Table 2. LDL-C/HDL-C ratio-specific characteristics among male participants at baseline (n=24566)

LDL-C/HDL-C ratio	N (%) or mean (SD)				p[a]
	Q1 <1.6 (n=2277)	Q2 ≥1.6-<2.1 (n=2125)	Q3 ≥2.1-<2.6 (n=1818)	Q4 ≥2.6 (n=2494)	
Age (years)	63.7 (11.6)	64.1 (11.5)	63.6 (11.6)	63.5 (11.4)	0.13
Body mass index (kg/m^2)	22.7 (2.8)	23.6 (2.9)	24.3 (2.9)	25.0 (2.8)	<0.01
Current smoking	758 (33.3)	624 (29.4)	523 (28.8)	809 (32.4)	<0.01
Regular alcohol consumption	1774 (77.9)	1346 (63.3)	1065 (58.6)	1091 (43.7)	<0.01
Systolic blood pressure (mmHg)	131.0 (20.3)	130.3 (19.4)	130.3 (19.5)	130.8 (19.1)	0.53
Diastolic blood pressure (mmHg)	78.2 (11.5)	78.0 (11.0)	78.2 (10.8)	78.6 (11.0)	0.44
Antihypertensive medication	566 (24.9)	516 (24.3)	453 (24.9)	605 (24.3)	0.27
Hemoglobin A1c (%)	5.02 (0.64)	5.12 (0.70)	5.18 (0.80)	5.23 (0.76)	<0.01
Hypoglycemic medication	87 (3.8)	90 (4.2)	80 (4.4)	115 (4.6)	0.19
Uric acid (mg/dL)	5.6 (1.4)	5.7 (1.3)	5.7 (1.3)	6.0 (1.4)	<0.01
Total cholesterol (mg/dL)	174.8 (29.0)	184.8 (28.5)	192.9 (28.6)	208.7 (32.3)	<0.01
Triglyceride (mg/dL)	94.3 (67.2)	112.8 (81.7)	129.9 (78.7)	158.9 (88.7)	<0.01
LDL-C (mg/dL)	85.7 (19.4)	107.2 (18.5)	119.7 (19.8)	139.0 (25.5)	<0.01
HDL-C (mg/dL)	71.1 (15.5)	58.3 (10.1)	51.4 (8.7)	43.3 (7.9)	<0.01
hs-CRP (mg/dL)	0.13 (0.44)	0.14 (0.57)	0.14 (0.44)	0.16 (0.45)	<0.01

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; SD, standard deviation; hs-CRP, high sensitivity C-reactive protein

[a]Significance was estimated using Kruskal-Wallis test for continuous items and Chi-square test or Fisher s exact test for categorical items.

with LDL-C/HDL-C ratio quartiles.

Fig. 1 shows disease-free survival curves for AMI among males based on LDL-C/HDL-C ratio quartiles. The disease-free survival rate for male participants with a LDL-C/HDL-C ratio of 2.6 or higher (fourth quartile) was significantly different from other quartiles. Multivariate-adjusted HR of Q4 was significantly higher than Q1 (p=0.03) (**Table 3-1**).

Multivariate-adjusted HR of Q3 for TC levels (HR=2.44, p=0.04) and Q4 for LDL-C levels (HR=2.50, p=0.04) were significantly higher and that of Q3 for HDL-C levels (HR=0.20, p=0.03) was significantly lower than Q1; however, a linear and obvious relationship was not observed across all quartiles (**Table 3-1**).

Table 3-2 shows hazard ratios for ischemic stroke based on lipoprotein levels and their ratio quartiles among males. For the LDL-C/HDL-C ratio, no significant relationship was found between quartiles and the risk of ischemic stroke [multivariate-adjusted HR of Q4=0.86 (p=0.56)]. Furthermore, no relationship was found between quartiles of LDL-C levels, HDL-C levels and the TC/HDL-C ratio, and the risk of ischemic stroke [multivariate-adjusted HRs of Q4=0.73 (p=0.27) for LDL-C levels, 0.90 (p=0.73) for HDL-C levels and 0.81 (p=0.47) for TC/HDL-C ratio]. Although multivariate-adjusted HR of Q3 for TC levels was significantly lower than Q1 (HR=0.55,

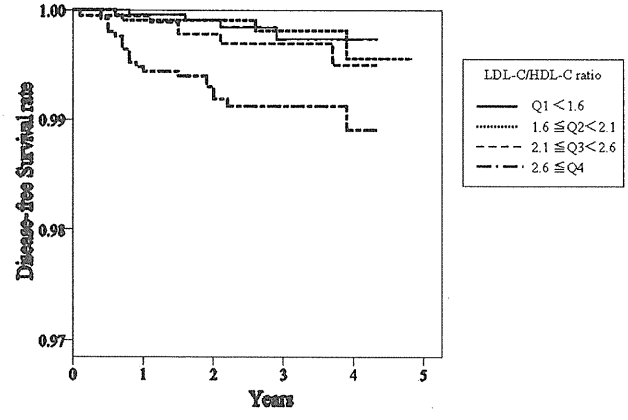

Fig. 1. Acute myocardial infarction-free rate for LDL-C/HDL-C ratio quartiles among male participants (n=8714, Cases=35).

p=0.04), a linear and obvious relationship was not observed across quartiles.

Table 3-3 shows hazard ratios for ischemic stroke for lipoprotein levels or their ratio quartiles among females. We found no significant relationship between quartiles of LDL-C/HDL-C ratio and risk of ischemic stroke [multivariate-adjusted HR of Q4=1.17 (p=0.69)]. In addition, we observed no relationship between the other quartiles [multivariate-adjusted

Table 3-1. Hazard ratios for acute myocardial infarction according to lipid level quartiles among male participants (n=8714, Mean age=63.7±11.5, Mean follow-up years=2.7±0.9)

	Number of events	Incidence rate[a]	Age-adjusted hazard ratios	95% CI	p	Multivariate- adjusted hazard ratios[b]	95% CI	p
LDL-C/HDL-C ratio								
Q1 <1.6	4/2277	0.28	1.00 (Reference)			1.00 (Reference)		
Q2 ≥1.6-<2.1	4/2125	0.32	1.08	0.27-4.33	0.91	0.99	0.25-3.96	0.98
Q3 ≥2.1-<2.6	6/1818	0.67	1.88	0.53-6.67	0.33	1.51	0.42-5.46	0.53
Q4 ≥2.6	21/2494	1.24	5.02	1.72-14.62	<0.01	3.50	1.15-10.64	0.03
TC levels (mg/dL)								
Q1 <180	12/3292	0.41	1.00 (Reference)			1.00 (Reference)		
Q2 ≥180-<200	6/2183	0.46	0.75	0.28-1.99	0.56	0.82	0.34-2.52	0.88
Q3 ≥200-<220	10/1651	1.33	1.63	0.71-3.78	0.25	2.44	1.01-5.91	0.04
Q4 ≥220	7/1588	1.00	1.17	0.46-2.98	0.74	1.81	0.68-4.77	0.23
LDL-C levels (mg/dL)								
Q1 <100	8/2884	0.36	1.00 (Reference)			1.00 (Reference)		
Q2 ≥100-<120	5/2427	0.31	0.72	0.23-2.19	0.56	0.64	0.21-1.96	0.43
Q3 ≥120-<140	9/1813	1.00	1.82	0.70-4.71	0.22	1.30	0.48-3.51	0.60
Q4 ≥140	13/1590	1.85	3.20	1.32-7.72	0.01	2.50	1.02-6.09	0.04
HDL-C levels (mg/dL)								
Q1 <50	24/3253	0.85	1.00 (Reference)			1.00 (Reference)		
Q2 ≥50-<60	7/2346	0.47	0.41	0.18-0.95	0.04	0.48	0.21-1.13	0.09
Q3 ≥60-<70	2/1647	0.27	0.16	0.04-0.67	0.01	0.20	0.05-0.86	0.03
Q4 ≥70	2/1468	0.33	0.19	0.05-0.81	0.02	0.27	0.06-1.19	0.08
TC/HDL-C ratio								
Q1 <2.8	4/2003	0.37	1.00 (Reference)			1.00 (Reference)		
Q2 ≥2.8-<3.4	3/2148	0.24	0.69	0.15-3.08	0.63	0.60	0.13-2.72	0.51
Q3 ≥3.4-<4.1	9/2066	0.77	2.18	0.67-7.07	0.20	1.52	0.45-5.19	0.50
Q4 ≥4.1	19/2497	1.13	4.05	1.38-11.90	0.01	2.82	0.91-8.72	0.07

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol
[a]Unadjusted incidence rate per 1,000,000 per year. [b]Age (10-year increase), current smoking, systolic blood pressure, body mass index, uric acid, and hemoglobin A1c were included in the Cox regression analysis.

HRs of Q4=0.73 (p=0.44) for TC levels, 0.68 (p=0.34) for LDL-C levels, 0.59 (p=0.19) for HDL-C levels and 1.43 (p=0.39) for TC/HDL-C ratio].

Discussion

This community-based, prospective cohort study conducted in a Japanese rural area showed that the LDL-C/HDL-C ratio at baseline was an independent predictor for future AMI among male participants, with a ratio of 2.6 or higher suggesting a risk of disease. Although an association between LDL-C and TC quartiles and the risk was observed, it may be weaker than LDL-C/HDL-C. On the other hand, we observed no obvious association between any lipoprotein level or their ratio quartiles and the risk of ischemic stroke in either sex. To the best of our knowledge, this is the first report to prospectively examine the association between the lipoprotein level or ratio quartiles and cardiovascular events, and to clarify the relationship between the LDL-C/HDL-C ratio and AMI among males in a rural Japanese community.

Several epidemiological studies have reported the LDL-C/HDL-C ratio to be an excellent predictor of coronary heart disease (CHD) risk[10, 12-14, 19]. In the Helsinki Heart Study[12], the LDL-C/HDL-C ratio was a strong predictor of CHD risk among participants with high triglyceride levels during 5-year follow up. Furthermore, the PROSPER study[13], which was a prospective cohort study that examined about 5,800 elderly participants over 3.7 years, suggested that increased CHD risk is associated with an elevated LDL-C/HDL-C ratio. In contrast, the Quebec Cardiovascular Study[11] showed that ratios of LDL-C/HDL-C and TC/HDL-C were associated with ischemic heart disease, and indicated that the TC/HDL-C

Table 3-2. Hazard ratios for ischemic stroke according to lipid level quartiles among male participants (n=8714, Mean age=63.7±11.5, Mean follow-up years=2.7±0.9)

	Number of events	Incidence rate[a]	Age-adjusted hazard ratios	95% CI	p	Multivariate- adjusted hazard ratios[b]	95% CI	p
LDL-C/HDL-C ratio								
Q1 <1.6	33/2277	2.36	1.00 (Reference)			1.00 (Reference)		
Q2 ≥1.6-<2.1	29/2125	2.36	0.93	0.57-1.53	0.78	1.02	0.61-1.71	0.94
Q3 ≥2.1-<2.6	21/1818	2.35	0.79	0.46-1.36	0.39	0.81	0.46-1.44	0.47
Q4 ≥2.6	31/2494	1.84	0.88	0.54-1.43	0.60	0.86	0.50-1.46	0.56
TC levels (mg/dL)								
Q1 <180	53/3292	1.84	1.00 (Reference)			1.00 (Reference)		
Q2 ≥180-<200	25/2183	1.94	0.69	0.43-1.12	0.13	0.69	0.42-1.13	0.15
Q3 ≥200-<220	16/1651	2.13	0.57	0.33-1.00	0.05	0.55	0.31-0.99	0.04
Q4 ≥220	20/1588	2.87	0.74	0.44-1.23	0.25	0.83	0.49-1.42	0.83
LDL-C levels (mg/dL)								
Q1 <100	45/2884	2.04	1.00 (Reference)			1.00 (Reference)		
Q2 ≥100-<120	32/2427	2.00	0.81	0.52-1.28	0.37	0.83	0.52-1.32	0.43
Q3 ≥120-<140	19/1813	2.11	0.66	0.39-1.13	0.13	0.65	0.37-1.12	0.12
Q4 ≥140	18/1590	2.57	0.76	0.44-1.31	0.32	0.73	0.42-1.27	0.27
HDL-C levels (mg/dL)								
Q1 <50	41/3253	1.45	1.00 (Reference)			1.00 (Reference)		
Q2 ≥50-<60	34/2346	2.28	1.17	0.74-1.84	0.51	1.18	0.74-1.87	0.49
Q3 ≥60-<70	23/1647	3.10	1.08	0.65-1.81	0.76	1.12	0.66-1.89	0.69
Q4 ≥70	16/1468	2.70	0.89	0.50-1.58	0.68	0.90	0.49-1.66	0.73
TC/HDL-C ratio								
Q1 <2.8	29/2003	2.68	1.00 (Reference)			1.00 (Reference)		
Q2 ≥2.8-<3.4	32/2148	2.55	0.99	0.60-1.64	0.97	0.99	0.59-1.67	0.97
Q3 ≥3.4-<4.1	22/2066	1.90	0.72	0.41-1.25	0.25	0.70	0.39-1.26	0.23
Q4 ≥4.1	31/2497	1.84	0.89	0.54-1.48	0.65	0.81	0.47-1.42	0.47

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol
[a]Unadjusted incidence rate per 1,000,000 per year. [b]Age (10-year increase), current smoking, systolic blood pressure, body mass index, uric acid, and hemoglobin A1c were included in the Cox regression analysis.

ratio might be a better indicator than the LDL-C/HDL-C ratio in males; however, a population-based cohort study from Framingham, Massachusetts reported that ratios of TC/HDL-C and LDL-C/HDL-C were positively associated with coronary heart disease risk in both sexes[5]. In the present study, multivariate-adjusted analysis showed that while the incidence of AMI was significantly associated with the LDL-C/HDL-C ratio, the magnitude of HR was almost identical in each quartile group of LDL-C/HDL-C and TC/HDL-C. Long-term observation may be needed to reveal an association between the LDL-C/HDL-C ratio or TC/HDL-C ratio and risk of future cardiovascular events.

We also observed a positive association between LDL-C levels and AMI. LDL-C is well known as an important risk factor for CVD. In the Suita study[20], which analyzed about 4,700 participants over a 11.9 years, the risk of myocardial infarction in the highest quartile of LDL-C (≥3.91 mmol/L) was 3.73 times higher than in the lowest quartile (<2.54 mmol/L) in males. Likewise, we showed that the highest quartile of LDL-C was significantly associated with the incidence of AMI. Upon additional analysis, no significant association was found between the LDL-C/HDL-C ratio and AMI after stratifying with the LDL-C median level [multivariate-adjusted HR of Q4 in high LDL-C=3.00 (p=0.56) and in low LDL-C=0.70 (p=0.66)]. The predictive value of LDL-C/HDL-C for AMI incidence may be evident with relatively high serum LDL-C (≥120 mg/L); however, LDL-C quartiles did not show a clear and linear trend compared to the LDL-C/HDL-C ratio. Moreover, TC quartiles did not show a liner a association in this study, although the third quartile of TC levels was significantly associated with the incidence; therefore, the

Table 3-3. Hazard ratios for ischemic stroke according to lipid level quartiles among female participants (n=15852, Mean age=60.7±11.7, Mean follow-up years=2.7±0.9)

	Number of events	Incidence rate[a]	Age-adjusted hazard ratios	95% CI	p	Multivariate-adjusted hazard ratios[b]	95% CI	p
LDL-C/HDL-C ratio								
Q1 <1.6	11/4025	0.26	1.00 (Reference)			1.00 (Reference)		
Q2 ≥1.6-<2.1	18/4408	0.35	1.25	0.59-2.65	0.56	1.13	0.53-2.42	0.76
Q3 ≥2.1-<2.6	16/3599	0.46	1.24	0.58-2.68	0.58	0.92	0.41-2.05	0.83
Q4 ≥2.6	23/3820	0.58	1.66	0.81-3.41	0.17	1.17	0.54-2.49	0.69
TC levels (mg/dL)								
Q1 <180	12/3484	0.38	1.00 (Reference)			1.00 (Reference)		
Q2 ≥180-<200	20/3638	0.56	1.55	0.76-3.17	0.23	1.39	0.66-2.94	0.39
Q3 ≥200-<220	19/3896	0.47	1.38	0.67-2.85	0.38	1.15	0.54-2.48	0.72
Q4 ≥220	17/4834	0.27	0.97	0.46-2.03	0.93	0.73	0.32-1.63	0.44
LDL-C levels (mg/dL)								
Q1 <100	12/3291	0.43	1.00 (Reference)			1.00 (Reference)		
Q2 ≥100-<120	21/4066	0.48	1.15	0.56-2.33	0.71	1.15	0.55-2.42	0.71
Q3 ≥120-<140	16/4301	0.32	0.78	0.37-1.66	0.52	0.74	0.34-1.61	0.45
Q4 ≥140	19/4194	0.39	0.94	0.45-1.94	0.86	0.68	0.31-1.49	0.34
HDL-C levels (mg/dL)								
Q1 <50	22/3345	0.74	1.00 (Reference)			1.00 (Reference)		
Q2 ≥50-<60	21/4453	0.39	0.79	0.43-1.43	0.43	0.85	0.45-1.59	0.61
Q3 ≥60-<70	15/3993	0.35	0.66	0.34-1.28	0.22	0.78	0.39-1.55	0.47
Q4 ≥70	10/4061	0.23	0.46	0.22-0.97	0.04	0.59	0.27-1.29	0.19
TC/HDL-C ratio								
Q1 <2.8	9/3728	0.24	1.00 (Reference)			1.00 (Reference)		
Q2 ≥2.8-<3.4	16/4559	0.29	1.20	0.53-2.73	0.66	1.10	0.48-2.54	0.82
Q3 ≥3.4-<4.1	19/3997	0.44	1.44	0.65-3.19	0.37	1.12	0.49-2.57	0.78
Q4 ≥4.1	24/3568	0.70	2.03	0.94-4.39	0.07	1.43	0.64-3.22	0.39

LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol
[a]Unadjusted incidence rate per 1,000,000 per year. [b]Age (10-year increase), current smoking, systolic blood pressure, body mass index, uric acid, and hemoglobin A1c were included in the Cox regression analysis.

LDL-C/HDL-C ratio may have greater clinical potential as a predictor of AMI than LDL-C or TC levels.

HDL-C is a well-known protective factor against CVD, and its levels are inversely associated with CHD[21]. We did not observe a clear association in the present study, which may have been due to the shorter follow-up period. Nevertheless, the LDL-C/HDL-C ratio at baseline was independently associated with future AMI, suggesting that the LDL-C/HDL-C ratio may better predict the outcome than HDL-C alone.

Interestingly, our results showed that the hazard ratio in male participants with a LDL-C/HDL-C ratio of 2.6 or higher was significantly higher than other quartiles. The Munster Heart Study (PROCAM)[14], which included middle-aged German men, showed a continuous and graded relationship between the LDL-C/HDL-C ratio and CHD mortality, with an increase in CHD deaths when the ratio was between 3.7 and 4.3. A clinical study of Japanese patients with suspected ischemic coronary disease evaluated the relationship between plaque formation and lipoprotein levels in coronary arteries using intravascular ultrasonography and found that the mean plaque area was significantly higher if the LDL-C/HDL-C ratio was at least 2.5[22]. It is possible that a LDL-C/HDL-C ratio of 2.6 or higher is a risk factor for AMI among Japanese males, and our results suggest that it is important to maintain an LDL-C/HDL-C ratio lower than 2.6 for primary prevention of AMI.

We did not find an association between the LDL-C/HDL-C ratio and ischemic stroke. Furthermore, there were mostly non-significant associations between other lipid profiles or their indices and ischemic stroke. The Cardiovascular Health study[23] reported a positive association between LDL-C and the risk of ischemic stroke, and the Oyabe study[24]

demonstrated an inverse relationship between HDL-C levels and ischemic stroke incidence; however, the Framingham study[25] and Hisayama study[26] did not report a clear association between the LDL-C level and the risk of ischemic stroke, and HDL-C levels were not associated with the risk of ischemic stroke in the Women's Health study[27]. Furthermore, LDL-C and HDL-C were not associated with ischemic stroke in the Atherosclerosis Risk in Community study[28]. Also, the NIPPON DATA 80[29, 30] reported that there was no relationship between ischemic stroke and TC levels. We propose three possible explanations for these discrepancies. First, these associations were heterogeneous across ischemic stroke subtypes, and lacunar infarction and cardioembolic infarction seem to be less associated with elevated LDL-C levels than atherothrombotic infarction[26]. It is probable that including these subtypes in the analysis masks the true association; therefore, the LDL-C/HDL-C ratio may not be clearly associated with the risk of total ischemic stroke in the present study. Ischemic stroke subtype-specific analysis may be needed to assess the potential relationship with the LDL-C/HDL-C ratio. Second, the follow-up period in our study was relatively short compared to previous studies. Long-term observation may reveal an association between the LDL-C/HDL-C ratio and risk of ischemic stroke. Finally, adjustment for confounding factors, especially blood pressure levels, might be insufficient in multivariate analysis. Ischemic stroke is likely to be influenced by blood pressure levels compared to AMI, and insufficient adjustment for blood pressure levels may mask the true association. To improve the accuracy of our findings, additional analysis stratified by blood pressure levels may be needed.

We found that the LDL-C/HDL-C ratio was associated with cardiovascular risk factors in both sexes. The Hisayama study[26] reported that LDL-C levels were linearly correlated with BMI, fasting blood glucose levels, and systolic and diastolic blood pressures, while HDL-C levels were inversely correlated with LDL-C levels. These factors are components of metabolic syndrome (MetS), which has received considerable attention because it is known to be a condition associated with a high risk for ischemic heart disease[31]. Furthermore, the LDL-C/HDL-C ratio was significantly correlated with hs-CRP levels, which is a circulatory inflammatory marker and well-known predictor of atherosclerotic disorders[32]; therefore, it is probable that the LDL-C/HDL-C ratio can assess the inflammation of blood vessels.

Our study has several limitations. The first is selection bias. Participants were selected from those who attended the annual health check-up, and they may have greater health awareness than the general population. Second, the follow-up period was relatively short (2.7 years) compared to previous cohort studies that observed outcomes for more than five years. Long-term observational studies may be needed to access causal associations between the LDL-C/HDL-C ratio and cardiovascular outcomes. Third, unknown sudden deaths were excluded from analysis, and it is possible that incidences were underestimated. Fourth, the present study investigated the outcome of total ischemic stroke. Ischemic stroke subtype-specific analysis may be needed in the future. Finally, we used LDL-C levels directly measured by a homogeneous assay in this study. It is possible that directly measured LDL-C levels are not as accurate as calculated LDL-C levels.

Conclusions

The LDL-C/HDL-C ratio at baseline was an independent predictor of future AMI among Japanese males. A ratio of 2.6 or higher may indicate non-fatal AMI risk, and might have the potential to assess the inflammation of blood vessels. In addition to other lipid profiles and ratios, our results indicate the utility of the LDL-C/HDL-C ratio as a predictor of AMI among men and the importance of lifestyle modification and better management of cardiovascular risks among people with high LDLC/HDLC ratios for primary prevention of future cardiovascular disease; however, given the relatively short follow-up period of this study, long-term studies may be needed to confirm our findings.

Conflict of Interest

The authors report no conflicts of interest.

Acknowledgements

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Serum Selenium Levels in Hemodialysis Patients Are Significantly Lower than Those in Healthy Controls

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Key Words
Hemodialysis · Selenium · Risk factors · End-stage renal disease

Abstract
Serum selenium levels have been thought to be decreased in hemodialysis patients; however, results of previous studies have been inconsistent. Population-based hemodialysis patients (n = 1,041) and randomly recruited healthy controls (n = 384) were enrolled. Serum selenium levels were determined by inductively coupled plasma mass spectrometry and compared in hemodialysis patients and controls using analysis of covariance after adjustment for confounding factors with $p < 0.1$ as the result of the multiple regression analysis. Age, serum albumin levels, hsCRP levels, LDLC levels, HDLC levels, regular drinking habit and hemodialysis treatment were significantly associated with serum selenium levels in multiple regression analysis. Multivariate-adjusted means (95% CIs) of serum selenium levels were 103 μg/l (101–105) in hemodialysis patients and 117 μg/l (114–121) in controls. Selenium levels in hemodialysis patients were decreased. Whether decreased serum selenium levels contribute to increased risks for morbidity and mortality in hemodialysis patients should be examined.

Introduction

Selenium deficiency contributes to an increased risk for the development of malignant neoplasms [1–3], viral infectious diseases [4–7] and cardiovascular diseases [3, 8, 9] in general populations. On the other hand, several studies have shown that serum levels of selenium in hemodialysis patients were lower than those in normal controls [10–14]. These findings suggest that decreased selenium levels in hemodialysis patients may contribute to increases in risks for morbidities from various diseases and that a decreased selenium level may be one of the unknown strong risk factors for death in hemodialysis patients.

However, there were inconsistent results and considerable wide variations of serum selenium levels in previous

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studies. The inconsistent results and wide variations were mainly due to small sample analysis and due to relatively inaccurate measurements by old equipment [15]. It is necessary to determine whether serum selenium levels are in fact decreased in hemodialysis patients compared to those in normal controls based on representative samples and a highly sensitive assay of selenium.

Subjects and Methods

Subjects

Eligible participants were adult hemodialysis patients who participated in the KAREN Study [16]. Data for 1,041 participants (663 men aged 22–91 years with a mean age of 61.2 ± 13.4 years, 378 women aged 25–88 years with a mean age of 61.1 ± 12.7 years) were analyzed in this study. Control subjects (193 men aged 40–78 years with a mean age of 57.6 ± 10.1 years, 191 women aged 41–77 years with a mean age of 56.9 ± 9.8 years) were recruited from two towns in the KAREN study area by stratified random sampling methods [17].

All participants gave written informed consent. 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.

Data Collection

The examinations in hemodialysis patients and controls consisted of a questionnaire, measurements of blood pressure and anthropometric data and blood tests. Predialysis blood sampling was carried out in hemodialysis patients [16] and overnight fasting samples were obtained from healthy controls [17]. Biochemical tests and blood counts were performed on the same day [16, 17]. The methods used for biochemical tests and blood counts have been previously reported in detail [16]. Residual sera were stored at −80°C in our laboratory until determination of selenium.

Selenium Determination

Frozen serum samples were unfrozen and each serum specimen (1 ml) was pipetted into a Teflon tube; then 3.0 ml of high-purity nitric acid was added. The tube was heated to 120°C for 12 h to completely decompose organic matter in the serum sample. The resultant solution was cooled and transferred into a Teflon beaker. The beaker was heated to 100°C until dryness. Dried samples were dissolved with 5 ml of 10% nitric acid and used for measurements. Selenium levels in sample solutions were determined using inductively coupled plasma mass spectrometry (Elan 6000, PerkinElmer Co., Ltd.). The within-run and total imprecision were determined according to the NCCLS Approved Guideline [18]. Two replicates of selenium measurements in mixed sera per day were carried out. The method produced a within-run standard deviation (SD) 3.9 μg/l at 139.8 μg/l. Total precision gave a SD of 6.2 μg/l at 139.8 μg/l.

Statistical Analysis

Mean ages (SDs) of hemodialysis patients and controls were compared by Student's t test. Sex- and age-adjusted means of continuous variables were compared between hemodialysis patients and controls using analysis of covariance (ANCOVA). The χ^2 test was used to compare frequencies among categories. Multiple regression analysis was performed for predicting serum selenium level using age, sex, serum albumin level, high-sensitivity C-reactive protein (hsCRP) level, low-density lipoprotein cholesterol (LDLC) level, high-density lipoprotein cholesterol (HDLC) level, regular drinking habit and hemodialysis treatment as explanatory variables. We calculated crude and adjusted mean levels of serum selenium in both hemodialysis patients and controls using ANCOVA separately by sex after adjusting for confounding factors with $p < 0.1$ as the result of multiple regression analysis (except for hemodialysis treatment). All p values were based on two-sided tests and p values <0.05 were considered to be statistically significant. The Statistical Package for Social Sciences (SPSS Japan Inc., version 15.0, Tokyo) was used for the analyses.

Results

Table 1 shows characteristics of hemodialysis patients and controls. Sex- and age-adjusted means of body mass index (BMI), serum albumin, total cholesterol levels, HDLC levels and LDLC levels in hemodialysis patients were significantly lower than those in controls. Adjusted geometric means of hsCRP levels in hemodialysis patients were significantly higher than those in controls. The proportion of patients having a regular drinking habit was significantly lower in hemodialysis patients than in controls.

Table 2 shows the results of multiple linear regression analysis for predicting serum selenium levels. Age, serum albumin levels, hsCRP levels, LDLC levels, HDLC levels, regular drinking habit and hemodialysis treatment were significantly associated with serum selenium levels. Collinearity between the explanatory variables was not found. Therefore, we used these variables except for hemodialysis treatment as explanatory variables in ANCOVA in order to calculate multivariate-adjusted means of serum selenium in hemodialysis patients and controls.

Table 3 shows crude and adjusted serum selenium levels both in hemodialysis patients and healthy controls. Crude means (SD) of serum selenium level (measured in micrograms per liter) were 100 (23) and 100 (21) in male and female hemodialysis patients, respectively, and they were 132 (23) and 125 (19) in male and female controls, respectively. Age-adjusted means (95% CI) of serum selenium level were 101 (99–103) and 101 (98–103) in male and female hemodialysis patients, respectively, and they were 131 (128–134) and 124 (121–127) in male and female controls, respectively. Multivariate-adjusted means (95%

Table 1. Baseline characteristics of hemodialysis patients and controls

Groups, n	Hemodialysis (1,041)	Controls (384)	p value
Serum selenium range, μg/l	18.41–226.22	56.12–215.62	
Mean age (SD), years	61.1 (13.1)	57.2 (9.9)	<0.001
Men, n (%)	663 (63.7)	193 (48.9)	<0.001
Sex- and age-adjusted means (95% CI)			
BMI	20.8 (20.6–21.0)	24.1 (23.8–24.4)	<0.001
Serum albumin, g/dl	3.8 (3.7–3.8)	4.4 (4.3–4.4	<0.001
hsCRP, mg/l	3.920 (3.46–4.38)	0.970 (0.13–1.80)	<0.001
Total cholesterol, mg/dl	155.4 (153.5–157.4)	197.8 (194.4–201.3)	<0.001
LDLC, mg/dl	85.2 (83.6–86.7)	122.4 (119.7–125.2)	<0.001
HDLC, mg/dl	47.2 (46.4–48.1)	53.0 (51.5–54.5)	<0.001
Current smoker, n (%)	278 (26.7)	100 (25.3)	0.3
Regular drinker, n (%)	74 (7.1)	131 (33.2)	<0.001

p values were calculated by Student's t test, χ^2 test or ANCOVA between hemodialysis patients and controls.
BMI = Body mass index; hsCRP = high-sensitivity C-reactive protein; LDLC = low-density lipoprotein cholesterol; HDLC = high-density lipoprotein cholesterol.

CI) of serum selenium level were 103 (102–105) and 103 (100–105) in male and female hemodialysis patients, respectively, and they were 119 (114–123) and 117 (112–122) in male and female controls, respectively. Serum selenium levels in hemodialysis patients were significantly lower than those in controls for both males and females even after adjustment for confounding factors ($p < 0.05$).

Table 2. Standardized regression coefficients by multiple linear regression analysis predicting serum selenium concentrations

	Standardized coefficients	p value	Variance inflation factor
Age	−0.123	<0.001	1.219
Men	0.027	0.309	1.309
BMI	0.027	0.313	1.360
Serum albumin	0.187	<0.001	2.044
hsCRP	−0.053	0.027	1.084
LDLC	0.097	<0.001	1.445
HDLC	0.077	0.006	1.464
Regular drinker	0.061	0.025	1.406
Hemodialysis	−0.234	<0.001	2.349

For abbreviations, see table 1.

Discussion

In this study, we estimated crude and adjusted means of serum selenium levels in both hemodialysis patients and controls. We revealed that serum selenium levels in hemodialysis patients were significantly lower than those in controls for both males and females. The results were robust even after adjustment for confounding factors.

A meta-analysis (consisting of 46 studies, n = 2,939; Europe: 47%, Asia: 30%, North America: 14%) showed results of comparisons of serum selenium levels between hemodialysis patients and controls [15]. Sample sizes of hemodialysis patients and controls ranged from 6 to 456 (median: 24) for hemodialysis patients and 5 to 490 (median: 28) for controls in this meta-analysis. There were 37 studies that showed lower selenium levels in hemodialysis patients, 8 studies that showed no differences between selenium levels in hemodialysis patients and controls, and one study that showed higher selenium levels in hemodialysis patients than in controls. Briefly, the results of the meta-analysis indicated that hemodialysis patients appeared to have lower levels of selenium than general populations. However, there were inconsistent results and considerable wide variations of serum selenium levels in those studies. The inconsistent results and wide variations were mainly due to small sample analysis and due to relatively inaccurate measurements by old equipment. Moreover, there have been no studies in which consideration was given to important confounders such as environmental selenium concentration (i.e. regional dif-

Table 3. Crude and adjusted serum selenium levels in hemodialysis patients and controls

Group	n	Serum selenium level, μg/l		
		crude mean ± SD	age-adjusted mean (95% CI)	multivariate-adjusted mean (95% CI)
HD				
Male	663	100 ± 23	101 (99–103)	103 (102–105)
Female	378	100 ± 21	101 (98–103)	103 (100–105)
Total	1,041	100 ± 22	101 (99–102)	103 (101–105)
Control				
Male	193	132 ± 23	131 (128–134)	119 (114–123)
Female	191	125 ± 19	124 (121–127)	117 (112–122)
Total	384	128 ± 21	128 (125–130)	117 (114–121)

Multivariate-adjusted means of serum selenium were estimated by analysis of covariance using age, serum albumin levels, hsCRP levels, HDLC levels, LDLC levels, and regular drinking habit as explanatory variables. For abbreviations, see table 1.

ference), sex-based differences and generation-based differences. Therefore, our study is the first study showing lower selenium levels in hemodialysis patients than in controls based on representative samples and a highly sensitive assay of selenium.

We determined serum selenium levels in this study. Generally, selenium levels in hair and nails are measured to determine past excessive exposure to selenium [19]. However, measurement of selenium levels in serum is useful for determining recent excessive exposure to selenium and detecting selenium insufficiency/deficiency [20]. Measurement of serum levels of selenium was therefore considered to be an ideal method for detecting selenium deficiency in hemodialysis patients in this study, and the serum selenium level is thought to be an ideal marker for determining selenium insufficiency/deficiency.

Conclusions

Serum levels of selenium in hemodialysis patients were significantly lower than those in healthy controls. Decreased selenium levels in hemodialysis patients may contribute to increases in risks for morbidities and mortalities from various diseases. We should examine in prospective longitudinal studies why serum selenium levels in hemodialysis patients are decreased and we should confirm whether decreased serum selenium levels in hemodialysis patients contribute to an increased risk for morbidity and mortality due to malignant, infectious and cardiovascular diseases.

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Disclosure Statement

None.

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Serum C-Reactive Protein Levels and Death and Cardiovascular Events in Mild to Moderate Chronic Kidney Disease

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SUMMARY

Chronic kidney disease (CKD) is associated with an increased risk of cardiovascular (CV) disease. Elevated circulating levels of high sensitivity C-reactive protein (hsCRP) have been suggested to be associated with high risk of CV disease. It is uncertain whether the CV risk in CKD can be stratified by hsCRP levels in the Japanese population. Baseline data including serum hsCRP and creatinine levels were determined in the general population. Estimated glomerular filtration rate (eGFR) was calculated using a modified MDRD equation, and CKD was defined as eGFR below 60 mL/minute/1.73m^2. We analyzed 1,074 male subjects with mild to moderate CKD (mean age, 70.4 years). CV events (stroke and myocardial infarction) and all-cause death were surveyed prospectively. The CKD subjects were followed for 5.1 years, and 72 CV events and 115 all-cause deaths were found (composite endpoint). After adjustment for established CV risk factors, hazard ratios (HRs) for the endpoint were significantly increased according to the hsCRP quintile ($P <$ 0.001), and HR for the highest (versus the lowest) quintile was 2.77 (95% CI; 1.61-4.77). These results suggest that serum hsCRP measurement is a useful tool for the risk stratification of CV events and death in CKD male subjects selected from the general population. (Int Heart J 2011; 52: 180-184)

Key words: Risk, Prediction, Stroke, Myocardial infarction, Mortality

Chronic kidney disease (CKD) is defined by a decreased glomerular filtration rate (GFR) and/or proteinuria.[1)] Several recent reports have shown that the risk for death is increased independently in subjects with mild renal dysfunction, compared with those who have preserved kidney function.[2,3)] The incidence of cardiovascular (CV) events in patients with CKD is higher than in those with normal kidney function,[2)] and it has been reported that CKD is an independent risk factor for CV events and CV death.[2,4)] Indeed, the incidence of CV events and death in Japanese patients with CKD is higher than that for individuals without CKD according to longitudinal studies.[5,6)]

On the other hand, serum high sensitivity CRP (hsCRP) has been reported to be related to the incidence of CV events in European and US general populations.[7-9)] The mean value of serum hsCRP in the Japanese general population tends to be lower than in other ethnic groups.[7-13)] Recently, the Hisayama cohort study has reported that elevated hsCRP in the Japanese adult population is a risk factor for ischemic stroke and coronary heart disease.[12,13)]

However, it remains unknown whether serum hsCRP levels increase the risk for CV events in individuals with CKD. Moreover, as the prevalence of CKD in the Japanese general population is greater than 10%[14)] and is higher than in the US population,[15,16)] it is uncertain whether the CV risk in CKD could be stratified by hsCRP levels in the Japanese population. The present longitudinal cohort study has therefore sought to ascertain whether increased serum hsCRP levels elevate the risk for CV events and all-cause death in CKD subjects selected from the general Japanese male population.

METHODS

Study subjects: We conducted a prospective community based cohort study of cardiovascular disease in Iwate Prefecture, which is located in the northern part of Honshu, Japan (Iwate-KENCO study). A total of 26,469 residents consented to participate in the study. The following participants were excluded from analysis: under 40 years of age (n = 1,100), female subjects (n = 16,508) (since females have a lower incidence of CV events), a history of myocardial infarction and stroke (n = 478), or missing data relating to blood sampling (n = 192), body mass index (BMI) (n = 13), or systemic blood pressure (n = 2).

The eGFR was calculated using an equation [eGFR (mL/

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minute/1.73 m^2) = 194 x $Cr^{-1.094}$ × $age^{-0.287}$] from the Modification Diet in Renal Disease Study (MDRD) for the Japanese population.[17] CKD was defined in the present study as eGFR < 60 mL/minute/1.73 m^2. Subjects with severe renal dysfunction (eGFR < 30 mL/minute/1.73 m^2) were excluded (n = 16). The final statistical analysis included 1,074 male subjects with CKD (mean age = 70.4 years). The study protocol was approved by the university ethics committee and the local hospital review committee. All participants provided written informed consent.

Measurements: All subjects used a self-report questionnaire to confirm their medical history, including the use (yes/no) of prescribed drugs for hypertension, diabetes, or hypercholesterolemia. Smoking status (current, past smoker, or nonsmoker) was also ascertained by questionnaire.

Systemic blood pressure in a sitting position after at least 5 minutes rest was measured by experienced research staff using an automatic digital device (BP-103i II, model 513000, Nippon Colin). Measurement was performed twice, with the mean value used for statistical analysis. Peripheral venous blood samples were taken from the upper arm with the subjects in a seated position to measure lipids, blood sugar, hemoglobin A1c, creatinine (Cr), and hsCRP. Serum Cr levels were measured by an enzymatic method (Hitachi 7700, Hitachi, Tokyo), and serum hsCRP levels were determined by turbidimetric immunoassay using a Nephelometer Analyzer II (Dade Behring).

Diabetes was defined as a nonfasting glucose concentration ≧ 200 mg/dL and/or HbA1c value ≧ 6.5% and/or use of antidiabetic agents including insulin. Hypertension was defined as systolic blood pressure ≧ 140 mmHg and/or diastolic blood pressure ≧ 90 mmHg and/or the use of antihypertensive medication. Hypercholesterolemia was defined as a total cholesterol concentration ≧ 240 mg/dL and/or use of lipid-lowering agents. BMI was calculated as weight (kg) divided by the square of height (m^2).

Endpoints: We defined the endpoint as the composite of CV event (stroke and myocardial infarction) and all-cause death. Incidents of hospitalized 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 myocardial infarction was based on the criteria of the MONICA study.[18] Stroke events were identified by accessing the Iwate Prefecture Stroke Registration Program, which included the entire area where the subjects lived; the details have been described previously.[19] 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. To verify the accuracy of the data, a physician or trained research nurse visited and checked the medical records of the referral hospitals.

The follow-up survey for acute myocardial infarction and stroke was carried out after the baseline study and between 2002 and 2004. Follow-up for stroke and myocardial infarction continued until March 2009. All-cause deaths and migrations were confirmed by official resident registration data issued by the local government offices (October, 2009).

Statistical analysis: Continuous variables are shown as the mean or median. CKD subjects were divided into quintiles according to their baseline serum hsCRP levels. To compare results among the hsCRP quintiles, one-way analysis of variance, Kruskal-Wallis test, and Pearson's chi-square test were used as appropriate. The event-free rate from entry into the study was estimated using the Kaplan-Meier method, followed by a trend test (log rank). The association between baseline serum hsCRP levels and the endpoint was evaluated. The hazard ratios (HR) of the hsCRP quintile for the endpoint were assessed using a Cox proportional hazards regression model. In this multivariable proportional-hazards regression model, adjustments were made in the analysis for age, hypertension, diabetes, hypercholesterolemia, BMI, and current smoking. We also used the Cox proportional hazards regression model for analysis of the linear trend among the hsCRP quintile as an ordinal variable. For analyses of CV incidence, person-years were censored at the date of CV events, 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 analyses were performed using SPSS software (Chicago, IL, USA). A significant difference was defined as $P < 0.05$.

Table. Baseline Data

	Total	hsCRP quintile and range					P*****
		Quintile 1 ≦ 0.2 mg/L	Quintile 2 0.3-0.4 mg/L	Quintile 3 0.5-0.7 mg/L	Quintile 4 0.8-1.5 mg/L	Quintlie 5 ≧ 1.6 mg/L	
n	1,074	173	227	224	233	217	
hsCRP* (mg/L)	0.6	0.2	0.3	0.6	1.0	2.8	
Age (years)	70.4	69.9	69.0	69.8	70.9	72.1	< 0.001
BMI (kg/m^2)	24.2	22.8	24.0	24.6	24.5	25.0	< 0.001
eGFR (mL/minute/1.73m^2)	53.3	53.8	53.7	53.7	53.2	52.8	0.359
Diabetes mellitus**	8.6%	6.4%	7.1%	7.6%	8.6%	13.8%	0.079
Hypertension***	56.6%	51.5%	51.5%	60.3%	60.1%	61.3%	< 0.05
(medication)	38.4%	32.9%	33.0%	37.1%	39.5%	48.4%	< 0.01
Hyperlipidemia****	11.9%	9.3%	11.9%	10.3%	11.6%	16.1%	0.219
(medication)	4.7%	5.2%	4.4%	2.7%	5.2%	6.0%	0.510
Smoking habits	21.7%	20.2%	20.3%	19.6%	22.8%	25.4%	0.439

*Median, **HbA1c ≥ 6.5% or BS ≥ 200 mg/dL and/or medications; ***BP ≥ 140 or 90 mmHg and/or medications; ****TC ≥ 240 mg/dL and/or medications; *****Differences among the CRP quintile.

Results

The prevalence of male CKD in the original cohort (Iwate- KENCO) study was 13.3%. As shown in the Table, in the CKD cohort, the percentage of cases of diabetes, hypertension, hypercholesterolemia, and current smoking were 8.6%, 56.6%, 11.9% and 21.7%, respectively. The mean eGFR was 53.3 mL/minute/1.73 m^2. Among the hsCRP quintiles, mean age and percentage use of antihypertensive agent were higher in the highest quintile compared to the lower quintiles. The percentage use of lipid-lowering agents did not differ significantly among the quintiles.

During the mean follow-up period of 5.1 years for the CKD cohort, 72 CV events (stroke = 55, myocardial infarction = 19) and 115 all-cause deaths were recorded. Kaplan-Meier curves for the event-free rate according to hsCRP quintiles in the CKD cohort are shown in Figure 1. The event-free rate was significantly lower in the 4th and 5th quintiles for hsCRP ($P <$ 0.0001 by log-rank test).

Cox regression analysis was performed to analyze the relationship between serum hsCRP and the outcome after adjustment for age, diabetes, hypertension, hypercholesterolemia, current smoking, and BMI (Figure 2). The HR for CV event and all-cause death obtained from the Cox proportional model for the 4th and 5th quintile for hsCRP was 1.78 (95% CI; 1.02-3.12) and 2.77 (95% CI; 1.61-4.77), respectively. The linear trend was significant ($P < 0.001$).

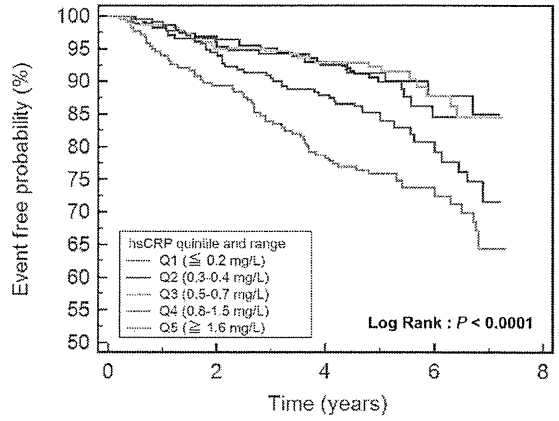

Figure 1. Kaplan-Meier curves of event-free probability according to the quintile of hsCRP value for cardiovascular events and all-cause death.

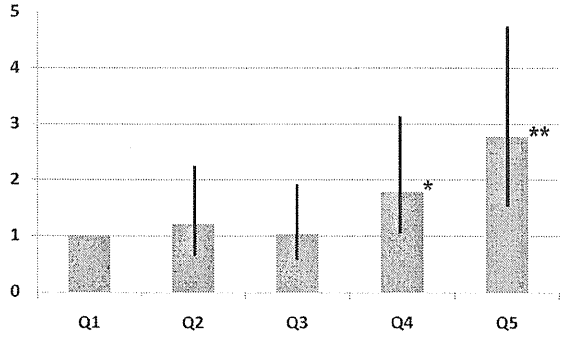

Figure 2. Hazard ratio and 95% CI for cardiovascular events and all-cause death adjusted by established risk factors. Cox's proportional hazard model after adjustment for classical cardiovascular risk factors (such as age, hypertension, hypercholesterolemia, diabetes mellitus, smoking habits and BMI). $^{*}P < 0.05$ (versus Q1). $^{**}P < 0.001$ (versus Q1).

Discussion

The present study demonstrates for the first time that in a CKD cohort defined by eGFR < 60 mL/minute/1.73 m^2 selected from a community based population, the subgroups with the higher hsCRP levels had a significantly greater risk of CV events (ie, myocardial infarction, stroke) and death compared to the low hsCRP subgroup. This relationship was robust even after adjustment for established CV risk factors such as age, hypertension, diabetes, hypercholestolemia, BMI, and current smoking. Our observations suggest that serum hsCRP levels are a useful tool for stratifying the risk of CV events and death within a CKD cohort selected from a general male population.

Go, *et al* reported that eGFR measured in subjects who underwent a health checkup was inversely correlated with mortality and incidence of CV events including stroke and heart failure, over a longitudinal follow-up for 3 years.[2] Similarly, the NIPPON DATA 90 study (a general population study with subjects recruited from various regions of Japan), reported that a decrease in eGFR (< 30 mL/minute/1.73 m^2) elevated the risk of all-cause death and CV death.[6]

The prevalence of CKD in the US population is about 8%, and has recently increased.[16] In contrast, the prevalence of CKD in Japanese is very high at about 20%, according to data on 52,759 subjects recruited from 7 regions around the country.[14] Particularly in men, the prevalence of CKD rises with aging in association with increased prevalence of hypertension and diabetes.[20] In order to prevent CV disease, it is therefore important that effective interventions be developed for the increasing number of individuals with CKD. It is also desirable that markers be developed to facilitate identification of the subgroup of those with CKD who are at increased risk of experiencing CV events.

On the other hand, it is known that inflammation is implicated in the progression of atherosclerosis, and that hsCRP is a useful biomarker for prediction of the risk of CV events such as coronary heart disease and stroke.[7-9] According to the results of a longitudinal follow-up for more than 10 years in a Japanese general population, men with high hsCRP levels (5th quintile versus 1st quintile) had a 6-fold higher risk of ischemic stroke.[13] It was also reported that the relative risk of coronary heart disease in the 3rd quintile of hsCRP was 3 times that in the 1st quintile.[12] We have previously reported that in men attending a multiphasic checkup, a positive relationship was observed between serum hsCRP levels and atherosclerotic plaque score in the carotid artery,[21] and the high hsCRP subgroup in the general population had a 2-fold higher risk of ischemic stroke and death.[19]

On the basis of these backgrounds, we established a hypothesis that among individuals with CKD, the risk of CV events is higher in the high hsCRP subgroup than the lower hsCRP subgroup. We have therefore sought to determine whether CV risk and death in subjects with mild to moderate

CKD (stage 3) could be stratified by serum hsCRP. As a result, we observed that the CKD subgroup with high hsCRP levels had a greater risk of CV events and all-cause death even after adjustment for confounding factors. Our findings suggest that inflammation may play a role in the progression of arteriosclerosis in CKD subjects.

A few reports have shown an association between CKD and serum hsCRP levels. According to a study of the relationship between serum hsCRP and CKD in African Americans, a positive correlation was seen between the presence of albuminuria and serum hsCRP levels in CKD subjects.[22] Menon, *et al* reported that high hsCRP levels (> 6.0 mg/L) are independently associated with onset of CV events in patients with predominantly nondiabetic kidney disease.[23] Soriano, *et al* also reported in a small number of CKD patients (n = 90) that the high hsCRP subgroup (> 10.5 mg/L) had significantly higher CV risk and mortality than the low hsCRP subgroup (< 8 mg/L).[24] However, no studies have yet explored whether serum hsCRP levels may be effective for stratifying CV risk within a large number of CKD subjects selected from a Japanese general population.

In the present study, all-cause death and CV events such as myocardial infarction and stroke were clearly prevalent in the higher hsCRP subgroups. These findings suggest that inflammation is associated with progression of atherosclerosis, and that hsCRP levels are a useful tool for stratifying the risk of CV events within a male CKD cohort selected from a general population. In the present study, the mechanisms by which inflammation increases CV events and death are not clear. However, there are several possible explanations for the fact that elevated serum hsCRP levels are associated with a high risk of CV events. A deterioration in renal function may accelerate inflammation activators such as homocysteine, oxidative stress, thrombogenic factors, apolipoprotein, and anemia.[25-28] In addition, it has been reported that inflammation is itself associated with progression of renal dysfunction and atherosclerosis.[29-31] Because renal dysfunction causes activation of macrophages and inflammatory cytokines such as interleukin-6 and tumor necrosis factor-alpha,[30-33] renal dysfunction and inflammation may conspire in a vicious cycle, injuring cerebral and coronary blood vessels and thus causing stroke and myocardial infarction.

The present study has several limitations. First, more than 35% of CKD subjects were receiving antihypertensive medications at baseline. Several types of antihypertensive drugs such as angiotensin-converting enzyme-inhibitors and angiotensin II receptor blockers reduce the onset of CV events. The present study could not examine what kind of antihypertensive drugs they take, and could not evaluate the effects of these drugs on the incidence of CV events. However, the percentage of subjects receiving antihypertensive drugs increased with quintiles of serum hsCRP (Table), which suggests that CKD subjects with higher serum hsCRP levels were more likely to receive these medications. This limitation may have underestimated the true association between serum hsCRP levels and CV events. Second, about 5% of the CKD subjects were receiving lipid-lowering agents. Statins have been reported to decrease serum hsCRP levels.[34] In the present study, however, there were no significant differences in the percentage of subjects receiving lipid-lowering agents among the quintiles. Third, in this CKD cohort, levels of hsCRP were obviously low compared to previously reported levels in other ethnic populations.[5,6,10,22-24] There were some reports that serum hsCRP levels in the Japanese population are clearly lower than in other races.[10,11] It is therefore uncertain whether the results found in the present study would be observed in other CKD populations with higher serum hsCRP levels. Fourth, although CKD in the present study was defined by reduced eGFR only, several studies have suggested that proteinuria or albuminuria is another diagnostic component for CKD.[35,36] In view of this, the prevalence of CKD and the relationship between the event risk and serum hsCRP might be varied according to the definition of CKD. Finally, this study clarified that Japanese adult men with CKD and high hsCRP levels had a greater risk of CV events and death than lower hsCRP subgroups. However, it is uncertain whether intervention to reduce serum hsCRP levels would decrease the incidence of CV events and death in CKD. One recent study has shown that statins lower CV events with a reduction in serum hsCRP levels in CKD subjects.[37] Further studies may be necessary to determine whether interventions to reduce serum hsCRP levels will decrease the prevalence of CV events and death in CKD subjects.

Conclusions: Serum hsCRP levels provide predictive information about CV events and all-cause death in men with CKD. This result implies that serum hsCRP measurement is a useful tool for the risk stratification of CV events and death in CKD male subjects.

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原著

透析患者の血清中ヒ素濃度の検討―健常対照との比較、血清ヒ素濃度が心筋梗塞ならびに虚血性脳卒中罹患リスクに与える影響

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要　約　【背景】高濃度のヒ素に曝露された地域住民の冠動脈疾患有病リスク・脳卒中有病リスクが高いことが報告されている。一方有機ヒ素を含む魚介類を多く摂取する日本人慢性維持透析患者では、有機ヒ素が排泄されずに体内に残存している可能性がある。本研究では、透析患者と無作為抽出された健常対照で血清中ヒ素濃度を比較した。次に血清中のヒ素濃度の高さが透析患者の心筋梗塞罹患リスク、脳卒中罹患リスクと関連しているのかを検討した。【方法】対象は平成15年に岩手県で開始した成人血液透析患者地域悉皆的コホート研究参加者1,214名中、登録調査時に血清中ヒ素濃度を測定した1,041名（男663名、女378名、平均年齢61.1歳）。健常対照は岩手県内の二つの町に居住する40-79歳の住民から年齢層化抽出法により選定した384人。血清ヒ素濃度はICP-MS（Elan 6000, Perkin Elmer Co Ltd.）を用いて測定し、透析患者と健常対照でそれぞれ年齢調整平均値を算出して比較した。透析患者の追跡調査は毎年透析施設を直接訪問して全ての透析患者診療記録を閲覧して死亡と心筋梗塞・脳卒中罹患を前向きに調査した。血清中ヒ素濃度で対象者を4分位に分けて粗死亡率、心筋梗塞と脳卒中の粗罹患率（/1000人年）を求めた。ヒ素濃度と死亡リスクならびに心筋梗塞罹患・脳卒中罹患との関係を多変量調整（性別・年齢・血圧・BMI・脂質異常・糖尿病・心筋梗塞既往・脳卒中既往・悪性新生物既往・CRP高値・アルブミン低値）トレンド検定で検証した。【結果】血清ヒ素濃度の性・年齢調整平均値（95％信頼区間）は、透析患者では42.4（40.1-44.6）μg/L、健常対照では11.6（7.82-15.4）μg/Lで透析患者の血清ヒ素濃度は有意に高かった。5年間の追跡調査（追跡調査総計4152人年、平均追跡期間3.9年）で382名の死亡、48名の急性心筋梗塞罹患、112名の虚血性脳卒中を確認した。4群の総死亡率（Q1/Q2/Q3/Q4（/1000人年））は93.3/89.7/79.5/106.0、心筋梗塞罹患率は4.0/10.6/15.6/15.0、虚血性脳卒中罹患率は、28.3/27.6/39.0/18.3であった。血清中ヒ素濃度が高いほど、急性心筋梗塞罹患リスクが有意に高くなっていた（多変量調整トレンド $p = 0.014$ ）。ヒ素濃度と総死亡リスク・虚血性脳卒中罹患リスクとの間に関係性はみられなかった。心筋梗塞既往例を除外した解析では、ヒ素濃度が高くなるほど心筋梗塞罹患リスクが高くなる関係はより明確になった（多変量調整トレンド $p = 0.009$）。【結論】透析患者は一般住民と比較して血清ヒ素濃度が高く、血清中ヒ素濃度が高くなるほど透析患者の心筋梗塞罹患リスクが高かった。日本人透析患者では体内にヒ素が過剰に蓄積している可能性があり、しかも過剰に蓄積したヒ素が心筋梗塞罹患リスクを上げている可能性がある。その因果関係について更なる検討が必要であるとともに、予防対策を講じる必要があると考えられた。

キーワード：透析患者，ヒ素，悉皆的コホート研究，心筋梗塞，虚血性脳卒中，カレン研究
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I. 緒　　言

透析患者は循環器疾患のハイリスク集団であり、一般住民に比べその循環器疾患有病リスク、発症リスク、循環器病死亡リスクはいずれも非常に高い[1]。しかし、一般住民で確立した循環器疾患の古典的危険因子の一部は（高血圧、高コレステロール血症、肥満）は、透析患者では予後に悪影響をもたらさないばかりか、一部の報告ではむしろ予後を改善する方向に働くことが観察されている[2]。また、上記の危険因子以外の古典的危険因子（年齢、男性、喫煙など）も、一般人に比べて予後への影響度は強くないことが示されており、透析患者では、古典的危険因子以外のリスク要因が死亡や循環器疾患発症に強く影響していることが示唆されている[3~5]。透析患者における特殊な状況は、古典的危険因子以外のリスク要因の確定を我々に求めているといえる。

一方高濃度のヒ素に慢性的に曝露された集団では、皮膚疾患、神経疾患、呼吸器疾患、一部の悪性新生物（皮膚がん、肺がん、膀胱がん）[6]の発症リスクが上がることが示唆されている。また高濃度ヒ素に慢性的に曝露された集団では、上記疾患発症リスク上昇とともに、冠動脈疾患[7~11]、下肢末梢動脈疾患[12]、脳卒中[13]などの心血管疾患発症リスクが高まることが指摘されている。透析患者は、心血管疾患発症リスクが非常に高い集団であり、危険因子として古典的な危険因子以外の要因が強く影響していることが示唆されていることから、高濃度ヒ素暴露地域で観察された循環器疾患危険因子としてのヒ素が、透析患者の循環器疾患罹患リスクに関係しているかどうかを検討する価値があると思われる。

健常人と比較して透析患者の生体内ヒ素が過剰に蓄積されているかどうかは欧米の報告では一定の見解が得られていない14)。しかし、有機ヒ素を多く含む魚介類を好んで食べる東アジア人はヒ素の食事による摂取量が多いことが知られ[15,16]、有機ヒ素が殆ど尿排泄されることを考慮すると[17]、腎機能が廃絶している日本人透析患者の体内にはヒ素が過剰に蓄積している可能性がある。また慢性的に過剰に蓄積したヒ素が透析患者の死亡リスクや疾患発症リスク（がんや循環器疾患）に影響を与えていることが判明した場合には、日本人の透析患者ではヒ素含有率の高い魚介類などの食事制限などを勧告し、透析患者の予後改善に寄与することを目指すべきである。

本研究では、従来明らかにされていなかった二つの点について焦点を当てて検討を行う。一つは、日本人透析患者の生体内ヒ素が過剰に蓄積しているのかを調べる目的で、多数の透析患者と代表性のある一般住民の血清のヒ素濃度を、精度の高い測定法を用いて測定してその違いを検討することである。もう一つは、すでに行われている地域悉皆的末期腎不全患者コホート研究データベースを用いて、コホート研究開始時の血清のヒ素濃度の高さが透析患者の予後に影響しているのかを前向き調査で明らかにすることである。今回の検討では、環境の高濃度ヒ素に曝露された台湾住民で観察された冠動脈疾患有病率と脳卒中有病率の高さに注目して、血清ヒ素濃度が総死亡に与える影響をみるとともに心筋梗塞症と虚血性脳卒中の罹患リスクに影響しているのかを検討した。

II. 方　　法

研究対象地域

今回の研究対象である透析患者はカレン研究（末期腎不全患者に対する多面的な取り組みにより循環器疾患発症リスクを割り出す研究：Kaleidoscopic Approaches to patients with end-stage RENnal disease, KAREN研究）に参加した成人血液透析患者である。カレン研究の対象地域は岩手県北部から県中央部で、平成14年当時の域内には38市町村が含まれ、総人口は939,448人である。図1の左側はカレン研究を実施した市町村（白塗りの区域）と参加した透析施設の存在地（白抜き番号表示）を示す。右側の図は健常対照を募っ

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*3 三愛病院泌尿器科
*4 結核予防会　第一健康相談所
*5 岩手医科大学医学部救急医学講座
*6 岩手医科大学医学部内科学講座、心血管・腎・内分泌内科分野
*7 岩手大学教育学部
受付日 2010年●月●日・受理日　2010年●月●日

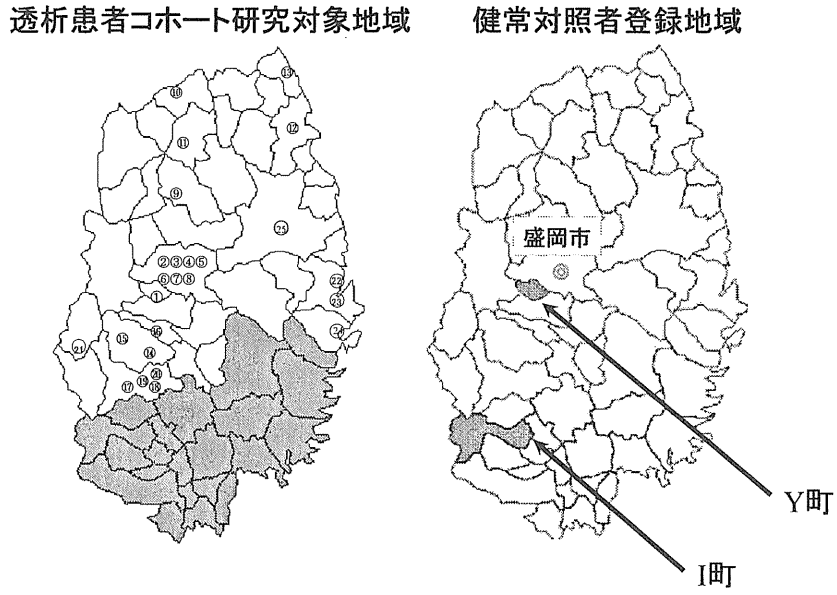

図1　カレン研究対象地域と参加施設ならびに健常対照参加者募集地域

図1は岩手県地図を示している。カレン研究は、左側の岩手県地図の中で、白く塗りつぶされた地域に存在する全ての透析施設(26施設)に研究参加を呼びかけ、25施設の協力を得て開始した。右側は健常対照参加者を募った二つの町の位置を示している。

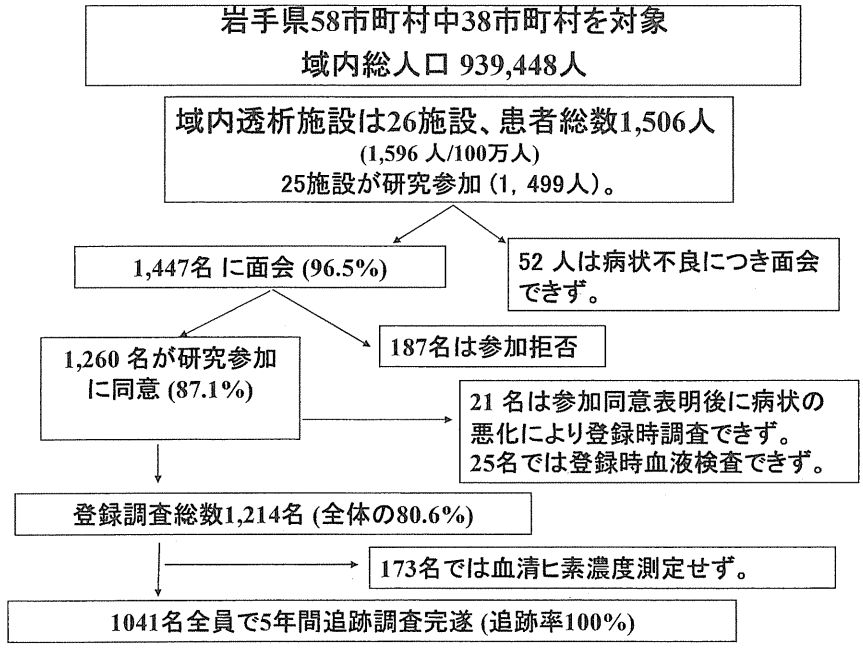

図2　カレン研究参加者選択手法と追跡調査内容の樹枝状図

岩手県中央部から北部にかけての総人口は2003年時点で94万人で、成人血液透析患者数は1,506名であった。インフォームドコンセントを実施できたのは1,447名で、最終的に1,214名の透析患者の登録が行われた。1214名中1041名で凍結保存された血清を用いて血清ヒ素濃度の測定を行った。5年間の追跡調査で1041名全員の生存状況・死亡年月日・死因・循環器疾患発症年月日を確認した。