartile groups according to intake of ALA existed only in male subjects. In female subjects, slightly elevated CRP levels in Q1 and equal levels in Q2, Q3, and Q4 categories were observed.

Table 5 shows adjusted geometric means of CRP level in the quartile groups separately by smoking status. Linear trends across quartile groups were shown in both smokers and ex-smokers in the male subjects. Multiple comparisons showed significant differences in CRP levels between the Q1 and Q2 categories, between the Q1 and Q3 categories, and between the Q1 and Q4 categories in male smokers (0.54 vs. 0.48, 0.48, 0.48, or 0.46, $p < 0.05$). The difference between CRP levels in the Q1 and Q4 categories was also significant in male ex-smokers. A significant difference was not

Table 5
Adjusted geometric means of CRP level in the quartile groups according to dietary intake of n-3PUFA separately by smoking status

Dietary intake of n-3 PUFA (% of total energy)		Q1 Men (0.15-1.0%) Women (0.24-1.2%)	Q2 Men (1.0-1.4%) Women (1.2-1.5%)	Q3 Men (1.4-1.7%) Women (1.5-1.9%)	Q4 Men (1.7-4.2%) Women (1.9-6.4%)	trend p
Male nonsmokers	(n)	336	374	411	426	
Adjusted CRP	(mg/L)	0.43 (0.38-0.48)	0.42 (0.38-0.46)	0.42 (0.38-0.46)	0.39 (0.35-0.43)	0.232
Male ex-smokers	(n)	323	306	320	312	
Adjusted CRP	(mg/L)	0.57 (0.51-0.64)	0.48 (0.43-0.54)	0.47 (0.42-0.52)	0.46 (0.41-0.51)	0.009
			$p < 0.05$			
Male smokers	(n)	429	407	357	350	
Adjusted CRP	(mg/L)	0.54 (0.51-0.58)	0.48 (0.46-0.51)	0.48 (0.45-0.51)	0.46 (0.43-0.49)	0.029
			$p < 0.05$			
				$p < 0.05$		
					$p < 0.05$	
Female nonsmokers	(n)	2,310	2,343	2,358	2,388	
Adjusted CRP	(mg/L)	0.44 (0.42-0.46)	0.43 (0.41-0.44)	0.41 (0.39-0.42)	0.41 (0.39-0.43)	0.005
Female ex-smokers	(n)	48	43	36	21	
Adjusted CRP	(mg/L)	0.43 (0.31-0.60)	0.47 (0.32-0.67)	0.52 (0.36-0.77)	0.68 (0.42-1.09)	0.097
Female smokers	(n)	101	74	66	52	
Adjusted CRP	(mg/L)	0.56 (0.44-0.71)	0.48 (0.37-0.63)	0.41 (0.31-0.55)	0.58 (0.43-0.79)	0.605

Data are expressed as adjusted geometric means (95% CI). Adjusted geometric means of CRP level for persons aged 60 years with BMI of 24 (kg/m^2), SBP of 128 (mmHg), HDLC of 56.0 (mg/L), LDLC of 117.0 (mg/L) HbA1c of 5.10 (%), intake of saturated fatty acid of 5.5% of total energy, and regular drinking (mean). 95% CI (confidence interval) is based on standard errors from analysis of covariance. p values were determined by analysis of covariance. Multiple comparisons were performed using Bonferroni's method.

found in CRP levels between the groups in male nonsmokers. Although differences in CRP levels between groups were small, a significant linear trend across the quartiles was found in female nonsmokers.

3. Discussion

The main findings of this study were (1) adjusted CRP levels were inversely associated with dietary intake of n-3PUFA or dietary intake of long-chain n-3PUFA for both the male subjects and female subjects, (2) the inverse relationship between adjusted CRP levels and dietary intake of n-3PUFA was more evident in smokers than in nonsmokers in the male subjects, and (3) male smokers taking a low dose of n-3PUFA (in the lowest quartile group) had significantly higher levels of adjusted CRP than those in other male subjects.

Although a linear trend between dietary intake of n-3PUFA and serum CRP levels exists, we also plotted the adjusted geometric mean CRP levels in 20 equally partitioned subgroups according to their intake of n-3PUFA in order to show a non-linear effect between CRP levels and intake of n-3PUFA. An interpolation curve obtained by using spline function showed a steep descent between categories 2 and 3 and a gradual descent between C3 and C20. The spline curve suggests that a cutoff point exists at the C3 point (i.e., 0.91% of energy) and this point possibly means n-3PUFA requirement to maintain attenuated inflammatory reaction (see additional figure as supplementary appendix).

This study also revealed gender-based differences in CRP levels. However, when subjects were limited to nonsmokers, the adjusted CRP levels were almost the same in the male and female subjects (0.42 mg/L in male nonsmokers vs. 0.44 mg/L in female nonsmokers). Considering the low rate of smoking among women and the high rate of smoking among men in this study, the sex-based difference in adjusted CRP levels is probably due to the difference in smoking prevalence between men and women.

The results of this study suggest that activated inflammation is independently attenuated by high-dose dietary intake of n-3PUFA, especially in male smokers. Since n-3PUFAs are precursors of anti-inflammatory eicosanoids (such as prostaglandin I3, prostaglandin E3, thromboxane A3, and leukotriene B5), n-3PUFAs are hypothesized to attenuate the inflammatory response [23,24]. Several studies have been carried out to determine the associations between dietary intake of n-3PUFA (or fish) and levels of inflammatory markers. Pischon et al. showed that dietary intake of n-3PUFA was inversely associated with plasma levels of soluble tumor necrosis factor (TNF) receptors and somewhat less with C-reactive protein in healthy men and women [23,25]. Ciubotaru et al. showed that dietary fish oil reduces levels of C-reactive protein and interleukin-6 in postmenopausal women undergoing hormone replacement therapy (HRT) [26]. These studies suggest that intake of n-3PUFA (or fish) decreases the levels of inflammatory cytokines and CRP levels in healthy subjects. The effect of n-3PUFA intake on serum CRP levels is more evident in high-risk subjects, such as women undergoing HRT. Some studies showed that both CRP levels and other inflammation-related markers were influenced by intake of n-3PUFA. However, we did not measure the levels of interleukins, TNF-alfa, or other inflammation-related agents such as matrix metalloproteinase or macrophage colony-stimulating factor, and we therefore can not discuss the possibility of effects on different inflammation-related pathways due to n-3PUFA.

Rodriguez et al. showed that the favorable effects of fish consumption were more evident in male smokers than that in male nonsmokers in Japanese Americans living in Hawaii [17]. Notably, the apparent preventive effects of fish consumption on cardiac events in their study and the apparent anti-inflammatory effect of dietary intake of n-3PUFA in our study are commonly observed in male smokers. Although the underlying biochemical mechanism was not discussed in their report, the anti-inflammatory effects of n-3PUFA are thought to contribute to the mechanisms that decrease cardiac events in heavy-smoking males.

Several limitations to our study should be noted. The cross-sectional design of the present study tolerates uncertainty of causal relationships. A single instance of blood sampling may be susceptible to short-term variation. Because determination of dietary variables, including fatty acid, was based on a self-administered food frequency questionnaire, information on dietary variables might have been overestimated or underestimated. Socio-economical status (SES) is one of the important confounding factors. However, there was little information about SES in this study and this is one of the limitations. Exercise habit is also one of the important factors that are associated with CRP levels. We performed regression analysis using exercise-related variables and other factors as explanatory variables in three ways: regular exercise, times per month, and quartile categories. There was no relationship between exercise habit and CRP levels in any of the three patterns. We thought that information about exercise in this study was not sufficient for adjusting for confounding effects. The small numbers of female ex-smokers (1.5%) and female nonsmokers (3%) limited statistical power to draw strong conclusions about these groups. The small number of individuals who consumed small amount of n-3PUFA was also one of the limitations.

Despite the lack of causal relationships, based on our findings and those of others, it is reasonable to conclude that sufficient dietary intake of n-3PUFA may attenuate inflammatory reaction and that this effect is more evident in high-risk populations such as male smokers.

Acknowledgements

The Iwate-KENCO study was supported by grants from the Open Translational Research Center Project, Advanced

Medical Science Center, Iwate Medical University, the Japan Arteriosclerosis Prevention Fund, the Ministry of Education, Culture, Sports, Science and Technology of Japan, and the Ministry of Health and Welfare of Japan. We express our gratitude to the staff of Iwate Health Service Association and the staff in all municipalities (Iwate Prefecture, Ninohe City, Ichinohe Town, Karumai Town, Kunohe Village, Yamada Town, Kawai Village, Miyako City, Niisato Village, Taro Village, Iwaizumi Town, Tanohata Village, Kuji City, Yamagata Village, Ohno Village, Fudai Village, and Taneichi Town).

M.O. had full access to all data in the study and takes responsibility for the integrity of the data and accuracy of the data analysis.

Appendix A

A.1. Members of Iwate-KENCO Study

Chairman: Akira Okayama (The First Institute of Health Service, Japan Anti-Tuberculosis Association, Tokyo)

Principal investigators: Akira Ogawa, Motoyuki Nakamura, YasuoTerayama, Kazuyoshi Itai, Toshiyuki Onoda, Masaki Ohsawa, Kozo Tanno, Kiyomi Sakata, Yuki Yoshida (Iwate Medical University, Morioka), Mitsumasa Tazawa (Morioka Public Health Care Center), Kazuko Kawamura (Iwate Health Service Association), Toru Kuribayashi (Iwate University, Morioka)

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2008.01.008.

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Atherosclerosis 204 (2009) 234–238

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Serum C-reactive protein levels can be used to predict future ischemic stroke and mortality in Japanese men from the general population

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ARTICLE INFO

Article history:
Received 11 March 2008
Received in revised form 18 July 2008
Accepted 31 July 2008
Available online 12 August 2008

Keywords:
Epidemiology
Inflammation
Stroke

ABSTRACT

Background: High C-reactive protein (CRP) levels have been reported to be associated with an increased risk of atherosclerotic cardiovascular events. The relationship of CRP levels to the risk of cerebrovascular events in the Japanese population, which has a lower prevalence of coronary artery disease and a lower CRP level than Western populations, has not been fully clarified. The present study examined the predictive value of serum high sensitivity CRP (hs-CRP) levels for future cerebrovascular events and mortality in the general Japanese population.

Methods: The subjects for this community-based, prospective cohort study were recruited from the general population (n = 7901, male only, mean age = 64.0 years). Serum hs-CRP levels and cardiovascular risk factors were determined at baseline. The mean follow-up period was 2.7 years. After excluding subjects with a cardiovascular history, the relationships between hs-CRP levels and cerebrovascular events and mortality were assessed.

Results: During follow-up, 130 participants had a first stroke (95 ischemic strokes), and 161 participants died. The hs-CRP tertile level was a significant predictor for a first ischemic stroke (3rd tertile, HR = 1.77: 95% CI, 1.04–3.03, compared with the 1st tertile), after adjustment for age and classical cardiovascular risk factors. Similar trends were observed for the prediction of all-cause mortality (3rd tertile, HR = 2.26: 95% CI, 1.49–3.42, compared with the 1st tertile).

Conclusion: CRP levels can be used to predict future ischemic stroke and mortality in Japanese men from the general population, independently from traditional cardiovascular risk factors.

1. Introduction

The degree of systemic inflammation that is represented by elevated high sensitivity C-reactive protein (hs-CRP) levels has been associated with an increased risk of cardiovascular events in studies conducted in the United States and Europe [1–3]. In the prospective Physicians' Health Study (PHS), elevated hs-CRP levels were associated with an approximately twofold increase in the risk of stroke [1].

We previously reported that, in apparently healthy males living in Japan, hs-CRP levels were closely associated with atherosclerotic changes as measured by carotid plaque formation [4]. Thus, the extent of inflammation may reflect the propensity of atherosclerotic lesions to precipitate clinical vascular events. However, the serum hs-CRP levels of the general Japanese population have been reported to be lower than those of other ethnic groups [5,6]. One must clarify whether associations between a future risk of cerebrovascular diseases and elevated hs-CRP levels also exist in a population that has a relatively lower hs-CRP level. Only one study has reported the association between hs-CRP and ischemic stroke in a rural area of Japan [7]. Therefore, we evaluated the ability of hs-CRP levels to predict future cerebrovascular events and mortality in a larger cohort of the general Japanese population.

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doi:10.1016/j.atherosclerosis.2008.07.040

2. Methods

2.1. Study subjects

The study subjects were recruited from the community-dwelling population living in the Ninohe, Kuji, and Miyako districts of Iwate in northern Japan (the Iwate-Kenpoku Cohort study). This study was conducted as part of a government-sponsored, multiphasic health checkup program aimed at the general population. Between April 2002 and January 2005, invitations to participate in this health checkup program were issued by government offices in 17 rural municipalities located in these districts; 26,469 individuals (9161 males) took part in the program and agreed to join the present study. Of these, 25,925 subjects (8957 males) had hs-CRP measurements. Subjects aged over 80 years (280 males) and those under 40 years (300 males), as well as those with a history of cardiovascular disease or stroke (527 males), were excluded. Thus, the data of 7901 males (mean age, 64.0 ± 9.7 years) were analyzed. Baseline clinical examinations included a standard 12-lead electrocardiogram, and a self-reported questionnaire was administered to document subjects' medical history and lifestyle. Hospital inpatients, persons who could not walk independently, and persons with recent inflammatory conditions, such as major trauma, surgery, or obvious acute infectious disease, were not included in the present study.

The study was approved by our institutional ethics committee, and all of the participants provided their written informed consent.

2.2. Risk factor definitions

The presence of baseline cardiovascular risk factors, including hypertension, diabetes mellitus, hypercholesterolemia, obesity, and smoking, was determined. Hypertension was defined as at least one of: systolic blood pressure ≥140 mmHg; a diastolic blood pressure ≥90 mmHg; or current antihypertensive therapy. Diabetes mellitus was defined as a history of a random blood glucose level ≥200 mg/dL or an HbA1c level ≥6.5% or current anti-diabetic therapy. Dyslipidemia was defined as a total cholesterol level ≥240 mg/dL or high density lipoprotein cholesterol level <40 mg/dL or current cholesterol-lowering therapy. Obesity was defined as a body mass index ≥25.0 kg/m^2. The estimated glomerular filtration rate (eGFR) was calculated using the modified equation of the Modification of Diet in Renal Disease (MDRD) study [8].

An electrocardiogram was not done in 225 males (2.8%). Body height or body weight was missing in 10 males, and blood pressure data were missing in 2 males. These participants were considered to have no risk factors such as atrial fibrillation, obesity, or hypertension if they had no history of atrial fibrillation or hypertension.

2.3. Blood samples and hs-CRP measurement

Blood samples were collected from an antecubital vein. The samples were collected into vacuum tubes containing EDTA or a serum separator gel (CRP, lipids). After sampling, tubes were stored immediately in an icebox and centrifuged at 1500 × g for 10 min within 8 h of collection. Aliquots of serum were stored at −20 °C, and routine hematology and biochemistry tests, including hs-CRP, were done within a few days after blood sampling. hs-CRP levels were determined using a highly sensitive immunonephelometric method with a coefficient of variation <5% (N Latex CRP, Dade Behring). The detection limit of CRP assay is 0.1 mg/L, and cases with levels below the limit of detection were considered as 0.1 mg/L.

2.4. Outcome measures

In this cohort study, the primary endpoint was all-cause death, as well as any non-fatal cardiovascular events, such as myocardial infarction, cerebral infarction, or other strokes. The dates of death and move-out were confirmed by the investigators reviewing population-register sheets in each local government. Persons who were known to be alive at the end of follow-up and those who had moved away from the study area were treated as censored cases.

Stroke events were identified by accessing the Iwate prefecture stroke registration program, which included the entire area where the subjects lived; details of this registry have been described previously [9]. Since 1991, the stroke registration program has been coordinated by the Iwate prefecture government and the Iwate Medical Association; the medical records of all medical facilities within the survey area are verified to ensure complete capture of all data. Incidents of acute myocardial infarction were identified by accessing data from the Northern Iwate Heart Disease Registry Consortium, which has been collecting data since 2002. The registration of acute myocardial infarction and sudden death was based on the criteria of the MONICA study [10]. To verify the accuracy of the data, a physician or trained research nurse visited and checked the medical records of the referral hospitals.

Females were excluded from the analysis due to a low incidence of ischemic stroke events (59 events in 15,457 females; 0.4%). For the same reason, coronary heart disease events (non-fatal myocardial infarction, 34 events in 7901 males; 0.4%) were also not analyzed.

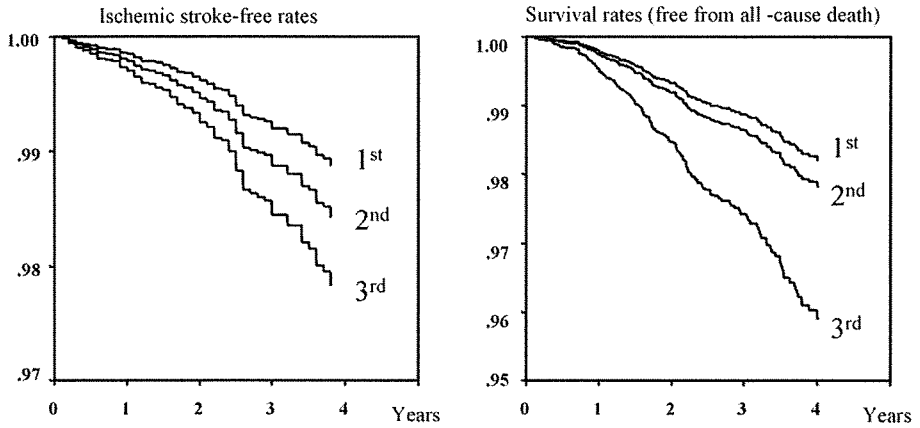

Fig. 1. Cumulative ischemic stroke-free rates and survival rates by age-adjusted Cox regression model for hs-CRP tertiles.

Table 1
Baseline clinical characteristics of all subjects with and without endpoints

	Ischemic stroke			All-cause death		
	(−)	(+)	p	(−)	(+)	p
No. of subjects	7806	95		7740	161	
Age (years)	63.9 ± 9.7	69.6 ± 7.2	<0.001	63.9 ± 9.7	69.8 ± 7.8	<0.001
Body mass index (kg/m^2)	23.9 ± 2.9	23.6 ± 3.0	0.20	24.0 ± 2.9	22.9 ± 3.0	<0.001
Systolic blood pressure (mmHg)	131 ± 19	139 ± 20	<0.001	131 ± 19	132 ± 20	0.31
Diastolic blood pressure (mmHg)	79 ± 11	80 ± 11	0.31	79 ± 11	76 ± 10	0.006
Hemoglobin A1c (%)	5.15 ± 0.74	5.28 ± 0.83	0.09	5.15 ± 0.74	5.30 ± 0.98	0.052
Serum creatinine (mg/dL)	0.82 ± 0.20	0.85 ± 0.16	0.15	0.82 ± 0.19	0.88 ± 0.42	0.18
eGFR (mL/min/1.73 m^2)	73.4 ± 15.1	69.2 ± 13.5	0.006	73.4 ± 15.0	70.2 ± 18.0	0.004
Uric acid (mg/dL)	5.73 ± 1.35	5.45 ± 1.51	0.038	5.72 ± 1.35	5.95 ± 1.59	0.16
Total cholesterol (mg/dL)	191 ± 32	188 ± 35	0.15	192 ± 32	181 ± 35	0.001
Triglyceride (mg/dL)	126 ± 84	123 ± 85	0.41	126 ± 84	121 ± 81	0.39
LDL-cholesterol (mg/dL)	114 ± 29	112 ± 31	0.25	114 ± 29	107 ± 32	0.023
HDL-cholesterol (mg/dL)	56 ± 15	56 ± 16	0.90	56 ± 15	53 ± 17	0.002
hs-CRP (mg/L)	0.54	0.80	<0.001	0.55	1.07	<0.001
Hypertension (%)	45.6	67.4	<0.001	45.7	54.0	0.038
Diabetes mellitus (%)	7.7	11.6	0.17	7.6	14.3	0.004
Dyslipidemia (%)	21.6	18.9	0.61	21.4	26.1	0.17
Obesity (%)	33.3	33.7	0.91	33.5	26.1	0.052
Atrial fibrillation (%)	2.6	15.8	<0.001	2.7	6.2	0.013
Current/past smoking (%)	62.2	75.8	0.007	62.2	68.9	0.085

hs-CRP, high sensitivity C-reactive protein; HDL, high density lipoprotein; LDL, low density lipoprotein; eGFR: estimated glomerular filtration rate.
Log-transformed values were used for comparisons of CRP levels.
Data are shown as mean ± S.D. hs-CRP are shown as geometric mean.

2.5. Statistical analysis

The cumulative survival curves (free of ischemic stroke or free of all-cause death) by hs-CRP tertile levels were determined according to the age-adjusted Cox model (Fig. 1). The proportionality assumptions of the hazard by hs-CRP tertile were verified by log minus log curves. To determine the relative risks for each hs-CRP tertile level, multivariate Cox proportional hazard models were used. Age and known cardiovascular risk factors were used, and age (10-year increase), systolic blood pressure, total cholesterol, high density lipoprotein cholesterol, uric acid, estimated glomerular filtration rate, body mass index, smoking, and presence of diabetes were forced into the multivariate adjusted model. One rural community ($n = 728$) was excluded from multivariate analysis because of missing data for serum uric acid, and cases having other missing data as random phenomena were also excluded. This multivariate analysis was finally performed for 7127 subjects. The results are expressed as the hazard ratio (HR) and the corresponding 95% confidence interval (CI). The analyses were performed using the SPSS statistical package, version 11.0.

3. Results

The mean follow-up period was 2.7 years. During follow-up, 130 subjects (1.6%) had a first stroke. Of these, 95 (1.2%) had an ischemic stroke; 161 (2.0%) died due to any cause; and 34 (0.4%) had a new onset, non-fatal myocardial infarction (MI). All of the non-ischemic strokes were the result of intracerebral or subarachnoid hemorrhages.

Baseline characteristics of the participants with and without ischemic stroke or all-cause death are shown in Table 1. Age, systolic and diastolic blood pressures, serum creatinine level, the prevalence of hypertension, atrial fibrillation, and smoking were higher in those with ischemic stroke than in those without. On the other hand, eGFR was lower in those with ischemic stroke than in those without. Similar results were obtained with respect to all-cause death. Some paradoxical relationships were found with respect to the uric acid level in participants with ischemic stroke, and the total cholesterol level and LDL level in those with all-cause death (Table 1).

The median serum hs-CRP level was 0.5 mg/L (95 percentile range: 0.1–4.3 mg/L) in males. This median hs-CRP level was lower than the levels reported in other populations in which hs-CRP levels were measured using the same assay methodology [1–3]. A total of 917 participants showed CRP levels ≤0.1 mg/L. Overall tertile ranges for the hs-CRP levels were: 1st, 0.1–0.3; 2nd, 0.4–0.7; and 3rd, ≥0.8 mg/L. Participants showing CRP > 10.0 mg/L comprised 1.7% of the study population. However, presence of acute infectious condition cannot be judged by CRP level alone, so making a cut-off level for infection is not possible. We therefore ventured to perform analyses without any exclusion criteria for high CRP level.

Table 2
Hazard ratios for first ischemic stroke and all-cause death by hs-CRP tertile levels

	hs-CRP tertile	Incidence of events/no. of subjects, n (%)	Age adjusted hazard ratios (95% CI)	p	Multivariate adjusted hazard ratios (95% CI)[a]	p
Ischemic stroke	1	22/2922 (0.75)	1.00 (reference)		1.00 (reference)	
	2	28/2296 (1.22)	1.41 (0.80–2.48)	0.24	1.30 (0.72–2.33)	0.39
	3	45/2683 (1.68)	1.95 (1.17–3.25)	0.010	1.77 (1.04–3.03)	0.037
All-cause death	1	36/2922 (1.23)	1.00 (reference)		1.00 (reference)	
	2	37/2296 (1.61)	1.22 (0.77–1.93)	0.40	1.15 (0.71–1.88)	0.57
	3	88/2683 (3.28)	2.32 (1.57–3.42)	<0.001	2.26 (1.49–3.42)	<0.001

hs-CRP, high sensitivity C-reactive protein; CI, confidence interval.
[a] Age (10-year increase), systolic blood pressure, total cholesterol, high density lipoprotein cholesterol, uric acid, estimated glomerular filtration rate, body mass index, smoking (current and past), and the presence of diabetes were forced into the Cox regression analysis model.

As shown in Fig. 1, first ischemic stroke-free survival was lower in the higher hs-CRP tertile level when adjusted for age ($p = 0.034$). Similar results were observed for all-cause death-free survival rates ($p < 0.001$). The proportionality assumptions of the hazard by hs-CRP tertiles for these outcomes were satisfied.

In the multivariate Cox regression analysis model adjusted by age, a significantly increased hazard ratio of ischemic stroke was found in the 3rd hs-CRP tertile (HR = 1.95, $p = 0.010$) compared to the 1st hs-CRP tertile. After adjustment for age (10-year increase) and other classical cardiovascular risk factors, such as systolic and diastolic blood pressures, total cholesterol, high density lipoprotein cholesterol, uric acid, eGFR, BMI, smoking (current and past), and the presence of diabetes, the estimated HRs were maintained in the 3rd hs-CRP tertiles (HR = 1.77, $p = 0.037$). The results of the analysis of all-cause death were similar (Table 2). When the presence of atrial fibrillation was included in the multivariate adjusted model for ischemic stroke, the statistical significance of the hs-CRP tertiles declined (3rd hs-CRP tertile, HR = 1.56, $p = 0.10$).

On the other hand, there was no significant association between the hs-CRP tertiles and strokes from any causes (trend $p = 0.19$) in the model adjusted by age and other classical cardiovascular risk factors.

4. Discussion

This prospective cohort study found that baseline serum hs-CRP level was an independent predictor for future ischemic stroke and all-cause mortality in an apparently healthy population. It is interesting that these results were obtained in the Japanese population, which has a lower median hs-CRP level than Western populations [4,5].

The major risk factors for stroke and cardiovascular disease, such as smoking, diabetes, and hypertension, are associated with higher hs-CRP levels [11,12]. These relationships could potentially explain the associations that have been found between hs-CRP level and stroke or mortality. However, since adjustment for such risk factors did not have a large effect on the associations, the traditional risk factors cannot completely explain the relationship between the hs-CRP level and ischemic stroke events.

Carotid plaque formation is a well-established predictor for future ischemic stroke in the general population [13,14]. Our previous data showed a close association between the hs-CRP level and the severity of carotid atherosclerosis as demonstrated by plaque formation in men [6]. The present prospective results show that future stroke events were related to elevated baseline hs-CRP levels; this finding appears to substantiate our previous cross-sectional data. Although a significant association between the hs-CRP level and carotid atherosclerosis was only seen in men in our previous data, the present study could not demonstrate a gender difference for the association between hs-CRP level and the study endpoint.

Atrial fibrillation has been known to be closely related with ischemic stroke due to cardiac thromboembolism. In the present study, the presence of atrial fibrillation was the strongest predictor for ischemic stroke in the same model of multivariate Cox regression analysis with various risk factors (HR = 5.13, 95% CI; 2.82–9.35, $p < 0.001$). It is considered natural that the significance of the hs-CRP tertiles declined when the presence of atrial fibrillation was included in the multivariate adjusted model for ischemic stroke.

In the present cohort, the association between elevated hs-CRP level and stroke was only present when the analysis was limited to the ischemic stroke subtype. In the present study's subjects, all non-ischemic strokes were intracranial hemorrhages, which are known to be caused by rupture of cerebral perforating arteries or an intracranial aneurysm. These pathological conditions develop primarily due to hypertension and small artery hyalinosis [15]. The relationship between cerebral aneurysm and atherosclerosis is not considered to be very strong [16]. Few large-scale prospective cohort studies have addressed stroke subtype.

The major results of our study are completely consistent with the findings of the Hisayama Study [7]. Although the novelty of our study may be lacking, we would raise some unique minor points of difference from the findings and design of the Hisayama Study. First, the presence of atrial fibrillation reduced the predictive power of CRP for ischemic stroke in our study. Second, hs-CRP measurement at baseline was planned a priori and the assay was performed immediately, without long-term cryopreservation. Third, registration of our study population was started in 2002. Compared with the survey in 1988 of the Hisayama study, many new anti-atherosclerotic agents such as strong statins, long-acting anti-hypertensive agents and angiotensin-receptor blockers were likely to be in more frequent use in our study population. Furthermore, our study population comprised older, more obese subjects compared with those in Hisayama Study. All of these characteristics are thought to represent a closer fit with modern Japanese society and community population.

It is possible that the hospital-based follow-up used in the present study was not completely reliable for detecting clinical events. However, an attempt was made to retrieve and view all medical charts from all hospitals and clinics located in the survey area, and the study included several remote teaching hospitals and tertiary referral medical centers. Furthermore, the population of the study district has been stable, with an annual variation rate of only 0.2%. Moreover, participants who developed cerebrovascular and cardiovascular diseases or fatal events had access to only a limited number of medical institutes. Therefore, most major clinical adverse events were likely to have been captured in the present study cohort.

Elevated hs-CRP levels did not reflect the presence of imminent diseases from which stroke events or all-cause deaths had not yet occurred, since the interval between baseline hs-CRP measurement and the ischemic stroke event or death was relatively long: a mean of 1.8 years for ischemic stroke events and a mean of 1.9 years for all-cause death.

Some study limitations should be noted. The results of this study are based on one baseline hs-CRP measurement. Subjects who had recent acute inflammatory conditions, other than a mild "common cold", were not included in the study. However, the subjects were not examined to determine whether any chronic infections, including silent infections such as periodontitis, bladder cystitis, and chronic bronchitis, were present. Chronic infections have been known to have a relationship with carotid atherogenesis [17]. The present study did not assess the use of drugs that can lower hs-CRP levels, such as rennin–angiotensin system inhibitors [18,19], statins [20], and thiazolidinedione [21]. However, it was unlikely that the frequency of the use of these medications was higher in event-free participants. Although imaging was used to verify all stroke cases who visited the hospital with typical symptoms of neurological deficit, patients with events who were not hospitalized or those who were hospitalized at hospitals located outside the area could not be captured in this study design. However, this occurred very infrequently. Finally, this study tested several possible outcomes, including stroke and coronary heart disease in each gender, and then reported the significant findings. The possibility thus remains that chance findings were responsible for the present results.

In conclusion, CRP levels can predict future ischemic stroke and mortality in Japanese males from the general population, independently from traditional cardiovascular risk factors other than atrial fibrillation.

Conflict of interest

The authors report no conflicts of interest.

Acknowledgments

This study was supported by a Grant from the Ministry of Health and Welfare of Japan (No. 17120501 Director: Akira Ogawa, MD), and the Open Translational Research Center Project of our university.

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Atherosclerosis 207 (2009) 298–303

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Predictive value of plasma B-type natriuretic peptide for ischemic stroke: A community-based longitudinal study

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ARTICLE INFO

Article history:
Received 27 January 2009
Received in revised form 16 April 2009
Accepted 20 April 2009
Available online 3 May 2009

Keywords:
Natriuretic peptide
Stroke
Population
Risk

ABSTRACT

Objective: Structural heart diseases including atrial fibrillation are precursors for ischemic stroke. Plasma B-type natriuretic peptide (BNP) has been reported to be increased in patients with several types of structural heart diseases. However, the predictive value of plasma BNP for ischemic stroke remains unknown. We have studied the predictive ability of plasma BNP for future development of stroke in community dwelling adults.

Methods: Subjects of this community-based study were recruited from the general population (n = 13,466). Plasma BNP levels and cardiovascular risk factors were determined at baseline. The incidence of ischemic stroke in the cohort was identified from regional stroke registry data. A multivariate Cox regression analysis was performed to analyze the relationship between plasma BNP levels and the risk of stroke.

Results: During a mean follow-up period of 2.8 years, 102 participants (65 males, 37 females) experienced a first ischemic stroke. In men, after adjustment for classical cardiovascular risk factors and atrial fibrillation, the hazard ratio (HR) for ischemic stroke was significantly elevated in the highest plasma BNP quartile (HR = 2.38; 95% CI = 1.07–5.29). In women, the relationship between plasma BNP levels and risk of ischemic stroke was of marginal significance after adjusting for the presence or absence of atrial fibrillation (HR = 3.03; 95% CI = 0.84–10.92, P = 0.09).

Conclusion: Elevated plasma BNP levels predict the risk of ischemic stroke within men from the general population.

1. Introduction

B-type natriuretic peptide (BNP) is a cardiac hormone secreted from the myocardium in response to changes in intracardiac volume and pressure [1,2]. Plasma BNP levels are known to be elevated in patients with symptomatic left ventricular systolic dysfunction [3,4] and correlate to New York Heart Association (NYHA) class as well as prognosis [5,6]. In addition, irrespective of the degree of left ventricular dysfunction, plasma BNP levels have been shown to be elevated in patients with various structural heart diseases including previous myocardial infarction, cardiomyopathy, valvular heart disease, hypertensive heart disease, and atrial fibrillation [3,7–13].

These structural heart diseases are precursors not only for heart failure, but also for ischemic stroke, and especially cardioembolic stroke [14]. However, there have been very few reports on the association between plasma BNP levels and the risk of stroke. The Framingham Heart Study [15] has described a 4.9-fold increase in the crude incidence of stroke or transient ischemic attack in the highest tertile of BNP levels compared to the lowest tertile. Kistorp et al. [16] reported that plasma levels of N-amino terminal fragment of the prohormone BNP (NT-proBNP) predicted the risk of stroke or transient ischemic attack, with a 3.6-fold increase in risk of stroke for participants with values above the 80th percentile vs those with values equal to or below the 80th percentile in the general population. However, the association between plasma BNP levels and risk of stroke subtypes remains unclear. The predictive value of plasma BNP measurement for ischemic stroke remains unknown.

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doi:10.1016/j.atherosclerosis.2009.04.029

We have studied the predictive ability of plasma BNP for future development of ischemic stroke in community dwelling adults.

2. Methods

2.1. Study population

The Iwate-Kenpoku Cohort (Iwate-KENCO) study was designed to prospectively investigate the risk of cardiovascular diseases including stroke and malignant tumor in the general Japanese adult population as described previously [17,18]. Subjects consisted of residents of the Ninohe, Kuji and Miyako districts in the northern Iwate prefecture, Japan. Between April 2002 and January 2005, 26,469 of these residents (men = 9161, women = 17,308) who were participating voluntarily in a multiphasic health checkup agreed to join the study (original cohort). The baseline survey included routine anthropometrical measurement, blood pressure measurement, ECG, routine laboratory assessment, a self-administered lifestyle questionnaire, and a food-frequency questionnaire. This study protocol was approved by our institutional ethics committee. All participants gave written informed consent.

Of the original cohort living in the Ninohe and Kuji districts (n = 15,927), 15,394 subjects (men = 5288, women = 10,106) underwent BNP measurement (BNP cohort). Subjects were excluded from this cohort on the basis of the following characteristics: age under 40 years (n = 575), history of cardiovascular or cerebrovascular events (n = 507), non-measurement of adjustment factors (n = 846). The final statistical analysis was therefore performed in 13,466 subjects (men = 4527, women = 8939, mean age = 62.7 years).

2.2. Outcome

In this cohort study, the primary endpoint was all-cause death, in addition to any nonfatal cardiovascular events such as myocardial infarction, cerebral infarction, or other strokes. Information about death and emigration was obtained from local government records. Stroke events were identified by accessing the Iwate prefecture stroke registration programme, which has been conducted since 1991 by the Iwate Medical Association with the support of the government of the Iwate prefecture [19]. Registration forms were submitted to the registration office of the Iwate Medical Association by mail when a patient with stroke was discharged from a medical facility. Diagnostic criteria for stroke used by the registry correspond with those published by the World Health Organization, based on a definition of sudden onset of neurological symptoms [20]. For diagnosis of stroke subtypes, computed tomography and/or magnetic resonance imaging were performed within each hospital. In order to improve accuracy of registration, trained research nurses checked medical charts in all hospitals located within these districts. Follow-up was conducted until August 2007.

2.3. Measurement

At the time of baseline survey, participants underwent anthropometrical measurement, ECG, blood pressure measurement, and routine laboratory assessment. In addition, a self-administered questionnaire was used to ascertain family history, symptoms, and lifestyle factors such as smoking habits, alcohol consumption, and exercise habits. A medical history including the status of drugs prescribed for hypertension, hyperlipidemia, diabetes, angina, myocardial infarction, congestive heart failure, and stroke was recorded by trained research staff. Using a 3-channel device, a standard 12-lead ECG was recorded in a supine position. Atrial fibrillation was defined by this 12-lead ECG at the time of baseline survey. Systolic and diastolic blood pressures were determined with an automatic device placed on the right arm of seated subjects who had rested in a sitting position for at least 5 min before measurement. Measurement was performed twice, with the mean value used for statistical analysis. Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, and/or current anti-hypertensive therapy. Hyperlipidemia was defined as total cholesterol level ≥240 mg/dL, and/or current lipid lowering therapy. Diabetes was defined as non-fasting glucose concentration ≥200 mg/dL, and/or glycosylated hemoglobin (HbA1c) value ≥6.5%, and/or current anti-diabetic therapy. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2). Smoking was defined as current smoker. Regular alcohol consumption was defined as drinking alcohol 5 days or more per week. Regular exercise was defined as exercising (at least 60 min) 8 days or more per month.

Venous blood samples for plasma BNP measurement were drawn from the antecubital vein of seated participants with minimal tourniquet use. Samples were collected into vacuum tubes containing ethylenediaminetetraacetic acid sodium. Tubes were stored in an icebox immediately after sampling and were transported to our laboratory within 8 h of collection. These were then centrifuged at 1500 × g for 10 min. After separation, plasma samples were stored frozen at −20 °C until the time of assay. Plasma BNP levels were measured by direct radioimmunoassay using monoclonal antibodies specific for human BNP (ShionoRIA BNP, Shionogi, Japan) within 4 months of separation. The intraassay and interassay coefficients of variation were 5% and 6%, respectively. The lower detection limit of the assay was 0.05 pg/mL. Enzymatic methods were used to measure serum total cholesterol levels, serum creatinine, and blood glucose. HbA1c was measured quantitatively with an HPLC method.

2.4. Statistical analysis

Participants were divided into quartiles according to their baseline plasma BNP levels. Continuous variables were expressed as mean ± SD. Group comparisons were based on the unpaired t-test and multiple group comparisons across BNP quartiles were based on the one-way analysis of variance. Because BNP values were not normally distributed, these were expressed as median and the Mann–Whitney U-test was used for comparison. Categorical parameters were expressed as proportions (percentage) and group comparisons were based on the chi-square test.

The ischemic stroke event free rates according BNP quartiles were estimated using the Kaplan–Meier method, followed by Log-rank test. A multivariate Cox regression analysis was performed to analyze the relationship between plasma BNP levels and risk of stroke. For all models, the hazard ratios were adjusted for age, BMI, blood hemoglobin levels, serum creatinine levels, presence or absence of hypertension, hyperlipidemia, diabetes, smoking, regular alcohol consumption, and regular exercise. The analysis was not adjusted for presence or absence of atrial fibrillation in Model 1 and was adjusted in Model 2. Additional multivariate Cox regression analysis using covariates in Model 1 was performed using 1 SD increments in natural logarithm-transformed BNP values. For the analysis of stroke incidence, person-years were censored at the date of stroke diagnosis, the date of emigration from the study area, the date of death, or the end of the follow-up period, whichever came first. All statistical analysis was performed using SPSS software, version 11.0. A significant difference was defined as $P < 0.05$.

3. Results

Baseline characteristics of participants by sex are shown in Table 1. The mean age of men was higher than that of women. The percentages of hypertension, diabetes, atrial fibrillation, smoking, regular alcohol consumption, regular exercise, and mean values for

Table 1
Comparison of baseline characteristics between men and women.

Characteristic	Men ($N=4527$)	Women ($N=8939$)	P-value
Age (years)	64.1 ± 10.3	62.0 ± 10.0	<0.001
Hypertension (%)	44.4	38.8	<0.001
Hyperlipidemia (%)	10.3	20.3	<0.001
Diabetes (%)	8.0	4.3	<0.001
Body mass index (kg/m^2)	23.9 ± 2.9	24.2 ± 3.4	<0.001
Smoking (%)	33.4	2.5	<0.001
Regular alcohol consumption (%)	47.4	4.2	<0.001
Regular exercise (%)	17.0	10.5	<0.001
Atrial fibrillation (%)	3.0	0.6	<0.001
Hemoglobin (g/dL)	14.6 ± 1.3	13.0 ± 1.1	<0.001
Creatinine (mg/dL)	0.82 ± 0.19	0.63 ± 0.12	<0.001
BNP (median) (pg/mL)	14.8	17.1	<0.001

Continuous variables are expressed as mean ± SD.
Comparison of BNP data are performed using a Mann-Whitney U test.

hemoglobin and serum creatinine were significantly higher in men. The percentage of hyperlipidemia and mean BMI were significantly higher in women. The median value for plasma BNP was higher in women.

Table 2 shows baseline characteristics among the BNP quartiles. In men, mean age and BMI and mean levels of hemoglobin, and serum creatinine were different among the BNP quartiles ($P<0.001$). Although the percentages of hypertension, hyperlipidemia, current smoking, and regular exercise were different ($P<0.001$), the percentages of diabetes and regular alcohol consumption did not differ among the BNP quartiles. In women, although mean age and mean levels of hemoglobin, and serum creatinine were different among the BNP quartiles ($P<0.001$), the mean BMI did not differ among the BNP quartiles. Although the percentages of hypertension, hyperlipidemia, diabetes, current smoking, and regular alcohol consumption were different ($P<0.05$), the percentage undertaking regular exercise did not differ among the BNP quartiles. Subjects with atrial fibrillation were concentrated in the highest BNP quartile in both men and women.

During a mean follow-up period of 2.8 years, 102 participants (65 males, 37 females) had a first ischemic stroke event. Ranges of BNP levels in men and women are shown in Table 2. The crude incidences of ischemic stroke (per 1000 person-years) among BNP quartiles in men and women are shown in Tables 3 and 4. The crude incidence of ischemic stroke in men was 2.76 per 1000 person-years in Q1 (the lowest quartile) and 12.51 per 1000 person-years in Q4 (the highest quartile). The crude incidence of ischemic stroke in women was 0.44 per 1000 person-years in Q1 and 2.95 per 1000 person-years in Q4. The crude incidence of ischemic stroke elevated in the highest quartile in both men and women.

The Kaplan–Meier curves for ischemic stroke event free rates according to BNP quartiles in men and women are shown in Fig. 1. The ischemic stroke event free rates differed significantly among the BNP quartiles in both men and women (men: $P<0.001$; women: $P<0.001$ by log-rank test).

Several studies have demonstrated that blood hemoglobin levels [21], renal function [22] and BMI [23] influence plasma BNP levels. For that reason, after adjustment for classical cardiovascular risk

Table 2
Comparisons of baseline characteristics among BNP quartiles.

BNP quartiles	Q1	Q2	Q3	Q4	P-value
Men					
Number of subjects	1131	1134	1129	1133	
Range of BNP levels (pg/mL)	<6.5	6.5–14.8	14.9–29.9	30.0<	
Age (years)	57.4 ± 10.1	61.9 ± 9.8	66.7 ± 8.3	70.3 ± 7.8	<0.001
Hypertension (%)	33.3	40.0	47.5	56.8	<0.001
Hyperlipidemia (%)	16.5	9.8	7.9	7.1	<0.001
Diabetes (%)	7.6	8.2	8.4	7.6	0.846
Body mass index (kg/m^2)	24.1 ±2.9	24.1 ±2.9	23.8 ± 2.9	23.6 ± 3.0	<0.001
Smoking (%)	40.4	34.9	31.6	26.6	<0.001
Regular alcohol consumption (%)	48.2	45.7	47.3	48.3	0.576
Regular exercise (%)	11.6	17.1	20.0	19.3	<0.001
Atrial fibrillation (%)	0.53	0.00	0.71	10.59	<0.001
Hemoglobin (g/dL)	15.0 ± 1.1	14.8 ± 1.1	14.5 ± 1.2	14.2 ± 1.4	<0.001
Creatinine (mg/dL)	0.80 ± 0.14	0.81 ± 0.16	0.83 ± 0.24	0.84 ± 0.19	<0.001
Women					
Number of subjects	2235	2228	2242	2234	
Range of BNP levels (pg/mL)	<8.9	8.9–17.0	17.1–30.4	30.5<	
Age (years)	57.6 ± 9.5	60.0 ± 9.8	62.8 ± 9.4	67.4 ± 8.7	<0.001
Hypertension (%)	28.6	33.4	40.7	52.4	<0.001
Hyperlipidemia (%)	24.3	20.4	20.2	16.3	<0.001
Diabetes (%)	5.0	3.1	4.1	4.9	0.006
Body mass index (kg/m^2)	24.3 ± 3.4	24.2 ± 3.3	24.0 ± 3.3	24.1 ± 3.5	0.206
Smoking (%)	3.9	2.4	2.2	1.5	<0.001
Regular alcohol consumption (%)	5.0	4.6	3.8	3.4	0.027
Regular exercise (%)	10.2	10.2	10.9	10.9	0.756
Atrial fibrillation (%)	0.09	0.05	0.04	2.24	<0.001
Hemoglobin (g/dL)	13.2 ± 1.1	13.1 ± 1.1	13.0 ± 1.1	12.8 ± 1.1	<0.001
Creatinine (mg/dL)	0.61 ± 0.10	0.63 ± 0.11	0.63 ± 0.10	0.66 ± 0.15	<0.001

Continuous variables are expressed as mean ± SD.

Table 3
The crude incidence and multivariate hazard ratio of ischemic stroke among BNP quartiles in men.

BNP quartiles	Q1	Q2	Q3	Q4	*P* for trend
Observed person-years	3619	3198	3116	3036	
Ischemic stroke					
Crude incidence (/1000 person-years)	2.76	2.19	3.21	12.51	
Multivariate HR (95%CI)					
Model 1	1.0 (ref.)	0.71 (0.27–1.89)	0.85 (0.34–2.12)	2.83 (1.29–6.20)	<0.001
Model 2	1.0 (ref.)	0.71 (0.27–1.88)	0.81 (0.33–2.03)	2.38 (1.07–5.29)	<0.005

For all models, the hazard ratios were adjusted for age, presence or absence of hypertension, hyperlipidemia, diabetes, smoking, regular alcohol consumption, and regular exercise. BMI, blood hemoglobin levels, and serum creatinine levels.
Model 1: The analysis was not adjusted for presence or absence of atrial fibrillation.
Model 2: The analysis was adjusted for presence or absence of atrial fibrillation.

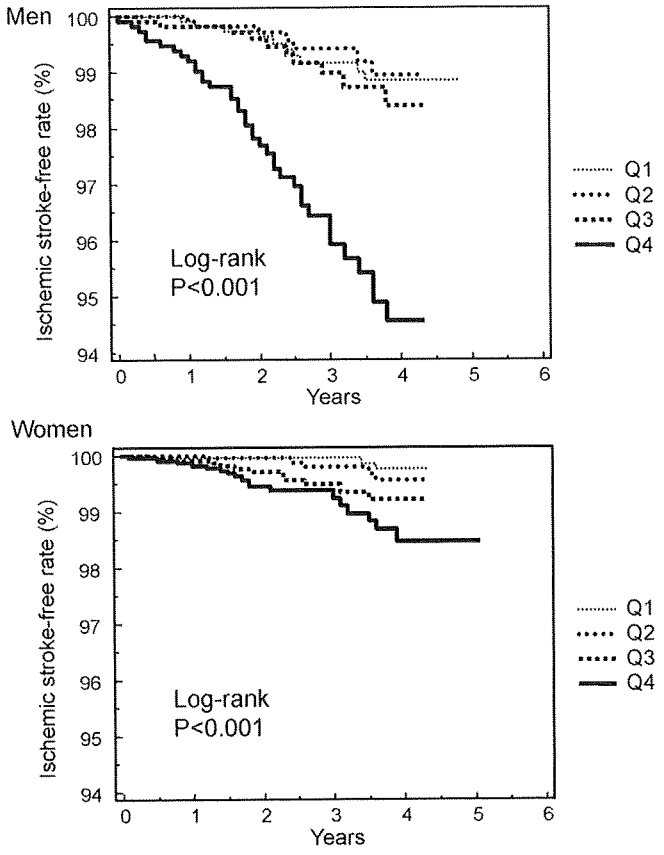

Fig. 1. Kaplan–Meier curves for ischemic stroke event free rate according to BNP quartiles by sex.

factors, blood hemoglobin levels, serum creatinine levels, and BMI, a multivariate Cox regression analysis was performed to analyze the relationship between plasma BNP levels and the risk of stroke. In men, the hazard ratio (HR) obtained from a Cox proportional model for ischemic stroke in the highest BNP quartile was significantly elevated in Model 1 (HR = 2.83; 95% CI = 1.29–6.20; Table 3). After also adjusting for the presence or absence of atrial fibrillation (Model 2), HR in the highest BNP quartile was still significantly elevated (HR = 2.38; 95% CI = 1.07–5.29; Table 3). The risk of incidence of ischemic stroke increased in association with BNP levels ($P < 0.01$). In women, HR for ischemic stroke in the highest BNP quartile was significantly elevated in Model 1 (HR = 3.61; 95% CI = 1.01–12.93; Table 4). After adjusting for the presence or absence of atrial fibrillation (Model 2), the relationship between plasma BNP levels and the risk of ischemic stroke was of marginal significance (HR = 3.03; 95% CI = 0.84–10.92, $P = 0.09$; Table 4).

An additional multivariate Cox regression analysis was performed using 1 SD increments in natural logarithm-transformed BNP values. Elevated plasma BNP levels were associated with an elevated risk of ischemic stroke in both men and women (HR = 1.70; 95% CI = 1.17–2.45 in men: HR = 1.69; 95% CI = 1.04–2.75 in women).

4. Discussion

There have been very few reports on the association between plasma BNP levels and the risk of stroke, [15,16] and the relationship with risk of stroke subtypes therefore remains unclear. The present study suggests that high plasma BNP levels predict the risk of ischemic stroke within the general Japanese population. Ischemic stroke is classified into atherothrombotic infarction, cardiogenic embolic infarction, and lacunar infarction. Several types of structural heart diseases including atrial fibrillation, which are associated with elevated plasma BNP levels, may be an important cause of ischemic stroke, especially cardioembolic stroke. In view of this, elevated plasma BNP levels may be a biomarker for high risk of ischemic stroke.

Cardiac disorders linked with ischemic stroke, especially cardioembolic stroke, are nonvalvular atrial fibrillation, acute

Table 4
The crude incidence and multivariate hazard ratio of ischemic stroke among BNP quartiles in women.

BNP quartiles	Q1	Q2	Q3	Q4	*P* for trend
Observed person-years	6794	6283	6188	6099	
Ischemic stroke					
Crude incidence (/1000 person-years)	0.44	0.80	1.78	2.95	
Multivariate HR (95%CI)					
Model 1	1.0 (ref.)	1.72 (0.41–7.25)	3.07 (0.84–11.16)	3.61 (1.01–12.93)	0.168
Model 2	1.0 (ref.)	1.79 (0.43–7.55)	3.15 (0.87–11.44)	3.03 (0.84–10.92)	0.269

For all models, the hazard ratios were adjusted for age, presence or absence of hypertension, hyperlipidemia, diabetes, smoking, regular alcohol consumption, and regular exercise. BMI, blood hemoglobin levels, and serum creatinine levels.
Model 1: The analysis was not adjusted for presence or absence of atrial fibrillation.
Model 2:The analysis was adjusted for presence or absence of atrial fibrillation.

myocardial infarction, ventricular aneurysm, and valvular heart disease. According to the Cerebral Embolism Task Force [14], nonvalvular atrial fibrillation is the most common cardiac disorder associated with embolic stroke, accounting for 45% of embolic strokes. Several previous studies have suggested that plasma BNP levels were significantly higher in patients with atrial fibrillation than in those without atrial fibrillation [11,13]. The Framingham Heart Study [15] has indicated that higher plasma BNP levels predict risk of atrial fibrillation. It is therefore possible that atrial fibrillation-related high plasma BNP levels are associated with increased risk of ischemic stroke. We therefore analyzed the relationship between plasma BNP levels and risk of ischemic stroke after adjusting for the presence or absence of atrial fibrillation. Even after this adjustment, HR was still significant in men. This suggests that there may be factors other than atrial fibrillation underlying the apparent relationship between plasma BNP levels and risk of ischemic stroke. As the present study did not perform echocardiography as a baseline examination, some subjects may have had asymptomatic structural heart disease (i.e. left ventricular dysfunction, valvular heart disease, or left ventricular hypertrophy) characterized by elevated plasma BNP [12] which would account for the significant relationship between plasma BNP levels and risk of ischemic stroke. This study was therefore unable to show a correlation between plasma BNP levels and risk of stroke independent of the presence of heart disease. However, it is difficult to perform echocardiography routinely for participants in a community-based multiphasic health checkup. A simple blood test for BNP is an ideal approach for selecting males at high risk for ischemic stroke within the general population. In addition, a previous study examining the relationship between traditional and nontraditional risk factors and the incidence of ischemic stroke subtypes has reported that left ventricular hypertrophy increases the risk not only of cardioembolic stroke but also of atherothrombotic stroke [24]. It follows that high plasma BNP levels may be associated with both cardioembolic and atherothrombotic stroke.

The present study has shown a median plasma BNP level of 14.8 pg/mL and the threshold plasma BNP levels associated with elevated risk of ischemic stroke of 30.0 pg/mL in men. The Framingham Heart Study [15] found a median plasma BNP level of 6.2 pg/mL and the threshold plasma BNP levels associated with elevated risk of stroke or transient ischemic attack of 20.0 pg/mL in a community-based male sample. Both studies have shown that excess risk is apparent at plasma BNP levels well below the thresholds currently used to diagnose heart failure [25].

A possible reason for the marginal significance of the relationship between plasma BNP levels and risk of ischemic stroke in women after adjusting for the presence or absence of atrial fibrillation may be the low incidence of stroke in the female cohort. The crude incidences of stroke in women were clearly lower than those in men, and thus, the statistical power to show any relationship between risk and incidence of stroke might be limited in women. As the statistical results concerning the relationship between plasma BNP levels and risk of stroke in women were not so robust, more events should be gathered to investigate the predictive power of plasma BNP with regard to stroke in women.

Although our study was a large, prospective community-based longitudinal study, several limitations must be considered when interpreting the results. Since ECG testing was performed only at the time of baseline survey, paroxysmal atrial fibrillation had not been detected and new incidence of atrial fibrillation was not captured after the baseline survey. Hence the impact of atrial fibrillation on the association between plasma BNP levels and risk of ischemic stroke may not have been accurately estimated. In addition, since the attending physicians participating in the registration survey were not all neurological specialists, the diagnosis of stroke subtypes was occasionally carried out by general physicians. However, since most of the patients registered were diagnosed using computed tomography or magnetic resonance imaging, the differential diagnosis between ischemic stroke and hemorrhagic stroke was made correctly.

In conclusion, this community-based study has shown that elevated plasma BNP levels predict the risk of ischemic stroke within Japanese men from the general population. This suggests that a simple blood test for BNP is an ideal approach for selecting men at high risk for ischemic stroke within the general population.

Acknowledgements

This study was supported in part by the Japan Arteriosclerosis Prevention Fund, and grants-in-aid for the scientific research fund of the Ministry of Education Science and Culture (18590786 and 20590836), and Ministry of Health and Welfare (17120501) of Japan, The authors thank the Iwate Stroke Registration Association for permission to use regional stroke registry data.

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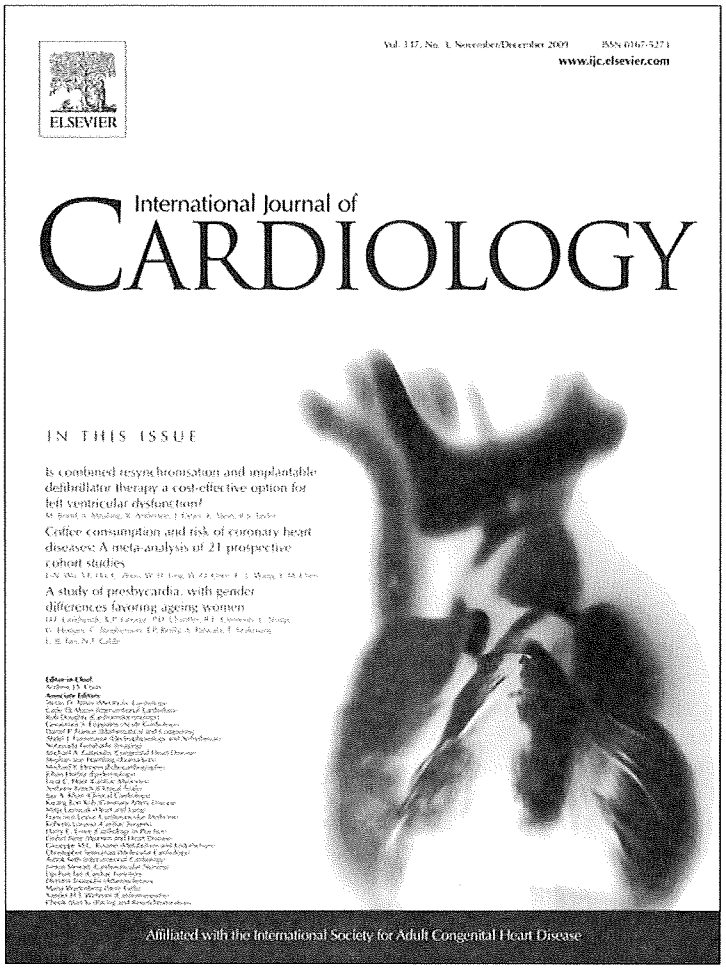

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International Journal of Cardiology 137 (2009) 226–235

International Journal of Cardiology

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Cardiovascular risk factors in the Japanese northeastern rural population ☆

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Received 7 December 2007; received in revised form 25 May 2008; accepted 28 June 2008
Available online 15 August 2008

Abstract

Background: People living in the northeastern part of Japan have high prevalences of hypertension and stroke. The current status of cardiovascular risk factors in them should be elucidated.
Methods: The survey was carried out from 2002 to 2004 in the northeastern part of the main island of Japan. A total of 26,472 Japanese men and women were enrolled (acceptance rate: 84.5%). Sex- and age-specific prevalences of cardiovascular risk factors were determined. Mean values of predictive markers (high-sensitivity C reactive protein (hsCRP), brain natriuretic peptide (BNP) and microalbuminuria) were also determined in each group. Risk factor-related variables in non-hypertensive subjects and hypertensive subjects were compared.
Results: Proportions of subjects with hypertension, diabetes and dyslipidemia were 46.0%, 7.6%, and 30.3%, respectively, in males and 38.6%, 4.0%, and 38.5%, respectively, in females. Mean values of hsCRP and BNP were 1.41 mg/L and 26.5 pg/mL, respectively, in males and 1.01 mg/L and 23.7 pg/mL, respectively, in females. Proportions of male and female subjects with microalbuminuria were 22.0% and 23.4%, respectively. These markers become higher with advance of age. Prevalence of atrial fibrillation was 1.56%, and it increased with advance of age in both men and women. High prevalences of cardiovascular risk factors in this area were found. Hypertensive subjects who did not take anti-hypertension medication accounted for about 20% of total subjects and their blood pressure remained poorly controlled.
Conclusion: Attention should be given to cardiovascular risk factors in the Japanese northeastern rural population.

Keywords: Cardiovascular risk factors; C-reactive protein; Brain natriuretic peptide; Microalbuminuria; Atrial fibrillation; The Iwate-KENCO study

1. Introduction

People living in the northeastern part of the main island of Japan (Tohoku area) have high prevalences of hypertension and stroke compared with those in people living in other areas [1,2] and they have a large intake of salt [3]. Attention should be given to the current status of cardiovascular risk factors in people living in this area of Japan.

☆ The study was supported by grants from the Open Translational Research Center Project, Advanced Medical Science Center, Iwate Medical University, the Japan Arteriosclerosis Prevention Fund, the Ministry of Education, Culture, Sports, Science and Technology of Japan, and the Ministry of Health, Labour and Welfare of Japan.

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doi:10.1016/j.ijcard.2008.06.052

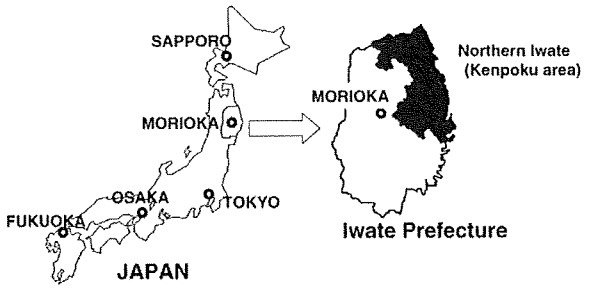

Fig. 1. The study area. This figure shows a map of Japan and a map of Iwate Prefecture. Iwate Prefecture is located in the northeastern part of the main island (Honshu Island) of Japan. The black area of Iwate Prefecture indicates the study area. Kenpoku means northern part of the prefecture in Japanese.

We have conducted a population-based prospective cohort study in the northeastern part of Japan. The aims of the present study were to determine the age- and sex-specific prevalences of cardiovascular diseases (CVD) and their risk factors by a cross-sectional analysis of data from the initial survey. We also compared cardiovascular risk factor-related characteristics in hypertensive subjects and non-hypertensive subjects to clarify the status of clustering risk factors in hypertensive subjects.

2. Subjects and methods

2.1. Study subjects

The "Iwate KENpoku COhort Study (Iwate-KENCO Study)" is a population-based prospective study in people living in the northeastern part of the main island of Japan (Fig. 1). The initial surveys were carried out from 2002 to 2004. Each survey was conducted from April to November. The study area is a typical rural area of Japan with a low move-out/move-in population, high proportion of people engaged in primary industry (18.4%) [4] and high proportion of elderly people (people aged 65 years or more: 26.2% of the total population). The study area consists of 17 municipalities, and the total population of the region in 2002 was 241,057. Invitations to multiphasic health screening were issued by government offices in the municipalities. A total of 31,318 people (11,003 men and 20,315 women) aged 18 years or older participated in annual health check-ups from 2002 to 2004 in the study area. Of those participants, 26,472 men and women gave written informed consent for participation in this study (acceptance rate: 84.5%). Sex- and age-specific numbers and proportions of participating subjects and acceptance rates are shown in Table 1. The study was approved by the Medical Ethics Committee of Iwate Medical University and conducted in accordance with the guidelines of the Declaration of Helsinki.

2.2. Measurements

Measurements of blood pressure were performed by well-trained staff. Weight was measured with an automated scale (TANITA digital scale Model BWB-200). Height was measured using a digital handle scale (YAGAMI model 48525YG-200D). Blood pressure was measured twice in the sitting position using an automatic device (BP-103i IIModel 513000, Nippon Colin, Komaki, Japan) after urination and a five-minute rest. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were each calculated as the mean of two measurements. Body mass index (BMI) was calculated as weight (kg) divided by the square of body height (m).

Self-administered questionnaires on demographic characteristics, history of cardiovascular disease and apoplexy, drug use, alcohol consumption, smoking, and exercise habit were used to collect individual information. Details were described previously [5].

A nutrition survey was carried out in each municipality. This survey was an optional survey and was carried out at each participant's own discretion (executing rate: 72.4%). Dietary habits during the previous month were assessed using a brief

Table 1
Age- and sex-specific numbers of participants, acceptance rates, and proportions of total population in the study area.

Age group	18–29	30–39	40–49	50–59	60–69	70–79	≥80	Total
Total population in the study area (*n*)	18,692	26,036	29,850	35,001	33,673	29,301	14,494	233,307
Participants of check-ups (*n*)	330	1302	3289	6449	11,038	8115	1078	31,318
Participants of the study (*n*)	266	1064	2793	5537	9376	6869	797	26,472
Acceptance rate (%)	80.6%	81.7%	84.9%	85.9%	84.9%	84.6%	73.9%	84.5%
Proportion of total population (%)	1.4%	4.1%	9.4%	15.8%	27.8%	23.4%	5.5%	11.3%
Total male population (*n*)	9326	12,940	15,019	17,113	15,081	12,475	4379	109,749
Participants of check-ups (*n*)	108	367	1005	1841	3930	3345	498	11,003
Participants of the study (*n*)	83	296	813	1520	3281	2863	385	9162
Acceptance rate (%)	76.9%	80.7%	80.9%	82.6%	83.5%	85.6%	77.3%	83.3%
Proportion of total population (%)	0.9%	2.3%	5.4%	8.9%	21.8%	22.9%	8.8%	8.3%
Total female population (*n*)	9366	13,096	14,831	17,888	18,592	16,826	10,115	123,558
Participants of check-ups (*n*)	214	935	2284	4608	7108	4770	580	20,315
Participants of the study (*n*)	180	768	1980	4017	6095	4006	412	17,310
Acceptance rate (%)	84.1%	82.1%	86.7%	87.2%	85.7%	84.0%	71.0%	85.2%
Proportion of total population (%)	1.9%	5.9%	13.4%	22.5%	32.8%	23.8%	4.1%	14.0%

Data are expressed as numbers or percentages.

self-administered diet history questionnaire (BDHQ). This is a 4-page structured questionnaire that consists of three sections: general dietary behavior and main cooking methods, consumption frequencies and amounts of intake of 5 alcoholic beverages, and frequencies of consumption of 50 selected food and nonalcoholic beverage items. Estimates of dietary intake of 48 food and beverage items, energy and nutrients were calculated using an ad hoc computer algorithm for the BDHQ, which was mainly based on the Standard Tables of Food Composition in Japan [6]. Results of validation study for the BDHQ were previously described in detail [7].

A resting 12-lead electrocardiogram was recorded in each participant after a five-minute rest. The electrocardiographic findings were independently evaluated by a trained clinical technician and a medical doctor in Iwate Health Service Association according to the original coding system developed by Iwate Health Association. In this study, sex- and age-specific prevalences of atrial fibrillation (AF) were determined. AF was defined according to the original coding system (including paroxysmal atrial fibrillation and atrial flutter).

2.3. Biochemical analyses

Casual blood samples were drawn from antecubital veins of seated participants. The samples were transported to a laboratory (Iwate Health Service Association) and analyzed on the same day.

Total cholesterol (TC) levels were determined by an enzymatic assay, triglyceride (TG) levels were determined by an enzyme-colorimetric assay, high-density lipoprotein cholesterol (HDLC) levels and low-density lipoprotein cholesterol (LDLC) levels were determined by a direct quantitative assay, and plasma glucose levels were determined by the hexokinase ultraviolet method. All of the above biochemical data were analyzed using an automated analyzer (HITACHI 7700). Glycosylated hemoglobin (HbA_{1c}) levels were determined by high-performance liquid chromatography using an automated analyzer (TOSOH HLC-723G7 Japan). Determinations of TC levels and HDLC levels were performed under the quality control program of the Center for Disease Control in the United States [5].

Serum levels of high-sensitivity C-reactive protein (hsCRP) were determined by the latex-enhanced immunonephelometric method (Dade Behring Diagnostics, Germany) with a threshold of 0.1 mg/L. In this estimation, hsCRP values under the minimum detectable level were regarded as being 0.1 mg/L. Plasma brain natriuretic peptide (BNP) levels were measured by a direct radioimmunoassay using monoclonal antibodies specific for human BNP (Shiono RIA BNP kit, Shionogi and Co., Ltd., Japan). Plasma BNP assays were performed for 65.6% of the subjects in the study. The method for measuring plasma BNP levels was previously described in detail [8].

Urine albumin was assessed quantitatively by an immunonephelometric method (N-antiserum albumin, Dade Behring) and urine creatinine was measured quantitatively by an enzymatic colorimetric test [9]. The urine albumin–creatinine ratio (UACR) was used since the accuracy of the ratio in comparison to a 24-hour urine sample has been demonstrated in previous studies [10,11].

2.4. Classification and definition

To examine to what extents traditional risk factors, dietary intake of nutrients and new predictive markers (hsCRP, BNP, and urine albumin) are associated with age in a cross-sectional analysis, we divided the participants into age-specific groups (18–29, 30–39, 40–49, 50–59, 60–69, 70–79, 80 years or older) for both sexes. Hypertension was defined as SBP being 140 mmHg or higher, DBP being 90 mmHg or higher, use of antihypertensive agents or a combination of these. Diabetes mellitus (DM) was defined as plasma glucose level being 200 mg/dL or higher, plasma HbA_1c level being 6.5% or higher, use of anti-diabetes agents or a combination of these. Dyslipidemia was defined as serum TC level being 220 mg/dL or higher, serum HDLC level being less than 40 mg/dL, use of anti-hyperlipidemia agents or a combination of these. In current drinkers, regular alcohol drinking was defined as drinking five days or more per week and occasional drinking was defined as drinking less than five days per week. In non-current drinkers, subjects were divided into past drinkers and non-drinkers. Regular exercise was defined as doing exercise for at least 60 min eight days or more per month, and exercise habit was defined as doing exercise for at least 60 min per month. Overweight was defined as BMI being 25 kg/m^2 or more and obesity was defined as BMI being 30 kg/m^2 or more.

In most previous studies, subjects with high CRP level (10 mg/L or higher) were excluded to avoid analysis of data from subjects who had developed apparent inflammatory disease [12]. Both mean hsCRP level in all subjects and that in subjects after excluding subjects with hsCRP levels greater than 10 mg/L are shown in this study. We defined high BNP level as 50 pg/mL or more according to our previous study [8]. Macroalbuminuria was defined as UACR being 300 mg/g or more, and microalbuminuria was defined as UACR being 30 mg/g or more and less than 300 mg/g. To estimate the proportion of participants with microalbuminuria, subjects with macroalbuminuria were excluded.

2.5. Statistical analysis

Prevalences of risk factors were determined in each age- and sex-specific group. Mean values (standard deviations) of risk factor-related variables were also determined in each group. Linear trend tests were used to examine the association between age and each variable after adjusting for other traditional risk factors (SBP, BMI, TC, HDLC, HbA_{1c}, and current smoking status). Comparisons of hsCRP levels, BNP levels, and urinary albumin levels in men and women were performed using the Mann–Whitney *U* test. The chi-square test was used to compare the proportions of subjects between the groups. Sex difference in the prevalence of AF was tested after direct age adjustment.

Age-adjusted SBP, TC, HDLC, LDLC, and HbA_{1c} were compared between the three groups according to presence of hypertension (non-hypertensive subjects, hypertensive subjects with medication, hypertensive subjects without medication) using analysis of covariance (ANCOVA). Prevalences of overweight, obesity, DM, and dyslipidemia were also compared between the three groups using age-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) by logistic regression analysis.

All p values were based on two-sided tests, and p values less than 0.05 were considered to be statistically significant. The Statistical Package for Social Sciences (SPSS Japan Inc., version 14.0, Tokyo) was used for the analyses.

3. Results

Table 2 shows age- and sex-specific characteristics of participants with regard to demographic, biochemical and comorbid conditions. SBP and HbA_{1c} levels were higher with advance of age in both sexes (trend $p<0.01$). Prevalences of hypertension and DM were higher with advance of age in both sexes (trend $p<0.01$). The proportions of subjects with hypertension were more than 50% in males aged 50 years or older and in females aged 60 years or older. The proportions of subjects with dyslipidemia were about 30% in middle-aged males and about 40% in females aged 50 years or older. Prevalences of myocardial infarction and stroke were very low in both sexes.

Table 3 shows age- and sex-specific proportions of subjects with a smoking habit, drinking habit, and exercise habit. The proportion of current smokers in males aged 49 years or younger was more than 50%. The proportion of current smokers was very low in the female subjects, but it exceeded 15% in females aged 39 years or younger. The proportion of regular drinkers in middle-aged male subjects

Table 2
Age- and sex-specific prevalences of cardiovascular diseases and mean levels of their risk factor-related variables.

Age group Men (n)		18–29	30–39	40–49	50–59	60–69	70–79	≥80	Total	Trend	Sex difference
Men (n)		86	214	813	1520	3281	2863	385	9162		
BMI	(kg/m^2)	22.4 (3.8)	24.2 (3.5)	24.1 (3.1)	24.3 (3.0)	24.1 (2.9)	23.6 (3.0)	23.0 (2.9)	23.9 (3.0)		‡
BMI≥25		25.6%	36.0%	34.9%	39.1%	36.3%	30.9%	21.3%	34.2%		
BMI≥30		5.8%	5.6%	4.2%	4.2%	2.8%	2.2%	0.8%	3.0%		
SBP	(mmHg)	114.2 (11.6)	119.9 (15.7)	122.1 (16.4)	127.5 (19.0)	131.9 (19.7)	133.8 (19.5)	136.9 (20.7)	130.7 (19.6)	†	‡
TC	(mg/dL)	171.7 (35.6)	192.3 (36.7)	197.1 (36.2)	195.8 (32.2)	191.4 (32.0)	188.0 (31.3)	184.2 (30.4)	191.1 (32.5)		
TG	(mg/dL)	122.4 (85.6)	144.0 (97.1)	154.4 (106.6)	135.7 (93.5)	124.6 (83.3)	113.1 (68.8)	104.3 (54.1)	125.1 (83.6)		
HDLC	(mg/dL)	53.7 (13.4)	55.3 (13.9)	56.4 (15.6)	56.8 (15.5)	56.1 (15.4)	55.5 (15.2)	54.3 (13.4)	56.0 (15.2)		
LDLC	(mg/dL)	102.1 (33.5)	116.7 (32.7)	117.3 (32.5)	116.3 (29.4)	113.4 (29.4)	111.9 (27.6)	109.7 (27.5)	113.6 (29.3)		
PG	(mg/dL)	92.8 (14.6)	99.0 (30.1)	107.8 (35.9)	113.4 (35.4)	115.8 (34.6)	116.6 (36.7)	117.6 (34.5)	114.4 (35.5)	†	‡
HbA_{1C}	(%)	4.68 (0.30)	4.81 (0.49)	4.99 (0.81)	5.12 (0.74)	5.18 (0.73)	5.20 (0.74)	5.17 (0.63)	5.14 (0.74)	†	
MI		0.0%	0.0%	0.0%	0.1%	0.8%	1.4%	1.3%	0.8%		‡
Stroke		0.0%	0.0%	0.1%	0.3%	0.4%	0.7%	0.3%	0.4%		‡
DM		0.0%	0.9%	3.8%	6.7%	8.4%	9.1%	7.8%	7.6%	†	‡
HTN		0.0%	10.7%	21.4%	35.4%	50.0%	55.9%	61.6%	46.0%	†	‡
DL		22.1%	30.4%	33.0%	33.3%	30.1%	29.0%	27.0%	30.3%		
Women (n)		180	620	1980	4017	6095	4006	412	17,310		
BMI	(kg/m^2)	21.7 (4.3)	22.5 (3.7)	23.4 (3.6)	24.0 (3.4)	24.3 (3.4)	24.3 (3.5)	24.0 (3.5)	24.0 (3.5)		
BMI ≥25	(%)	13.9%	22.1%	28.0%	35.1%	39.9%	40.4%	34.8%	36.5%		
BMI ≥30	(%)	6.7%	4.8%	5.3%	5.5%	5.5%	6.0%	3.5%	5.5%		
SBP	(mmHg)	102.1 (11.1)	107.5 (14.1)	115.1 (16.8)	121.9 (19.3)	127.9 (19.4)	132.3 (19.6)	135.3 (20.7)	125.2 (20.1)	†	
TC	(mg/dL)	167.8 (29.0)	176.5 (30.0)	192.3 (31.6)	209.6 (32.7)	209.4 (30.8)	206.3 (30.3)	201.2 (33.1)	205.0 (32.4)		
TG	(mg/dL)	75.7 (69.3)	89.3 (62.5)	98.2 (77.4)	112.1 (68.3)	117.5 (64.6)	117.5 (62.7)	113.2 (54.5)	112.5 (66.9)		
HDLC	(mg/dL)	62.7 (14.6)	63.3 (14.1)	63.6 (14.5)	63.0 (14.4)	60.4 (14.2)	59.6 (14.3)	58.6 (13.4)	61.2 (14.4)		
LDLC	(mg/dL)	95.1 (26.7)	100.8 (26.1)	113.1 (28.2)	126.1 (29.7)	127.0 (27.8)	124.8 (27.0)	121.5 (28.1)	123.3 (28.9)		
PG	(mg/dL)	90.7 (11.9)	94.1 (14.3)	100.7 (22.2)	104.4 (25.0)	108.0 (26.9)	110.9 (28.3)	116.6 (33.6)	106.5 (26.5)	†	
HbA_{1C}	(%)	4.65 (0.28)	4.75 (0.41)	4.88 (0.52)	5.08 (0.64)	5.16 (0.66)	5.21 (0.62)	5.23 (0.72)	5.10 (0.63)	†	
MI		0.0%	0.0%	0.0%	0.0%	0.2%	0.6%	1.7%	0.3%		
Stroke		0.0%	0.2%	0.2%	0.2%	0.3%	0.2%	0.7%	0.2%		
DM		0.0%	0.2%	1.8%	3.0%	4.3%	5.9%	7.5%	4.0%	†	
HTN		0.6%	4.2%	12.3%	28.5%	43.5%	58.7%	63.8%	38.6%	†	
DL		8.9%	9.4%	20.9%	41.0%	44.2%	42.2%	35.9%	38.5%		

Data are expressed as means (standard deviations) or percentages. †, $p<0.05$ by linear trend test. ‡ means significantly higher than that in the other sex, and p value (<0.05) was estimated by Student's t-test or the chi square test. Abbreviations: BMI, body mass index; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; HDLC, high-density lipoprotein cholesterol level; LDLC, low-density lipoprotein cholesterol level; PG, casual plasma glucose; HbA_{1c}, percentage of glycosylated hemoglobin; MI, myocardial infarction; DM, diabetes mellitus; HTN, hypertension; DL, dyslipidemia.

Table 3
Age- and sex-specific cardiovascular risk factors: proportions of subjects with smoking, alcohol drinking, and exercise habits.

Age group	18–29	30–39	40–49	50–59	60–69	70–79	≥80	Total	Trend	Sex difference
Men (*n*)	86	214	813	1520	3281	2863	385	9162		
Smoking status										
Current	57.0%	58.9%	55.0%	41.4%	27.6%	21.9%	16.6%	31.1%	†	‡
Ex-smoker	4.7%	14.0%	23.0%	25.5%	31.0%	38.0%	37.1%	31.2%	†	‡
Non-smoker	38.4%	27.1%	22.0%	33.2%	41.5%	40.1%	46.2%	37.8%		
Drinking habit										
≥5 days/week	26.7%	51.4%	55.2%	54.9%	46.1%	38.1%	29.4%	45.1%		‡
<5 days/week	32.6%	26.6%	23.1%	22.8%	24.0%	20.7%	17.7%	22.6%		‡
Non-drinker	39.5%	17.8%	19.4%	18.0%	21.1%	28.3%	40.0%	23.6%		
Ex-drinker	1.2%	4.2%	2.2%	4.3%	8.9%	12.9%	13.0%	8.8%		‡
Exercise habit										
≥60 min*8 times/month	17.4%	8.4%	5.3%	9.8%	20.0%	21.2%	22.9%	17.2%		‡
≥60 min/month	37.2%	29.9%	25.7%	30.2%	40.6%	42.9%	41.6%	38.0%		
Women (*n*)	180	620	1980	4017	6095	4006	412	17,310		
Smoking status										
Current	21.7%	15.2%	7.0%	3.4%	1.1%	0.7%	0.0%	2.9%	†	
Ex-smoker	11.7%	9.4%	4.4%	1.2%	0.6%	0.5%	0.5%	1.6%	†	
Non-smoker	66.7%	75.5%	88.6%	95.4%	98.4%	98.8%	99.5%	95.5%		
Drinking habit										
≥5 days/week	7.8%	11.9%	9.8%	4.5%	3.0%	1.9%	2.9%	4.2%	†	
<5 days/week	34.4%	36.5%	27.4%	19.1%	11.4%	6.7%	4.4%	14.9%	†	
Non-drinker	48.9%	48.1%	60.9%	74.4%	84.5%	90.4%	91.3%	79.3%		
Ex-drinker	8.9%	3.5%	1.9%	2.0%	1.1%	0.9%	1.5%	1.5%		
Exercise habit										
≥60 min*8 times/month	11.1%	6.3%	7.1%	10.8%	12.1%	10.2%	11.4%	10.6%		
≥60 min/month	26.1%	26.1%	27.1%	33.9%	35.8%	33.0%	29.6%	33.1%		

Proportions are expressed as percentages.
†and ‡ are explained in Table 2.

was more than 50%. The proportions of subjects doing regular exercise were 17.2% in males and 10.6% in females. Among persons aged 30 to 59 years, the proportion of subjects doing regular exercise was less than 10% in both sexes.

Table 4 shows age- and sex-specific mean levels of daily dietary intake of nutrients. The most notable characteristic in this study population is a very high level of dietary intake of salt in middle-aged and elderly people. Dietary intake of salt was about 13 g/day in males aged 39 years or younger and became higher with advance of age, exceeding 16 g/day in males aged 60 years or older. Dietary intake of salt was about 10 g/day in females aged 39 years or younger. It also became higher with advance of age and exceeded 13 g/day in females aged 60 years or older.

Mean dietary intake of carbohydrate (percent of total energy) was about 55% in both sexes. Mean dietary intake of fat was about 23% in males and it was 25% in females. Dietary intake of saturated fatty acid was about 6% in males and it was about 7% in females. Dietary intake of monounsaturated fatty acid was about 8% in males and it was about 9% in females. Ratios of n-6PUFA to n-3PUFA in the diet were 3.3 in males and 3.4 in females. The ratio exceeded 4.0 in subjects aged 39 years or younger in both sexes, but the ratio became lower with advance of age (trend $p<0.05$).

Table 5 shows age- and sex-specific mean levels of hsCRP, BNP, urinary albumin , and UACR. Mean hsCRP levels were 0.92 mg/L in males and 0.75 mg/L in females after excluding subjects with apparent inflammation. Levels of hsCRP were positively associated with age in both sexes (trend $p<0.01$). Levels of hsCRP in males were higher than those in females ($p<0.05$).

BNP levels were positively associated with age in both sexes (trend $p<0.01$). Crude BNP levels were higher in men than women in total subjects ($p<0.01$), but they were lower in male subjects aged less than 60 years than in females aged less than 60 years ($p<0.01$). Our data showed that about 16% of total subjects aged 60–69 years, 20% of total subjects aged 70–79 years and more than 40% of total subjects aged 80 years or more had BNP levels of 50 pg/mL or higher in both sexes.

Mean crude urinary albumin concentration and mean UACR in the male subjects were 45.6 mg/L and 54.7 mg/g, respectively, and those in females were 25.2 mg/L and 39.5 mg/g, respectively. Macroalbuminuria was seen in 3.1% of total male subjects and in 1.6% of female subjects. After excluding subjects with macroalbuminuria, proportions of subjects with microalbumimnuria were less than 10% in the 18 to 39 years age group and 10–20% in the 40 to 59 years age group in both sexes. Both prevalence of microalbuminuria and