Association between Serum C-reactive Protein Levels and Microalbuminuria: A Population-Based Cross-Sectional Study in Northern Iwate, Japan

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Abstract

Objective **The presence of microalbuminuria is a renal marker of vascular endothelial damage, and is an independent and strong predictor of increased risk for cardiovascular mortality and morbidity. Elevated circulating C-reactive protein (CRP) levels have recently been reported to be a novel cardiovascular risk factor, and it has been suggested that this acute-phase protein impairs vascular endothelial function. The aim of the present study was to determine whether serum CRP level is a dependent or an independent risk factor of microalbuminuria in the general population.**

Methods **Subjects of this cross-sectional study were apparently healthy individuals drawn from the general Japanese population (mean age, 62; men, 2,236; women, 4,217). Serum CRP levels were determined using a highly sensitive kit and urine albumin-creatinine ratio (UACR) was calculated using a single urine sample. Multivariate logistic regression analysis was used to determine which risk factors (ie, age, hypertension, diabetes, obesity, hypercholesterolemia, smoking, and CRP) might predict the presence of microalbuminuria.**

Results **In addition to classical cardiovascular risk factors such as age, hypertension, diabetes and obesity, serum CRP levels are also significantly correlated with microalbuminuria in men (odds ratio=1.42, 95% CI= 1.13–1.79; $p<0.01$) and women (odds ratio=1.25, 95% CI=1.05–1.49; $p<0.01$). When subjects with diabetes were excluded from the analysis, serum CRP levels continued to be a significant predictor for microalbuminuria (odds ratio=1.35, 95% CI=1.06–1.73; $p<0.05$ for men: odds ratio=1.23, 95% CI=1.03–1.47; $p<0.05$ for women).**

Conclusions **The present study has shown that low-grade inflammation as represented by high sensitivity CRP levels may be significantly related to the presence of microalbuminuria. This suggests that microalbuminuria may be a useful marker representing systemic low-grade inflammation as well as being an established cardiovascular risk factor in apparently healthy individuals.**
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Key words: C-reactive protein, microalbuminuria, general population, risk, atherosclerosis

Introduction

Several epidemiological and clinical studies have demonstrated that the presence of microalbuminuria is an independent and strong predictor of increased risk for cardiovascular mortality and morbidity in certain high-risk groups such as patients with diabetes and hypertension (1–3). In the general population, Hillege et al have recently reported that increased urinary albumin excretion as well as microalbuminuria were associated with increased cardiovascular and non-cardiovascular mortality, independent of other classical cardiovascular risk factors (4). These observations suggest that microalbuminuria is the renal expression of general vascular damage and a marker of atherosclerosis not only in specific subjects with an accumulated cardiovascular risk but also in a subjects with relatively lower risks.

Several nested case-control studies have shown that circulating levels of C-reactive protein (CRP) are correlated with an increased risk of myocardial infarction (5), stroke (6), pe-

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ripheral arterial disease (7), and sudden cardiac death (8). A recent study has shown that in a general female population serum CRP levels are a strong predictor of cardiovascular events independent of traditional major risk factors (9). In in vitro studies, exogenously applied CRP has been reported to impair vascular endothelial expression of nitric oxide synthase (10, 11), and to upregulate endothelial expression of chemokines and adhesion molecules (12, 13). As the presence of microalbuminuria is a marker of vascular endothelial damage (14–16), it might be expected that the degree of urinary albumin excretion is enhanced by elevated circulating levels of CRP.

However, few previous studies have examined the relationship between microalbuminuria and serum CRP levels in the general population. In order to search for a new cardiovascular risk screening strategy, it will be necessary to examine the relationships among established risk factors and contemporary candidates. This cross-sectional study therefore examined the correlation between microalbuminuria and serum CRP levels after adjustment for established risk factors in apparently healthy Japanese men and women.

Table 1. Comparisons of Baseline Characteristics between Men and Women

	Men	Women
Number	2,236	4,217
Age (yrs)	62.7±10.2	61.8±9.7
Systolic BP (mmHg)	128.6±18.9	125.7±19.9
Diastolic BP (mmHg)	77.2±10.5	73.8±10.8
Hypertension (%)	40.1	37.3
Body mass index (kg/m^2)	23.7±2.9	24.1±3.4
Obesity (%)	8.1	16.9
Smoker (%)	35.9	2.1
Diabetes (%)	7.2	4.6
Serum creatinine (mg/dl)	0.81±0.15	0.63±0.11
Serum total cholesterol (mg/dl)	196.0±33.2	209.9±31.7
Hypercholesteloremia (%)	6.7	13.5
Antihyperlipidemic drug (%)	3.7	8.1
Microalbuminuria (%)	19.2	19.8

BP: blood pressure, UACR: urine albumin-creatinine ratio.

Methods

Study population

The Iwate-Kenpoku cohort (Iwate-KENCO) study is designed to prospectively investigate the risks of acute myocardial infarction, chronic congestive heart failure, stroke, and malignant tumor in a general adult population in northern Japan. This study was conducted as a part of a government-regulated multiphasic health checkup for the general population over 40 years of age, based on the act of Health and Medical Service Act for the Elderly (1981). The study design incorporated a baseline survey which consisted of a self-administered questionnaire on lifestyle, a food-frequency questionnaire, blood pressure measurement, ECG, anthropometrical measurement, and collections of blood and urine. A follow-up survey assessing mortality, migration, and the incidence of cardiovascular diseases, stroke and cancer is planned to be carried out after the baseline study. The survey population in this study included 4 rural communities in Ninohe district (Ichinohe town, Ninohe city, Kunohe village, and Karumai town) in the northern Iwate prefecture, Japan. This region has a resident population of 38,987 adults over the age of 40 years (male; 18,096: female; 20,891). Invitations to participate in the multiphasic health screening programme were issued by government offices in each community. In 2002, 3,878 males and 6,978 females (total, 10,856) aged 40 years and over took part in the programme. Of these participants, 9,051 (male; 3,227, female; 5,824) agreed to join the study (acceptance rate; 83.4%). This study protocol was approved by our university ethics committee and local institutional review committees. All participants gave written informed consent.

Subjects were excluded from the present analysis if their specimens were incomplete or they did not agree to the withdrawal of additional samples for urinary albumin or serum CRP measurement. Subjects without anthropometrical data were also excluded. In addition, subjects with the following characteristics were excluded; history of cardiovascular events such as stroke (n=238) or acute myocardial infarction (n=42), hematuria determined by a dipstick test (n=633), menstruating (n=56), micturition pain (n=18), and over 80 years of age (n=321). For the final statistical analysis, those with macroalbuminuria as defined by a urinary albumin-to-creatinine ratio (UACR) of ≥300 mg/g were excluded (17). The final statistical analysis was therefore performed in 2,236 men and 4,217 women (Table 1).

Measurements

Subjects used a self-reported questionnaire to document medical history including status (yes or no) of prescribed drugs for hypertension, diabetes, hypercholesterolemia, micturition pain (yes or no), stroke, angina, and myocardial infarction. Family history, clinical symptoms, smoking habits (current or non-smoker), alcohol intake (yes or no), and nutrient intake were also assessed by a questionnaire developed by the study committee. Systolic and diastolic blood pressures were determined with an automated sphygmomanometer (BP103i II, Nippon Colin, Komaki, Japan), placed on the right arm of seated subjects who had rested in a sitting position for at least 5 minutes before the measurement. Measurement was performed twice, with the mean value used for statistical analysis. Hypertension was defined as systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg, and/or the use of antihypertensive medication. Body height was measured with participants in stocking feet and weight was measured wearing light clothing. Body mass index was calculated as weight (kg) divided by the square of height (m^2). Obesity was specified as body mass index ≥27.8 kg/m^2 in men, and ≥27.3 kg/m^2 in women

(18). Diabetes was ascertained either by self-reported physician diagnosis or by a measured non-fasting glucose concentration ≥200 mg/dl and/or HbA1c value ≥6.5%.

Non-fasting blood samples were drawn from the antecubital vein of seated participants with minimal tourniquet use. Samples were collected into vacuum tubes containing ethylenediaminetetraacetic acid (glucose, HbA1c), or a serum separator gel (CRP, lipids). Tubes were stored immediately after sampling in an icebox and were transported to the central laboratory (Iwate Health Service Association) within 8 hours of collection. They were then centrifuged at 1,500 *g* for 10 minutes. After separation, the serum samples were stored at 4°C, and measurements were then performed. Otherwise, samples were stored frozen at −20°C until the time of assay. High sensitivity serum CRP levels were measured by the latex-enhanced immunonephelometric method (N Latex CRP, Dade Behring). Both within- and between-assay quality control procedures were used and the coefficient of variation (CV) of the method was less than 2%. The value in the calibrator was assigned from CRM 470 (IRMM, Geel, Belgium), an international plasma protein reference material. The minimal detectable concentration of this assay system was 0.10 mg/*l*. Enzymatic methods were used to measure serum total cholesterol levels, and serum creatinine. Blood glucose was determined using an enzymatic method (Kanto Kagaku, Tokyo, Japan). Glycosylated hemoglobin (HbA1c) was measured quantitatively with an HPLC method (Tosoh, Tokyo, Japan). Hematuria was semi-quantified by a dipstick test and was defined by more than +1.

A spot urine was collected, cooled and transported to the Iwate Health Service Association laboratory during each afternoon and analyzed on the same day. 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. The UACR was used since the accuracy of the ratio in comparison to 24-hour urine sample has been demonstrated in previous papers (19, 20). The sensitivity limit for albumin was 6 mg/*l*. Interassay and intraassay CV were both within 5%. Subjects showing levels below the sensitivity limit were regarded as 'no microalbuminuria' irrespective of their urine creatinine concentration.

Statistical analysis

We used Student's test to evaluate differences in means and chi-square tests to evaluate differences in proportions. Trends analysis was used to test for associations between increasing levels of UACR and known risks of microalbuminuria and serum CRP levels were divided into UACR quartiles. To define microalbuminuria in random urine specimens, we used the UACR cutoff point recommended by the American Diabetes Association (17) and the National Kidney Foundation (UACR >30 mg/g) (21). For comparison of mean values among quartiles one-way analysis of variance was used. To test the independent relationships among serum CRP, microalbuminuria, and several continuous or categorical parameters (age, hypertension, obesity, hypercholesterolemia, smoking, diabetes), a multivariate logistic regression analysis was used to estimate odds ratio and 95% confidence intervals. Since serum CRP levels were not normally distributed, the value was logarithmically transformed for the above mentioned statistical analysis. All statistical analysis was performed using SPSS software (Chicago, Illinois, USA). A significant difference was defined as $p<0.05$.

Results

The characteristics of the population by sex are listed in Table 1. Subject ages ranged from 40 to 79 years for both sexes. Although obesity was more common among women, current smoking and drinking were significantly higher in men. Serum total cholesterol levels, percentages of hypercholesterolemic subjects and anti-hyperlipidemic drug use were higher in women compared to men.

The distribution of serum CRP levels and UACR among study subjects was highly skewed toward the lower levels (Figs. 1 and 2). The 25th, 50th, and 75th percentile values of CRP were 0.20, 0.50, and 1.0 mg/*l* in men, and 0.20, 0.40, and 0.80 mg/*l* in women. The 25th, 50th, and 75th percentile values of UACR were 7.1, 11.5, and 22.8 mg/g in men, and 8.7, 14.1, and 24.9 mg/g in women.

Table 2 shows several cardiovascular risk factors among UACR quartile groups in men. Age, systolic blood pressure, diastolic blood pressure, and serum CRP were elevated with increasing levels of UACR. In women, age, systolic blood pressure, diastolic blood pressure, body mass index, incidence of obesity, and serum CRP levels were increased with UACR levels, whereas the percentage of current alcohol drinker was decreased (Table 3).

In a logistic model for the prediction of microalbuminuria, age, obesity, hypertension, diabetes, and serum CRP levels were independently associated with the presence of microalbuminuria in both sexes (Table 4). When subjects with diabetes were excluded from the analysis, serum CRP levels continued to be a significant predictor for microalbuminuria in both sexes. However, when subjects with obesity and/or diabetes were excluded, a significant relationship between microalbuminuria and serum CRP was observed only in men. As several kinds of antihypertensive medications have been reported to reduce urinary albumin excretion in patients with essential hypertension (22, 23), analysis was performed after exclusion of subjects taking any antihypertensive medications. The relationship between serum CRP levels and microalbuminuria was persistent.

To eliminate the effect of estrogen replacement therapies on plasma CRP levels (24), subjects (n=40) who had been visiting a gynecologist (and were therefore possible users of estrogen) were excluded from the analysis. Despite this exclusion the observed relationship between serum CRP levels and microalbuminuria was still significant (odds ratio=1.251, 95% CI=1.053–1.487; $p<0.02$). In addition, to eliminate the

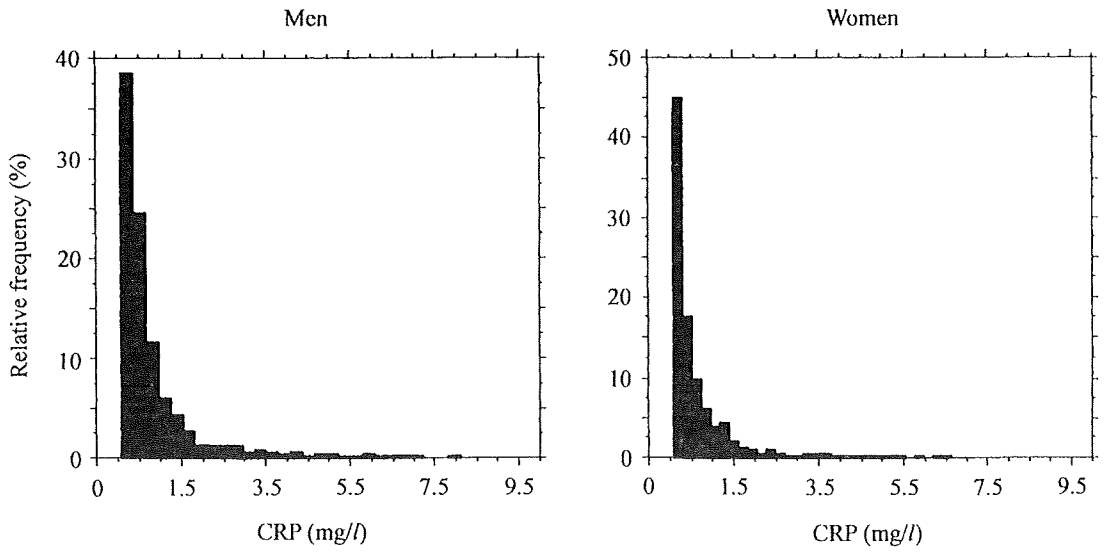

Figure 1. Distribution of C-reactive protein levels in apparently healthy men (left) and women (right) recruited from the general population aged 40 to 79 years.

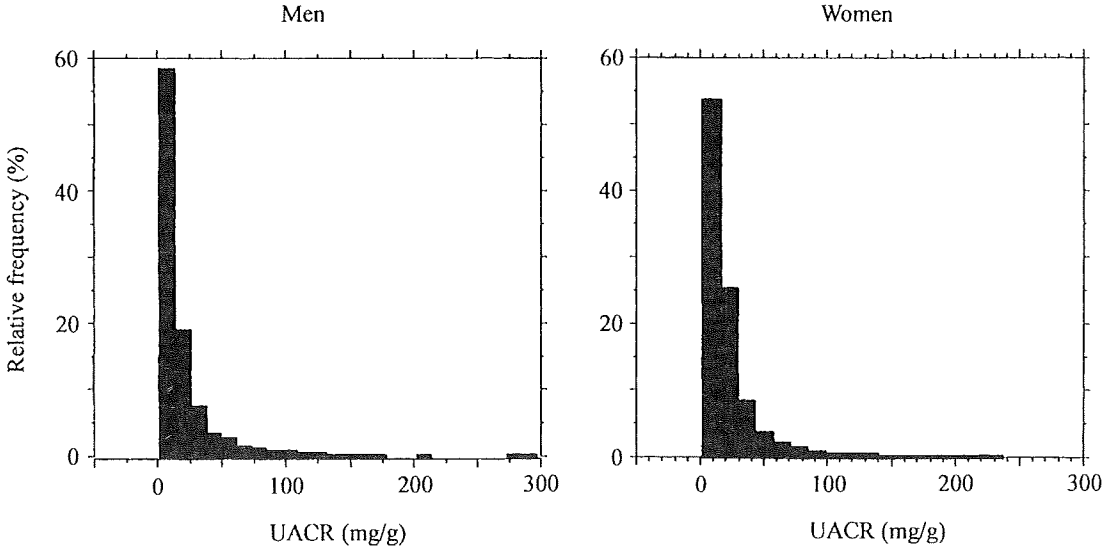

Figure 2. Distribution of urinary albumin-creatinine ratio in apparently healthy men (left) and women (right) recruited from the general population aged 40 to 79 years.

effect of statins on serum CRP levels (25, 26), the same multivariate regression analysis was performed after exclusion of subjects taking anti-hyperlipidemic drugs (men, n=82; women, n=343). Once again, the effect of CRP on microalbuminuria remained significant in both men (odds ratio=1.479, 95% CI=1.168–1.873; $p<0.01$) and women (odds ratio=1.270, 95% CI=1.060–1.522; $p<0.01$).

Discussion

After adjustment for several confounding factors related to albuminuria using a multivariate analysis, we found that serum CRP levels are significantly associated with microalbuminuria in the general population. This correlation was obvious even after exclusion of subjects with diabetes. This observation suggests that low-grade inflammation as assessed by high serum sensitive CRP levels may play a role

Table 2. Comparisons of Baseline Characteristcs among Quartile Groups Classified by UACR Levels in Men

	UACR quartile groups				p value
	Q1	Q2	Q3	Q4	
Number	558	555	563	560	
Age (yrs)	59.6±10.8	62.2±9.9	63.8±10.0	65.3±9.3	<0.001
Systolic BP (mmHg)	121.9±17.0	126.2±18.0	129.7±18.6	136.4±19.1	<0.001
Diastolic BP (mmHg)	74.4±9.7	76.4±10.7	77.4±10.6	80.5±10.2	<0.001
Body mass index (kg/m^2)	23.4±2.6	23.5±2.8	23.6±2.8	24.3±3.3	<0.001
Obesity (%)	5.2	6.5	7.6	13.2	<0.001
Diabetes (%)	2.7	5.4	8.3	12.1	<0.001
Smoker (%)	35.8	34.4	38.4	35	ns
Drinker (%)	67.4	68.8	69.3	70.2	ns
Hypercholesteloremia (%)	5.9	6.1	6.7	7.9	ns
C-reactive protein (mg/*l*)	0.92±2.20	0.87±1.42	0.98±1.92	1.76±5.95	<0.001*

BP: blood pressure, UACR: urine albumin-creatinine ratio, *analysis was done after log transformation.

Table 3. Comparisons of Baseline Characteristcs among Quartile Groups Classified by UACR Levels in Women

	UACR quartile groups				p value
	Q1	Q2	Q3	Q4	
Number	1,041	1,072	1,050	1,054	
Age (yrs)	58.1±10.1	60.7±9.7	63.5±9.0	65.1±8.7	<0.001
Systolic BP (mmHg)	118.9±17.8	122.6±18.2	126.9±19.1	134.5±21.0	<0.001
Diastolic BP (mmHg)	70.5±10.0	72.2±10.2	74.4±10.3	78.0±11.3	<0.001
Body mass index (kg/m^2)	23.6±3.2	23.8±3.2	24.2±3.4	24.8±3.7	<0.001
Obesity (%)	12.9	12.8	19	22.8	<0.001
Diabetes (%)	3	3.6	4.3	7.7	<0.001
Smoker (%)	2.9	2.7	1.6	1.2	<0.01
Drinker (%)	22.5	19.8	15.2	15.5	<0.001
Hypercholesteloremia (%)	11.7	12.9	14.4	15.2	<0.05
C-reactive protein (mg/*l*)	0.78±1.63	0.85±1.95	1.05±3.16	1.16±2.92	<0.001*

BP: blood pressure, UACR: urine albumin-creatinine ratio, *analysis was done after log transformation.

in the induction of microalbuminuria, and that urinary albumin excretion rate reflects not only established cardiovascular risk factors but also systemic low-grade inflammation in an apparently healthy population. In fact, several studies have shown that microalbuminuria is evident in subjects with central obesity without an apparent relationship to hypertension and diabetes (27, 28).

When subjects with obesity and/or diabetes were excluded from the present analysis, we found that the association between serum CRP levels and microalbuminuria persisted only in the male cohort. Although the reason for the gender difference in the correlation after exclusion of diabetes and obesity remains unknown, the impact of low-grade inflammation on vascular endothelial dysfunction, an important factor in the development of microalbuminuria (16), may be obscure in women with low cardiovascular risk factors. In fact, several clinical and experimental studies have shown that endothelial nitric oxide production/release is higher in females (29, 30). The differing nature of endothelial function between men and women may be a reason for the observed gender differences in non-obese and non-diabetic subjects.

The percentage of subjects receiving anti-hypercholesterolemic agents in this study cohort was approximately five percent. This class of the drug has been reported to decrease serum CRP levels in subjects with hypercholesterolemia (25, 26). It follows that overall serum CRP levels in users of these drug might be decreased, modifying the relationship between serum CRP and microalbuminuria. However, when subjects receiving anti-hypercholesterolemic agents were excluded from the analysis, the original results persisted.

Several studies have demonstrated that estrogen replacement therapies increase serum CRP concentrations (24). This effect may also alter the relationship between serum CRP and microalbuminuria in women. Although we did not check for the use of estrogen in our participants, the prevalence of hormone replacement therapy use in a community-based

Table 4. Multiple Logistic Regression Model to Predict Microalbuminuria

	Men			Women		
	Odds ratio	(95% CI)	p value	Odds ratio	(95% CI)	p value
All subjects (men, n=2,236; women, n=4,217)						
Age	1.025	(1.013, 1.038)	<0.001	1.029	(1.020, 1.039)	<0.001
Obesity	1.988	(1.402, 2.819)	<0.001	1.251	(1.024, 1.527)	<0.05
Smoking	1.145	(0.903, 1.452)	ns	0.981	(0.519, 1.855)	ns
Hypertension	2.582	(2.060, 3.238)	<0.001	2.616	(2.212, 3.093)	<0.001
Hypercholesterolemia	1.132	(0.740, 1.728)	ns	1.038	(0.831, 1.295)	ns
Diabetes	2.082	(1.454, 2.983)	<0.001	1.689	(1.226, 2.326)	<0.001
C-reactive protein	1.424	(1.130, 1.794)	<0.01	1.256	(1.058, 1.491)	<0.01
Subjects without diabetes (men, n=2,076; women, n=4,021)						
Age	1.025	(1.012, 1.038)	<0.001	1.030	(1.020, 1.040)	<0.001
Obesity	1.967	(1.350, 2.865)	<0.001	1.263	(1.026, 1.555)	<0.05
Smoking	1.142	(0.887, 1.470)	ns	1.000	(0.528, 1.894)	ns
Hypertension	2.813	(2.213, 3.576)	<0.001	2.618	(2.201, 3.114)	<0.001
Hypercholesterolemia	1.146	(0.735, 1.788)	ns	0.980	(0.776, 1.239)	ns
C-reactive protein	1.356	(1.063, 1.730)	<0.02	1.233	(1.030, 1.476)	<0.05
Subjects without diabetes and obesity (men, n=1,916; women, n=3,363)						
Age	1.030	(1.016, 1.044)	<0.001	1.034	(1.023, 1.045)	<0.001
Smoking	1.173	(0.900, 1.528)	ns	0.924	(0.428, 1.991)	ns
Hypertension	2.666	(2.072, 3.432)	<0.001	2.654	(2.188, 3.219)	<0.001
Hypercholesterolemia	1.218	(0.749, 1.980)	ns	0.996	(0.760, 1.306)	ns
C-reactive protein	1.403	(1.086, 1.811)	<0.01	1.135	(0.928, 1.389)	ns
Subjects without antihypertensive medications (men, n=1,803; women, n=3,293)						
Age	1.024	(1.010, 1.038)	<0.001	1.029	(1.018, 1.040)	<0.001
Obesity	2.036	(1.307, 3.171)	<0.002	1.313	(1.013, 1.701)	<0.05
Smoking	1.062	(0.807, 1.396)	ns	1.070	(0.536, 2.138)	ns
Hypertension	2.808	(2.156, 3.658)	<0.001	2.798	(2.271, 3.447)	<0.001
Hypercholesterolemia	1.118	(0.675, 1.853)	ns	1.221	(0.936, 1.593)	ns
Diabetes	2.711	(1.763, 4.168)	<0.001	1.858	(1.223, 2.823)	<0.01
C-reactive protein	1.547	(1.177, 2.035)	<0.01	1.272	(1.032, 1.568)	<0.05

Japanese population has been reported to be very low compared to other countries (31). Because estrogen replacement therapies are usually prescribed by gynecologists after hysterectomy in this country, we elected to exclude subjects attending gynecologists from the analysis. Despite this exclusion the relationship between CRP and microalbuminuria remained significant, suggesting that it is unlikely that hormone replacement therapies significantly modify the association.

It is possible that the observed correlation between microalbuminuria and serum CRP in the general population may be due to bias in the selection of subjects from the general population. However, the present study covered about 20% of the age-matched population in the area. Moreover, frequencies of hypertension and history of stroke, the distribution of plasma total cholesterol, random blood glucose level and body mass index of this cohort were comparable to those from a recent national healthy survey in a randomly selected Japanese adults population (32).

The limitation of the present study is that serum CRP concentrations seen in percentile levels such as 25th, 50th and 75th in this study population are clearly lower than those seen in populations of differing ethnicity (33). However, our observation is consistent with a recent Japanese nationwide epidemiological study (34). The prevalence of microalbuminuria in the present study may be relatively higher than that seen in other races (35), although few studies have examined the prevalence of microalbuminuria in general populations with a mean age over 60 years. It may be speculated that a higher prevalence of microalbuminuria with lower serum CRP levels is a specific characteristic of the Japanese population. It may therefore be uncertain to generalize from the present results to other populations.

In conclusion, the present study has shown that low-grade inflammation as represented by serum high sensitivity CRP levels may be significantly related to the presence of microalbuminuria in the general population. This suggests that the presence of microalbuminuria may be a useful marker representing low-grade systemic inflammation as well as established cardiovascular risk factors in apparently healthy individuals.

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Original Article

Cardiovascular Risk Factors in Hemodialysis Patients: Results from Baseline Data of Kaleidoscopic Approaches to Patients with End-stage Renal Disease Study

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BACKGROUND: The prevalence of cardiovascular risk factors and the prevalence of comorbidities in adult hemodialysis patients in Japan are not fully understood.
METHODS: In "Kaleidoscopic Approaches to Patients with End-stage Renal Disease Study" (The KAREN Study, 2003), trained research stuff examined 1,214 adult hemodialysis patients (mean age, 61.2 years; 779 males and 435 females) of 1,506 patients in northern areas of Iwate Prefecture. Cardiovascular risk factors and the prevalence of comorbidities in hemodialysis patients were compared with those in the general population using direct age-adjustment methodology and standardized morbidity ratios (SMRs).
RESULTS: In hemodialysis patients, common causes of end-stage renal disease were chronic glomerulonephritis (29.8%), diabetic nephropathy (24.5%), and other diseases. Prevalence and SMR of myocardial infarction were 5% and 9.6, respectively, and those of stroke were 13% and 5.7. The prevalences of hypertension and diabetes mellitus were 87% and 29%, respectively. Mean systolic blood pressure and mean diastolic blood pressure were 155 mmHg and 85 mmHg, respectively. Mean levels of total serum cholesterol, high-density lipoprotein cholesterol, and albumin in patients with end-stage renal disease were lower than those of the general population (160.6 vs. 203.3 mg/dL, 48.5 vs. 59.7 mg/dL, and 3.7 vs. 4.4 g/dL, respectively). Mean levels of C-reactive protein were higher than those of the general population (3.80 vs. 1.16 mg/L).
CONCLUSION: Hemodialysis patients have a high prevalence of cardiovascular risk factors and comorbidities. Levels of nutrition-related markers were lower, and C-reactive protein levels were higher, in hemodialysis patients than in the general population.
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Key words: the KAREN Study, Renal Dialysis, Risk Factors, Population, Cross-Sectional Studies.

More than 200,000 patients (1,722 per million) with end-stage renal disease (ESRD) underwent maintenance renal replacement therapy in Japan in 2002.1 In the United States, the prevalence of patients with ESRD was 1,403 per million in 2001.[2] The incidence and prevalence of ESRD, especially in diabetic and elderly patients, have been increasing over the past two decades in both countries.[1,2]

ESRD patients have a high mortality rate. The crude annual mortality rate of patients with ESRD has remained unchanged for the last ten years in Japan, at around 9%.[1] The high mortality rate of patients with ESRD is partly attributable to their high incidence of cardiovascular disease (CVD).[3]

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Little is known, however, about CVD risk factors in Japanese ESRD patients. One population-based study in ESRD patients was carried out more than ten years ago and reported that the prevalence of coronary artery disease in patients with ESRD was 1.4% in the 1970's and 2.5% in the 1980's.[4,5] Prevalences of CVD risk factors and cardiovascular comorbidities in recent years, however, have not been determined despite an increase in the numbers of diabetic and elderly patients.

The aim of this study was to reveal the prevalence of CVD risk factors in hemodialysis patients using a population-based study. We also compared the prevalence of CVD risk factors in hemodialysis patients with those of the general population.

METHODS

Setting of the Study

We have conducted the "Kaleidoscopic Approaches to patients with end-stage RENal disease Study" (the KAREN Study). The KAREN Study is a population-based prospective study designed to determine the effects of risk factors on CVD morbidity and mortality in ESRD patients. The study region is a section of northern Iwate Prefecture located in the northern part of the main island of Japan. The study area consists of 38 municipalities with a total population of 939,448 in 2002. There are 26 dialysis institutes in this region.

A preliminary survey to determine the number of dialysis patients in this area was carried out by sending facsimiles or letters to 26 dialysis institutes in April 2003. All the 26 institutes informed us of their numbers of ESRD patients, which totaled 1,506 adult hemodialysis patients. The prevalence of hemodialysis was 1,596 per million, and 6% of ESRD patients were undergoing peritoneal dialysis. Directors of 25 institutes, in which 1,499 hemodialysis patients were undergoing hemodialysis therapy, agreed to participate in the study.

Initial investigations for the KAREN study began in June 2003 and finished in March 2004. Annual checks of patients' medical records were scheduled to ascertain interim cardiovascular and cerebrovascular events, and will be continued for at least five years. This study was approved by the Medical Ethics Committee of Iwate Medical University and conducted in accordance with the guidelines of the Declaration of Helsinki.

Subjects

We used baseline data from the KAREN Study for cross-sectional analysis. There were 1,499 adult hemodialysis patients in the KAREN Study, though we were not able to contact 52 of these patients because of serious physical conditions or mental disorders. We obtained written informed consent for participation in the study from 1,260 patients (acceptance rate: 87.1%). Baseline examinations were not conducted in 21 of the 1,260 patients because of deterioration in their general conditions. In the end, we enrolled 1,239 patients in our study.

In the cross-sectional analysis, we excluded data from 25 patients because blood samples were not obtained. Data from 1,214 patients (80.6% of the total patients, aged 22 to 95 years, 779 males and 435 females) were used for analysis.

Research Staff and Data Collection

The KAREN staff includes two physicians (an urologist and a cardiologist), eight nurses, and 22 assistants. Assistants were recruited on an area-by-area basis, and they were involved in obtaining informed consents, checking questionnaires, and measuring blood pressure and body height. All the research staff were trained and approved before conducting the survey. A coordinating center was set up in the Department of Hygiene and Preventive Medicine, School of Medicine, Iwate Medical University, Morioka City, Iwate Prefecture, Japan.

Paper forms were brought to the coordinating center by the KAREN staff. Blood test data were sent to the coordinating center electronically, and only staff with permission was able to enter the room and edit data.

Initial Examinations

The baseline examination consisted of a questionnaire, measurements of blood pressure and anthropometric data, medical information reviews, and blood tests.

(1) Questionnaire

Each participant was asked to complete a questionnaire during a hemodialysis session. The questionnaire consisted of 24 questions regarding past history, family history, medication history, alcohol drinking habits, smoking habits, sleeping time per day, occupational status, the number of housemates, food preferences, and self-assessment of personality. The KAREN staff helped disabled patients fill out questionnaires, without manipulation of responses.

(2) Blood Pressure and Anthropometric Data

KAREN research staff took all measurements of body height and blood pressure. Body weight was measured using an automated scale at each institute before dialysis. Body height was measured as the length from between the heels to the centriciput point, in the supine position, using a metallic tape measure. Blood pressure was measured in the contralateral arm in patients with patent arteriovenous fistulae or grafts. Pre-dialysis blood pressure was measured twice in the supine position using an automatic device (BP-103i II Model 513000, Nippon Colin, Komaki, Japan) after a five-minute bed rest prior to cannulation. Post-dialysis blood pressure was measured in the supine position in a similar manner after a five-minute bed rest immediately following removal of the cannulae. 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 dry weight (kg) divided by the square of body height (m).

(3) Reviews of Medical Records

The two physicians and eight nurses visited the 25 institutes and reviewed patients' medical records and treatment regimens. They recorded patients' characteristics such as age, sex, past history,

family history, date hemodialysis was initiated, length of hemodialysis sessions, number of hemodialysis sessions per week, prescribed dry weight, inter-dialysis weight gain at the beginning of the week, cause of ESRD, diabetic status (based on past or current use of hypoglycemic agents), previous extremity amputation, comorbid conditions, current medications, falls in blood pressure (both falls in SBP of 30 mmHg or more and falls in SBP to below 90 mmHg) during hemodialysis sessions or the use of a vasopressor agent during hemodialysis sessions, blood pressure elevation (elevation in SBP of 30 mmHg or more) during hemodialysis sessions, use of erythropoietin, and other hemodialysis regimens.

(4) Blood Test Data

Pre-dialysis blood sampling was carried out at the beginning of hemodialysis sessions by the dialysis nursing staff. Blood samples were drawn from arteriovenous fistulae or grafts through dialysis cannulae into vacuum tubes containing EDTA or a serum separator gel or citrate. The blood samples were transported to a laboratory (Mitsubishi Kagaku Bio-Clinical Laboratories, Inc., Morioka branch office) and analyzed the same day.

Levels of total cholesterol, triglyceride, uric acid, and creatinine were measured by enzymatic assays. The urease GLC method was used to determine levels of Blood urea nitrogen (BUN). High-density lipoprotein (HDL) cholesterol levels were determined by a direct quantitative assay, while concentrations of sodium ion, chloride ion, and potassium ion were determined using electrodes. Serum levels of calcium were determined by the o-cresolphthalein complexone method. Total protein levels were determined by the biuret method, and serum albumin levels were determined by the bromcresol green method. All of the above biochemical data were analyzed using an automated analyzer (AU5232, Olympus Corp., Tokyo, Japan). Low-density lipoprotein (LDL) cholesterol levels were determined by a direct quantitative assay, and serum phosphate levels were determined by an enzymatic assay. These biochemical data were analyzed using an automated analyzer (AU800, Olympus Corp., Tokyo, Japan). Plasma glucose levels were determined by an enzymatic assay using an automated analyzer (H-7150 Hitachi High-Technology Corp., Tokyo, Japan). Glycosylated hemoglobin (HbA_{1c}) levels were determined by a latex agglutination turbidimetric immunoassay using an automated analyzer (JCA-BM9030, JEOL Ltd, Tokyo, Japan). Serum levels of C-reactive protein (CRP) were determined by the latex-enhanced immunonephelometric method (Dade Behring Diagnostic, Germany). Combined blood cell counts were determined using automated blood cell counters (Sysmex XE-2100 and Sysmex SE-9000, Sysmex, Kobe, Japan). Determinations of total cholesterol levels and HDL cholesterol levels were performed under the quality control program of the Centers for Disease Control and Prevention in the United States through the Osaka Medical Center for Health Science and Promotion, Japan.[6]

Data Handling and Classification

We determined causes of ESRD and comorbid conditions primarily according to diagnostic criteria (Table 1).[7,8] To compare characteristics of patients with similar causes of ESRD, patients were divided into three groups: a chronic glomerulonephritis group, a diabetic nephropathy group, and an other renal diseases group.

Habitual smoking was defined as currently smoking. Regular alcohol drinking was defined as drinking five or more days per week. Pulse pressure (PP) was defined as the difference between SBP and DBP, and delta SBP was defined as pre-dialysis SBP minus post-dialysis SBP. We defined persons with HDL cholesterol levels of less than 40 mg/dL as persons with low HDL cholesterol levels. We defined persons with CRP levels of more than 10 mg/L as persons with high CRP levels.

The Iwate KENCO Study is a population-based study that has been carried out in the general population in the same area as the KAREN Study.[9,10] We compared CVD risk factors and cardiovascular comorbid conditions in hemodialysis patients in the KAREN Study to those in the general population, as determined in the Iwate KENCO Study.

Statistical Analysis

Continuous variables are expressed as means ± standard deviation, and the Student's t test or the chi square test was used to compare two groups. The Mann-Whitney U test was used for skewed data (TG levels and CRP levels). One-way analysis of variance (ANOVA) or the Kruskal-Wallis test (TG levels and CRP levels) was used to compare three or more groups. Multiple comparisons were performed using Bonferroni's method, and age-adjusted values were calculated by the direct method based on data from the Iwate KENCO Study. Standardized morbidity ratios (SMRs) of myocardial infarction, stroke, hypertension, and diabetes mellitus in hemodialysis patients were also calculated based on data from the Iwate KENCO Study.

A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS(r) software (SPSS, Japan Inc., Version 11.0).

RESULTS

Table 2 shows patient characteristics. The mean age of the 1,214 patients (779 males and 435 females) was 61.2 ± 13.0 years (range, 22 to 95 years). The mean age at the start of hemodialysis was 54.2 ± 15.8 years, and the mean duration of hemodialysis was 7.0 ± 6.7 years. These numbers are similar for both male and female patients. Mean BMIs were 21.2 ± 2.9 in the male patients and 20.2±3.1 in the female patients.

The most common cause of ESRD was chronic glomerulonephritis (29.8%), and the second-most common cause was diabetic nephropathy (24.5%). The etiology was unknown in 24.9% of the patients. The proportions of patients with myocardial infarction, stroke, and peripheral arterial disease were 5%, 13%, and 16%, respectively. The proportions of smokers were 39.5% of

Table 1. Criteria for determining the causes of end-stage renal disease and comorbid conditions in hemodialysis patients.

Causes of end-stage renal disease	
Chronic glomerulonephritis (CGN)	1 Hematuria 2 Proteinuria (2+, 3+) 3 Sustained renal insufficiency The diagnosis of CGN required that all three above-mentioned criteria or pathology be diagnosed by biopsy.
Diabetic nephropathy (DMN)	1 Clinically diagnosed as diabetes mellitus 2 Proteinuria (≥ 300 mg/day) or edema or hypertension or renal insufficiency The diagnosis of DMN required that both above-mentioned criteria or pathological diagnosis be confirmed by biopsy.
Hypertensive nephrosclerosis	1 Proteinuria (±, +) 2 Hypertension 3 Sustained renal insufficiency The diagnosis of hypertensive nephrosclerosis required that all above-mentioned 3 criteria or pathological diagnosis by biopsy.
Polycystic kidney disease	The diagnosis of polycystic kidney disease required that image modalities (CT, US or MRI) identify multiple cysts in both kidneys.
Lupus nephritis	1 Clinically diagnosed as systemic lupus erythematosus Sustained renal insufficiency 2 The diagnosis of lupus nephritis required that both above-mentioned criteria and pathological diagnosis be confirmed by biopsy.

Comorbid conditions	
Myocardial infarction	1 Evolving Q-wave (at least 2 lead) myocardial infarction 2 Cardiac enzymes elevation: more than twice the normal range 3 Sustained chest pain lasting at least 30 minutes The diagnosis of myocardial infarction required two of the above-mentioned criteria.
Peripheral arterial disease	1 History of bypass surgery or angioplasty 2 Ankle-arm systolic blood pressure ratio of ≤ 0.8. 3 Exertional leg pain relieved by rest plus claudication diagnosed by physician. The diagnosis of peripheral artery disease required one of the above-mentioned criteria or image modality identification.
Stroke	1 Abrupt onset of new neurologic deficit lasting at least 24 hours, with specific localizing findings comfirmed by physician 2 Without evidence for underlying nonvascular cause. The diagnosis of stroke required both 1 and 2 criteria or image modality identification (CT or MRI).
Hypertension (HTN)	1 Anti-hypertension medication 2 Systolic blood pressure ≥ 140 mmHg 3 Diastolic blood pressure ≤ 90mm Hg The diagnosis of HTN required one of the above-mentioned criteria
Diabetes mellitus (DM)	1 Past or current use of hypoglycemic agents 2 Casual plasma glucose ≥ 200 mg/dL 3 HbA_{1c} ≥ 6.5% The diagnosis of DM required one of the above-mentioned criteria.
Dyslipidemia	1 Past or current use of anti-hyperlipidemia agents 2 Serum total cholesterol level ≥ 220 mg/dL 3 Serum low-density lipoprotein cholesterol level ≥ 140 mg/dL 4 Serum high-density lipoprotein level ≤ 40 mg/dL The diagnosis of dyslipidemia required at least one of the above-mentioned criteria.

CT: computed tomography
US: ultrasonography
MRI: magnetic resonance imaging

Table 2. Characteristics of patients in the KAREN Study.

		Total	Male	Female	p-value
Number		1214	779	435	
Age	(year)	61.2 ± 13.0	61.1 ± 13.1	61.4 ± 12.7	NS
Age at starting hemodialysis	(year)	54.2 ± 15.8	54.1 ± 16.0	54.3 ± 15.3	NS
Body Mass Index	(kg/m^2)	20.8 ± 3.0	21.2 ± 2.9	20.2 ± 3.1	< 0.001
Duration of hemodialysis	(year)	7.0 ± 6.7	6.9 ± 6.9	7.1 ± 6.5	NS
Sessions of hemodialysis	(/week)	2.88 ± 0.35	2.89 ± 0.33	2.85 ± 0.39	NS
Length of a hemodialysis session	(hour)	3.73 ± 0.64	3.80 ± 0.65	3.62 ± 0.54	< 0.001
Cause of end-stage renal disease	(%)				
Glomerulonephritis		29.8	29.1	31.0	NS
Diabetic nephropathy		24.5	27.5	19.3	0.002
Hypertensive nephrosclerosis		9.8	9.9	9.7	NS
Polycystic kidney		3.5	3.2	4.1	NS
Other minor diseases		7.4	6.4	9.2	0.036
Unknown		24.9	23.9	26.7	NS
Comorbid condition					
Myocardial infarction		5.2	5.4	4.8	NS
Stroke		13.1	13.1	13.1	NS
Peripheral artery disease		16.1	16.2	16.1	NS
Habits	(%)				
Currently smoking		28.2	39.5	7.8	< 0.001
Regular drinking		6.9	9.1	3.0	< 0.001
Hypertension	(%)	87.1	88.2	85.3	NS
Anti-hypertension medication	(%)	68.5	70.6	64.6	0.036
Number of prescribed drugs		1.36	1.41	1.28	0.070
Pre-systolic blood presxure	(mmHg)	155 ± 24	155 ± 23	155 ± 25	NS
Pre-diastolic blood pressure	(mmHg)	85 ± 13	85 ± 14	85 ± 134	NS
Post-systolic blood pressure	(mmHg)	142 ± 26	143 ± 25	140 ± 28	0.041
Post-diastolic blood pressure	(mmHg)	80 ± 14	80 ± 14	79 ± 14	NS
Delta-systolic blood pressure	(mmHg)	13 ± 23	11 ± 22	15 ± 23	0.012
Diabetes mellitus	(%)	29.1	32.1	23.7	< 0.001
HbA_{1c}	(%)	4.68 ± 0.95	4.69 ± 0.93	4.65 ± 0.98	NS
Plasma glucose	(mg/dL)	128.3 ± 54.8	129.6 ± 57.6	126.0 ± 49.4	NS
Dyslipidemia	(%)	43.1	46.1	37.2	< 0.001
Total cholesterol	(mg/dL)	154.9 ± 35.6	148.1 ± 33.6	166.9 ± 36.0	< 0.001
Triglyceride	(mg/dL)	108.6 ± 67.7	106.6 ± 72.3	112.3 ± 58.3	< 0.001*
High-density lipoprotein (HDL) cholesterol	(mg/dL)	47.0 ± 15.3	45.1 ± 14.9	50.4 ± 15.4	< 0.001
Low-density lipoprotein (LDL) cholesterol	(mg/dL)	84.9 ± 27.0	81.0 ± 26.2	91.8 ± 26.9	< 0.001
% of low HDL cholesterol (< 40mg/dL)	(%)	35.9	42.0	25.1	< 0.001
Nutrition-related data					
Total protein	(g/dL)	6.5 ± 0.5	6.5 ± 0.5	6.4 ± 0.5	0.001
Serum albumin	(g/dL)	3.7 ± 0.4	3.8 ± 0.4	3.7 ± 0.4	0.011
Blood urea nitrogen	(mg/dL)	71.2 ± 15.7	70.9 ± 15.4	71.8 ± 16.1	NS
Serum creatinine	(mg/dL)	11.0 ± 2.8	11.5 ± 3.0	10.1 ± 2.2	< 0.001
Inflammatory markers					
White blood cell count	(/μL)	5732 ± 1739	5891 ± 1765	5446 ± 1654	< 0.001
C-reactive protein (CRP)	(mg/L)	4.01 ± 9.26	4.27 ± 8.40	3.54 ± 10.62	NS*
% of high CRP (> 10 mg/L)		9.4%	10.7%	7.0%	0.025

Data are expressed as means ± standard deviation, or percentages.

P-values were obtained by a Student's t test, the chi square test, or the Mann-Whitney U test (triglyceride levels and CRP levels).

*: p-values by the Mann-Whitney U test.

Table 3. Characteristics of patients in groups according to cause of end-stage renal disease.

		Chronic glomerulonephritis (a)	Diabetic nephropathy (b)	Others ©	p-value	multiple comparisons or χ^2 test a vs b	a vs c	b vs c
Number		362	298	554				
Male/Female		227 / 135	214 / 84	338 / 216	0.006	*		*
Age	(year)	57.7 ± 12.9	62.8 ± 11.0	62.5 ± 13.6	< 0.001	**	**	
Age at starting hemodialysis	(year)	48.1 ± 15.9	59.2 ± 11.3	55.5 ± 16.6	< 0.001	**	**	**
Body Mass Index	(kg/m^2)	20.5 ± 2.8	21.3 ± 3.0	20.8 ± 3.1	0.002	**		**
Duration of hemodialysis	(year)	9.6 ± 7.7	3.7 ± 3.3	7.1 ± 6.7	< 0.001	**	**	**
Sessions of hemodialysis	(/week)	2.91 ± 0.33	2.84 ± 0.37	2.87 ± 0.36	NS			
Length of a hemodialysis session	(hour)	3.80 ± 0.61	3.69 ± 0.63	3.71 ± 0.62	0.039	*		
Comorbid condition	(%)							
Myocardial infarction		5.5	4.4	5.4	NS			
Stroke		10.8	14.1	14.1	NS			
Peripheral artery disease		19.1	15.1	14.8	NS			
Hypertension	(%)	83.4	95.3	85.2	< 0.001	*		*
Anti-hypertension medications	(%)	63.8	82.9	63.7	< 0.001	*		*
Number of prescribed drugs		1.23 ± 1.18	1.78 ± 1.29	1.23 ± 1.18	< 0.001	**		**
Pre-systolic blood presxure	(mmHg)	150 ± 23	166 ± 25	152 ± 22	< 0.001	**		**
Pre-diastolic blood pressure	(mmHg)	86 ± 13	85 ± 13	84 ± 14	0.048		**	
Pre-pulse pressure	(mmHg)	64 ± 16	81 ± 18	68 ± 16	< 0.001	**		**
Post-systolic blood pressure	(mmHg)	137 ± 26	153 ± 27	140 ± 24	< 0.001	**		**
Post-diastolic blood pressure	(mmHg)	80 ± 15	80 ± 13	79 ± 14	NS			
Post-pulse pressure	(mmHg)	56 ± 16	73 ± 19	60 ± 13	< 0.001	**		**
Delta-systolic blood pressure	(mmHg)	13 ± 20	13 ± 25	12 ± 22	NS			
Diabetes mellitus	(%)	5.2	100	6.5	< 0.001	*		*
HbA_{1c}	(%)	4.34 ± 0.61	5.60 ± 1.13	4.41 ± 0.66	< 0.001	**		**
Plasma glucose	(mg/dL)	111.8 ± 35.5	169.6 ± 69.8	116.9 ± 43.8	< 0.001	**		**
Dyslipidemia	(%)	43.1	56.4	41.3	< 0.001	*		*
Total cholesterol	(mg/dL)	155.1 ± 32.1	152.9 ± 37.9	155.8 ± 36.5	NS			
Triglyceride	(mg/dL)	109.3 ± 59.2	116.7 ± 81.2	103.8 ± 64.4	0.024†	‡		‡
High-density lipoprotein (HDL) cholesterol	(mg/dL)	47.4 ± 16.2	44.5 ± 14.4	48.0 ± 15.0	0.005	**		**
Low-density lipoprotein (LDL) cholesterol	(mg/dL)	84.7 ± 25.0	83.7 ± 27.5	85.6 ± 27.9	NS			
% of low HDL cholesterol (< 40mg/dL)	(%)	36.7	44.0	31.0	< 0.001			*
Habits								
Currently smoking	(%)	28.4	29.2	27.5	NS			
Regular drinking	(%)	9.1	7.0	5.4	NS			
Nutrition-related data								
Total protein	(g/dL)	6.5 ± 0.5	6.5 ± 0.5	6.5 ± 0.5	NS			
Serum albumin	(g/dL)	3.8 ± 0.4	3.7 ± 0.4	3.8 ± 0.4	0.001	**		**
Blood urea nitrogen	(mg/dL)	72.1 ± 14.8	68.7 ± 15.4	71.9 ± 16.2	0.007	**		**
Serum creatinine	(mg/dL)	11.8 ± 2.8	9.8 ± 2.5	11.2 ± 2.7	< 0.001	**		**
Inflammatory markers								
White blood cell count	(/μL)	5682 ± 1783	6087 ± 1658	5572 ± 1728	< 0.001	**		**
C-reactive protein (CRP)	(mg/L)	3.87 ± 9.56	4.43 ± 9.36	3.87 ± 900	NS†			
% of high CRP (> 10 mg/L)	(%)	9.1	11.1	8.5	NS			

Data are expressed as means ± standard deviations, or as percentages.

P-values were obtained by ANOVA, the chi square test, or the Kruskal-Wallis test (†).

*: $p < 0.05$, **: $p < 0.01$, by the multiple comparison test (Bonferroni method) or the chi square test.

Table 4. Comparison of prevalence, comorbidity, anthropometrical data, and blood sampling data in end-stage renal disease (ESRD) patients in the KAREN Study with those of the general population, using the Iwate KENCO Study.

	Male				Female				Total			
	General population	ESRD patients			General population	ESRD patients			General population	ESRD patients		
Number	4029	779			7338	435			11367	1214		
Comorbidity												
	prevalence	prevalence	SMR	(95% CI)	prevalence	prevalence	SMR	(95% CI)	prevalence	prevalence	SMR	(95% CI)
Myocardial infarction	0.81%	5.5%	8.0	(5.6, 10.4)	0.18%	4.9%	28.2	(16.2, 40.3)	0.40%	5.2%	9.6	(6.7, 12.3)
Stroke	4.05%	13.1%	3.6	(2.9, 4.4)	1.66%	13.1%	8.3	(6.1, 10.5)	2.50%	13.1%	5.7	(4.8, 6.6)
Hypertension	23.0%	88.2%	4.3	(4.0, 4.6)	23.2%	85.3%	3.8	(3.4, 4.2)	23.2%	87.1%	4.0	(3.8, 4.3)
Diabetes mellitus	6.92%	32.1%	5.1	(4.5, 5.7)	3.65%	23.7%	6.8	(5.5, 8.1)	4.81%	29.5%	6.5	(5.8, 7.2)
% high C-reeactive protein (> 10mg/L)	1.44%	10.9%	8.4	(6.6, 10.2)	1.17%	6.99%	6.1	(3.9, 8.3)	1.27%	9.47%	7.8	(6.4, 9.3)
Anthopometrical and blood sampling data (mean value)												
		Age-adjusted mean			Age-adjusted mean				Age- and sex-adjusted mean			
Body Mass Index	(kg/m^2)	23.7	21.2		24.0	20.1			23.9	20.5		
Systolic blood pressure	(mmHg)	130	155		126	155			127	155		
Diastolic blood pressure	(mmHg)	77	85		74	85			75	85		
Total cholesterol	(mg/dL)	193.5	149.1		208.1	166.9			203.3	160.6		
HDL cholesterol	(mg/dL)	56.2	45.1		61.5	50.4			59.7	48.5		
Serum albumin	(g/dL)	4.4	3.8		4.4	3.7			4.4	3.7		
C-reactive protein	(mg/L)	1.39	4.27		1.04	3.54			1.16	3.80		

Data are expressed as means or percentages or standardized morbidity ratios (SMRs).

SMR: standardized morbidity ratios

CI: confidence interval

male patients and 7.8% of female patients, while 9.1% of the male patients and 3.0% of the female patients were regular alcohol drinkers.

The majority (87.1%) of patients had hypertension, and 78.6% of the patients with hypertension took anti-hypertension medication. Pre-dialysis SBP and DBP were similar in the male patients and female patients. Post-dialysis SBP in the female patients was significantly lower than that of the male patients. About one-third (29.1%) of the patients had diabetes mellitus; the percentage of male patients with diabetes mellitus was higher than that of female patients with diabetes mellitus, but the mean levels of plasma glucose and HbA1c were similar in both male and female patients. The proportion of patients with dyslipidemia was 43.1%, and 83.1% of the patients with dyslipidemia had low HDL cholesterol levels. Mean levels of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides in the female patients were higher than the corresponding levels in male patients. Mean levels of CRP were 4.27 mg/L in the male patients and 3.54 mg/L in the female patients. CRP levels were higher than 10 mg/L in 10.7% of the male patients and in 7.0% of the female patients.

Table 3 shows characteristics of patients in the three renal disease groups. The mean age at the beginning of hemodialysis in the diabetic nephropathy group was higher than the mean ages of the other two groups, and the mean BMI and percentage of male patients in the diabetic nephropathy group were higher than those of the other two groups. Mean duration of hemodialysis differed between the three groups, with the shortest in the diabetic nephropathy group and the longest in the chronic glomerulonephritis group.

In the diabetic nephropathy group, all blood pressure parameters except for DBP remained high regardless of intensive anti-hypertension medication. The percentage of patients with dyslipidemia and the mean levels of triglycerides were higher, and the mean levels of HDL cholesterol were lower, in the diabetic nephropathy group relative to the other two groups. Nutrition-related parameters such as mean levels of serum albumin, BUN, and creatinine were lower in the diabetic nephropathy group than in the other groups. The mean white blood cell count in the diabetic nephropathy group was higher than in the other two groups.

Table 4 compares comorbid conditions, BMI, blood pressure, lipid levels, albumin levels, and CRP levels in hemodialysis patients to those of the general population. SMRs of myocardial infarction and stroke were 9.6 (8.0 in males and 28.2 in females) and 5.7 (3.6 in males and 8.3 in females), respectively. Mean levels of total serum cholesterol, HDL cholesterol, albumin, and BMI in the ESRD patients were lower than in the general population. Both the mean levels of CRP and the percentage of patients with high CRP levels were higher than in the general population.

DISCUSSION

The KAREN Study was designed as a population-based prospective study to assess the effects of risk factors on CVD morbidity and mortality in ESRD patients under a quality control program, and the study covered more than 80% of hemodialysis patients in the area of interest. Analysis of baseline data from the KAREN Study revealed CVD risk factors and cardiovascular comorbidities in hemodialysis patients.

About 90% of the hemodialysis patients in the KAREN Study had hypertension, and 78.6% of the hypertensive patients took anti-hypertension medications. More than 40% of the patients in the KAREN Study had dyslipidemia. More than 80% of patients with dyslipidemia had low HDL cholesterol levels, a similar percentage to that found in a previous study.[11]

Diabetic nephropathy accounted for 25% of all causes of ESRD and 29% of the KAREN Study patients had diabetes mellitus. The proportion of patients with hypertension and mean WBC count were higher, and levels of nutrition-related markers were lower, in the diabetic nephropathy group than in the other two groups. These conditions may be related to the poor prognoses for patients with diabetic nephropathy.[12,13]

The Okinawa Dialysis Study (OKIDS) revealed cardiovascular comorbid conditions in ESRD patients more than ten years ago,[4,5] but the prevalence of cardiovascular comorbidity for each renal disease subgroup was not shown. One benefit of our study was that it revealed comorbid conditions of hemodialysis patients for each renal disease group. The prevalences of myocardial infarction and stroke were similar between the renal disease groups. The cardiovascular comorbidities in the diabetic nephropathy patients were not different from those in patients with other renal diseases, a finding that disagrees with the results of an ESRD study in the United States.[13] The percentage of smokers in the KAREN Study (28%) is reflective of the general population of Japan.[9,14] Further studies are needed to determine whether smoking contributes to the high risk of CVD in ESRD patients in Japan, and efforts should be increased to encourage ESRD patients to stop smoking.[15]

The percentage of patients with diabetic nephropathy in the KAREN Study was similar to that of the Japanese Society for Dialysis Therapy (JSDT) Survey.[1] However, the proportion of patients with chronic glomerulonephritis in the KAREN Study was lower, and the percentage of patients with hypertensive nephrosclerosis in the KAREN Study was higher, than those in the JSDT Survey.[1]

In the current study, we identified causes of ESRD using information from medical records. The most common reason for classifying patients as unknown etiology was insufficient information regarding whether onset of proteinuria preceded that of hypertension. The low rate of diagnostic renal biopsy (10.4%) also made differential diagnosis difficult. Thus, it is possible that some patients with chronic glomerulonephritis or hypertensive nephrosclerosis should have been classified as patients with unknown etiology.

The percentage of patients with chronic glomerulonephritis was higher, and the percentage of patients with hypertensive nephrosclerosis was lower in the KAREN Study than those

reported by United States Renal Data System (USRDS).[2] The prevalence of hypertension was similar, the prevalence of myocardial infarction was lower, and the prevalence of stroke was higher in the KAREN Study. These results seem to reflect a high prevalence of stroke and a low prevalence of myocardial infarction in the Japanese general population relative to the general American population.[16,17]

In this study, the prevalence of CVD comorbidities was higher, albumin levels were lower, and CRP levels were higher in hemodialysis patients than in the general population. The lower albumin levels in hemodialysis patients may contribute to the high incidence of CVD.[18] Serum CRP levels in hemodialysis patients were significantly higher than those in the general population (1.80 mg/L vs. 0.84 mg/L) even after removal of subjects with apparently elevated CRP levels (10+ mg/L), and the high risk for CVD in hemodialysis patients might be partly explained by the large percentage of subjects with low-grade inflammation.[19-22]

It has been shown that traditional risk factors were not associated with the development of CVD in hemodialysis patients.[23] Some authors reported that malnutrition, inflammation, and atherosclerosis were closely linked in ESRD patients, and suggested that malnutrition and inflammation are stronger predictors than are traditional risk factors for hemodialysis patients.[23,24]

Instead of collecting isolated cases, we collected prevalent cases of hemodialysis in our study. This approach may fail to detect cases that are more serious. We were unable to make appointments with 52 patients because of serious physical conditions, and initial investigations were not conducted for 21 patients because of deteriorated health. Patients who would not give informed consent were probably in poorer condition. These factors might have reduced the number of serious cases of ESRD in our study; thus, the results obtained of our study might represent results for ESRD patients in relatively good condition. The prevalence of risk factors and comorbidities might therefore be underestimated.

We compared comorbid conditions in ESRD patients with the general population. In the Iwate KENCO Study, comorbid conditions were assessed using self-reported questionnaires, while in the KAREN Study, they were assessed using patients' medical records. This difference may artificially exaggerate differences in the prevalences of comorbidities between hemodialysis patients and the general population.

In conclusion, hemodialysis patients have a high prevalence of cardiovascular risk factors and comorbidities. Levels of nutrition-related markers were lower, and CRP levels were higher, in hemodialysis patients relative to the general population.

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APPENDIX

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CRP levels are elevated in smokers but unrelated to the number of cigarettes and are decreased by long-term smoking cessation in male smokers

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Abstract

Background. It is not clear whether there is a dose–response relationship between the number of cigarettes smoked per day and CRP level and whether there is a relationship between the length of smoking cessation and CRP level.

Methods. Geometric mean levels of CRP were compared in smoking status groups for 1926 men aged 40 to 69 years using analysis of covariance.

Results. After adjusting for several confounding factors, geometric mean levels of CRP (mg/L) were significantly different among the three smoking status groups (0.41 in non-smokers, 0.57 in current smokers, 0.48 in past smokers, $P < 0.05$). A linear trend was not found in the relationship between CRP level and number of cigarettes smoked per day. The mean CRP level in the long cessation (≥5 years) group was significantly lower than that in the short cessation (<5 years) group (0.45 vs. 0.58, $P < 0.05$) and similar to that in the non-smokers group (0.45 vs. 0.41, NS).

Conclusions. CRP levels in current smokers are elevated but unrelated to the number of cigarettes smoked per day. In past smokers, long-term smoking cessation may contribute to the reduction in risk of development of cardiovascular diseases through inflammatory mechanisms.

Keywords: Smoking cessation; C-reactive protein; Cross-sectional study; Cardiovascular disease; Iwate-KENCO study

Chronic inflammation plays a pivotal role in the development of atherosclerosis [1]. Traditional risk factors are thought to induce inflammatory reaction and to cause the development of atherosclerosis [2]. Cigarette smoking is thought to be one of the major factors responsible for promotion and progression of atherosclerosis [3–5], although the mechanisms underlying the pathophysiology of atherogenesis have not been elucidated. Thus, several studies have focused on the association between smoking and inflammatory response [6–8].

C-reactive protein (CRP) is one of the most widely used inflammatory markers because of its high level of accuracy and its availability. High-sensitivity assays for CRP that provide information on low-grade inflammation [9] have recently become available. Epidemiological studies have revealed that increased serum CRP level is positively associated with risk of development of cardiovascular diseases [6,7,10–16].

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However, it has not been determined whether a dose–response relationship between the number of cigarettes smoked and CRP level exits [6–8]. It has also not been determined whether there is a relationship between the length of smoking cessation period and serum CRP level [8,17].

In this study, we examined the association between number of cigarettes smoked per day and serum CRP levels and the association between length of the smoking cessation period and serum CRP levels in apparently healthy Japanese men.

Subjects and methods

Study subjects

This study is a part of the ongoing Iwate-KENCO Study (Iwate KENpoku COhort Study), which has been carried out since 2002 in Iwate Prefecture, Japan. The study area consists of four municipalities (Ninohe City, Ichinohe Town, Karumai Town and Kunohe Village) with a total population in 2002 of 62,665, including 13,046 men aged 40 to 69 years. Invitations to multiphasic health screening were issued by government offices in each community. In 2002, 2337 (17.9%) of the 13,046 men aged 40 to 69 years participated in annual health checkups. Of those participants, 1950 men gave written informed consent for participation in this study (acceptance rate: 86.9%).

Nineteen subjects with CRP levels greater than 10 mg/L were excluded to avoid analysis of data from subjects who had developed acute inflammatory disease [18]. Five subjects were excluded because of lack of anthropometrical data. The remaining 1926 men were enrolled in this study.

This study was approved by the Medical Ethics Committee of Iwate Medical University and conducted in accordance with the guidelines of the Declaration of Helsinki.

Measurements

Measurements of blood pressure were performed by well-trained staff. Participants were asked to avoid eating or exercise 30 min before measurements. 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 5-min 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 dietary information were used to collect individual information. In this questionnaire, current smokers were asked about the number of cigarettes smoked per day and duration of smoking. Past smokers were asked about the number of cigarettes smoked per day and age at which they had stopped smoking.

Laboratory methods

Casual blood samples were drawn from antecubital veins of seated participants with minimal tourniquet use into vacuum tubes containing EDTA (glucose, HbA1c) or a serum separator gel (CRP, lipids). The samples were transported to a laboratory (Iwate Health Service Association) and analyzed.

Serum levels of CRP were determined by the latex-enhanced immunonephelemetric method (Dade Behring Diagnostic, Germany) with a threshold of 0.1 mg/L. In this estimation, CRP values under the minimum detectable level were regarded as being 0.1 mg/L. 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 (HbA1c) 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 through the Osaka Medical Center for Health Science and Promotion, Japan.

Data handling and classification

To examine the relationships between CRP level and cardiovascular risk factors, participants were divided into quartile groups according to CRP level. To examine the relationship between the pack-years of smoking and CRP level, current smokers were subdivided into three groups according to pack-years of smoking. To examine the relationship between number of cigarettes smoked per day and CRP level, current smokers were also subdivided into three groups according to number of cigarettes smoked per day: a light smoker group (1–19 cigarettes/day), moderate smoker group (20–29 cigarettes/day) and heavy smoker group (≥30 cigarettes/day). To examine the relationship between length of smoking cessation period and CRP level, past smokers were subdivided into two groups according to length of smoking cessation period: a short cessation period group (no smoking for less than 5 years) and a long cessation period group (no smoking for 5 years or more).

Several studies have shown that alcohol intake [19,20] and exercise [21,22] are associated with serum CRP level. Regular drinking was defined as drinking 5 days or more per week and exercise habit was defined as doing exercise at least 60 min per month.

Statistical analysis

One-way analysis of variance (ANOVA) was used to test differences among three groups or more. Multiple comparisons were performed using Bonferroni's method. Comparisons of skewed data were performed using the Mann–Whitney *U* test. Multiple linear regression analysis was performed using natural logarithm-transformed CRP (ln CRP) as a dependent variable and smoking status patterns (light smoker, moderate smoker, heavy smoker and past smoker), age, BMI, systolic blood pressure and levels of HbA1c, HDLC and LDLC, which were significantly related to CRP level in univariate analysis, as independent variables.

After adjusting for several confounding factors (those significantly related to ln CRP levels in a multiple regression analysis), geometric mean levels of CRP were compared using analysis of covariance (ANCOVA). Linear trends between number of cigarettes smoked per day and geometric mean levels of CRP and between pack-years of smoking and geometric mean levels of CRP were examined after adjusting for major confounders. A P value less than 0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS software (version 11.0, Chicago, IL).

Results

Characteristics according to smoking status are shown in Table 1. Age, BMI, HDLC levels and prevalence of exercise habit in current smokers were lower than those in non-smokers. The percentage of current smokers who were regular drinkers was higher than the percentage of non-smokers who were regular drinkers. Mean levels of crude CRP were 0.80 mg/L in non-smokers, 0.87 mg/L in past smokers and 0.98 mg/L in current smokers. Multiple comparisons using the Mann–Whitney *U* test showed that serum CRP levels in current smokers were significantly higher than those in non-smokers ($P < 0.01$). The mean CRP levels in past smokers were intermediate between those in non-smokers and those in current smokers.

Characteristics according to quartile groups of CRP levels are shown in Table 2. Age, BMI, SBP, DBP, prevalence of smokers and levels of TC, TG, LDLC, plasma glucose and HbA1c were increased significantly with increase in CRP level. HDLC levels were inversely associated with CRP levels ($P < 0.01$).

Table 3 shows the results of multiple linear regression analysis using ln CRP as a dependent variable and using smoking status patterns (past smoker, light smoker, moderate smoker and heavy smoker) as independent variables. The three patterns of current smoking status were significantly related to ln CRP levels, while the standardized coefficients were similar. Past smoking status was also significantly related to ln CRP level. The levels of HDLC, LDLC and HbA1c were also related to ln CRP level. The high levels of correlation among the explanatory variables seem to exist. We also performed a multiple regression model using the products of pairs of explanatory variables as independent variables for adjusting for interactions among explanatory variables. The results were not changed even after adjusting for interactions among explanatory variables. And analysis of residuals showed the robustness of the multiple regression model.

Table 1
Descriptive characteristics of 1926 men aged 40–69 years with CRP levels less than 10 mg/L according to smoking status

	Non-Smoker	Past smoker	Current smoker	*P* value
Number	661	503	760	
AGE (years)	60.2 (7.4)	59.4 (8.4)	56.1 (8.6)	<0.001
BMI (kg/m^2)	24.0 (2.8)	24.2 (2.9)	23.5 (3.1)	<0.001
SBP (mm Hg)	128.9 (19.1)	129.3 (19.4)	126.0 (19.6)	0.003
Regular drinker	41.3%	50.1%	58.8%	<0.001*
Exercise habit	32.3%	37.9%	26.6%	<0.001*
TC (mg/dL)	197.4 (33.8)	201.6 (32.8)	195.9 (34.6)	0.012
TG (mg/dL)	133.8 (91.5)	151.0 (119.2)	152.0 (95.5)	0.001
HDLC (mg/dL)	57.8 (14.8)	58.0 (15.7)	55.8 (14.9)	0.010
LDLC (mg/dL)	117.9 (31.2)	120.0 (29.6)	117.3 (32.7)	0.305
Plasma glucose (mg/dL)	114.8 (38.7)	112.9 (34.0)	115.1 (43.3)	0.604
HbA1c (%)	5.07 (0.78)	5.11 (0.76)	5.12 (0.87)	0.528
CRP (mg/L)	0.79 (1.20)	0.87 (1.24)	0.98 (1.30)	0.022

Data are expressed as means (standard deviation) or percentages. *P* values for comparison among three groups by ANOVA.
Abbreviations: TC, total cholesterol level; TG, triglyceride level; HDLC, high-density lipoprotein cholesterol level; LDLC, low-density lipoprotein cholesterol level; HbA1c, percentage of glycosylated hemoglobin; SBP, systolic blood pressure.
* *P* values for χ^2 test among three groups.

Non-adjusted and adjusted geometric mean levels of CRP are shown in Table 4. Adjusted mean CRP levels were significantly different among three groups (Non-smoker vs. Current smoker, $P < 0.01$; Non-smoker vs. Past smoker, $P = 0.04$; Past smoker vs. Current smoker, $P < 0.01$). Adjusted mean CRP levels were different in the short cessation group and long cessation group (0.45 vs. 0.58, $P < 0.05$). Adjusted mean CRP level in the long cessation group was similar to that in the non-smoker group (0.45 vs. 0.41, NS).

A significant linear trend was not observed either in the relationship of adjusted CRP levels among subgroups according to the number of cigarettes smoked per day or in the relationship of CRP levels among subgroups according to the pack-years of smoking.

Discussion

The main findings of this study were (1) CRP levels were elevated in current smokers regardless of the number of cigarettes smoked per day both before and after adjusting for major confounders and (2) there were significant differences between adjusted CRP levels in the short cessation group and long cessation group.

Inconsistent results have been reported for the relationship between number of cigarettes smoked per day and CRP