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The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [28].

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Prehypertension Subtype With Elevated C-Reactive Protein: Risk of Ischemic Stroke in a General Japanese Population

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BACKGROUND
Prehypertension (PreHT) and low-grade inflammation are both known to be related to the incidence of cardiovascular events. This cohort study investigated whether the high-risk group for future ischemic stroke among PreHT subjects can be predicted by stratification of high-sensitivity C-reactive protein (hsCRP).

METHODS
A total of 22,676 subjects aged 40–80 years from the general population who had no cardiovascular history underwent baseline measurement of serum hsCRP, and were followed for the incidence of ischemic stroke.

RESULTS
During the mean follow-up period of 2.7 years, 143 subjects had a first ischemic stroke. In a Cox multivariable model after adjustment for cardiovascular risk factors, there was no significant difference in hazard ratio (HR) for incidence of ischemic stroke between the normotension (NT) and PreHT subjects (HR = 1.72, 95% confidence interval (CI): 0.93–3.18, vs. NT subjects). In contrast, the HR for incidence of ischemic stroke in PreHT subjects with higher hsCRP levels (≥0.5 mg/l in men, ≥0.4 mg/l in women, more than median levels according to sex) was increased compared to NT subjects with lower hsCRP levels (HR = 2.63, 95% CI: 1.11–6.24). Moreover, the HR for incidence of ischemic stroke in PreHT subjects with lower CRP levels (HR = 0.91, 95% CI: 0.31–2.73) did not differ from that in NT subjects with lower hsCRP levels.

CONCLUSIONS
This study showed that, in a Japanese general population, hsCRP was a marker for relatively short-term risk of ischemic stroke in PreHT subjects.

Keywords: *blood pressure; cerebral infarction; cohort study; hypertension*

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A new blood pressure (BP) category (prehypertension (PreHT)—defined as a systolic BP (SBP) level of 120–139 mm Hg and/or a diastolic BP (DBP) level of 80–89 mm Hg) was introduced by the Seventh Joint National Committee on the Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-7) in 2003.[1,2] PreHT has been demonstrated to be associated with an increased incidence of cardiovascular disease (CVD).[3–5] It has been suggested by the Atherosclerosis Risk in Communities (ARIC) study that elimination of PreHT would prevent 30% of new cardiovascular events.[6] The JNC-7 report has recommended lifestyle modifications including dietary changes and exercise to achieve BP goals. However, the JNC-7 also discussed the possibility that dealing with large numbers of prehypertensive individuals might impose an excessive burden on clinicians and lead to an excessive administration of antihypertensive drugs. The PreHT group, which has been ever reported about an association with CVD, was a heterogeneous cohort of individuals with varying risk profiles for CVD. Identification of the high-risk group for future CVD among PreHT individuals would therefore enable interventions to be targeted with greater accuracy. The risk of CVD among PreHT individuals was recently reported to be more pronounced in blacks and in those with diabetes mellitus (DM) or high body mass index (BMI).[6,7] However, information about high-risk groups for CVD among PreHT individuals is limited.

Inflammation plays an important role in atherosclerosis. Several epidemiological studies have demonstrated that high-sensitivity C-reactive protein (hsCRP) levels, as a predictive marker of atherosclerosis, were positively associated with the risk of ischemic heart disease and ischemic stroke.[8–10] We have therefore hypothesized that elevated hsCRP levels are associated with an increased risk of future ischemic stroke in PreHT

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individuals. We thus assessed the association between PreHT and the risk of ischemic stroke among middle-aged and elderly adults in the community, and estimated the usefulness of stratification by hsCRP level for predicting the risk of ischemic stroke in subjects with PreHT.

METHODS

Study participants. The Iwate-KENCO study cohort is a population-based prospective study in Japanese residents of the Ninohe, Kuji, and Miyako districts in the northern part of Iwate prefecture, northeast of Honsyu, Japan.[11,12] Participants were recruited from a government-regulated multiphasic health checkup. According to annual statistical data for 2003 issued by the Iwate prefecture government, this area had a resident population of 241,057 with 26% over the age of 65 years. In this region, there are nine public hospitals with admission facilities. Participants who suffered from cerebrovascular disease usually had access to only these medical institutes. Of 31,318 (11,003 men) individuals aged ≥18 years who took part in the health checkup program between April 2002 and January 2005, 26,469 (9,161 men) individuals consented to participate in this cohort study (85%). After exclusion of participants with previous myocardial infarction ($n = 119$), previous stroke ($n = 872$), or missing data ($n = 1,456$) at baseline, 22,676 subjects (7,666 men and 15,010 women) aged 40–80 years were analyzed for the study.

Follow-up. Diagnosis of stroke was based on the criteria established for the Monitoring System for Cardiovascular Disease commissioned by the Ministry of Health and Welfare.[13] These criteria correspond with those published by the World Health Organization[14] and stroke was defined as the sudden onset of neurological symptoms. Stroke was classified as either ischemic or hemorrhagic based on brain imaging including computed tomography or magnetic resonance imaging. Hospitalized patients with newly diagnosed stroke were registered from April 2002 to August 2007. Patients with transient ischemic attack and traumatic hemorrhagic stroke were excluded from registration. Registration was initially performed by attending physicians at each hospital. Furthermore, to ensure complete capture of all registrations, physicians or trained research nurses visited and checked the medical charts and/or discharge summaries at referral hospitals within the study area. A stroke registration program has been coordinated mainly by the government of Iwate prefecture and the Iwate Medical Association.[15] The study was approved by our institutional ethics committee.

Data collection. Participants completed a self-report questionnaire to document their medical history including current medications and lifestyle factors such as smoking habits and alcohol intake. Baseline clinical examinations including standard 12-lead electrocardiogram were performed. BP was measured twice using an automatic digital sphygmomanometer after 5 min of rest in a sitting position, and the average of these two values was used for analysis. Blood samples were drawn from an antecubital vein, and routine hematology and biochemistry data were obtained. Serum hsCRP levels were measured using the Behring latex-enhanced CRP assay on a Behring nephelometer BN-100 (Dade Behring, Deerfield, IL). BMI was calculated by dividing weight (in kilograms) by the square of height (in meters).

Risk factor definition. According to BP levels, participants were classified into the following three groups: normotension (NT) defined as SBP < 120 mm Hg and DBP < 80 mm Hg; PreHT defined as SBP ≥120 mm Hg but <140 mm Hg or DBP ≥80 mm Hg but <90 mm Hg; hypertension (HT) defined as SBP ≥140 mm Hg, DBP ≥90 mm Hg or current use of antihypertensive agents. We further divided the PreHT group into two subcategories according to hsCRP levels (above or below median hsCRP value for each gender). DM was defined as a random blood glucose level ≥200 mg/dl, hemoglobin A_{1c} level ≥6.5%, or current antidiabetic therapy. The estimated glomerular filtration rate was calculated using the modified equation presented in the Japanese Modification of Diet in Renal Disease (MDRD) study.[16] Renal dysfunction was defined as an estimated glomerular filtration rate <60 ml/min/1.73 m^2.

Statistical analysis. Data are presented as mean ± s.d. or percentage. All data were analyzed with SPSS statistical software, version 11.0 (SPSS, Chicago, IL). Comparison of continuous variables at baseline among the three BP categories of NT, PreHT, and HT was performed by one-way analysis of variance with the Scheffe's *post hoc* test. Furthermore, the *t*-test was used to compare means of continuous variables between the six subcategories of NT, PreHT, HT and with/without above median hsCRP levels according to sex. The Kruskal–Wallis analysis and Mann–Whitney *U*-test were used for comparisons of hsCRP levels among the three BP categories and the six subcategories, respectively. χ^2-Test was used for comparison of categorical variables. The Kaplan–Meier method with log-rank test was used to compare ischemic stroke-free rates among the three BP categories and the six BP subcategories stratified by median hsCRP levels. A Cox proportional hazards model was constructed including these categories and adjusting for sex, baseline age (in 10-year increments), BMI, total cholesterol, high-density lipoprotein cholesterol, smoking habits, regular alcohol intake, presence of DM, atrial fibrillation (Af) and renal dysfunction. Hazard ratios (HRs) and 95% confidence intervals (CIs) for ischemic stroke were estimated from Cox models constructed using dummy variables for PreHT, HT with NT as a reference, or for the five BP subcategories with NT with below median hsCRP levels as a reference. $P < 0.05$ was considered to be statistically significant.

RESULTS

PreHT was found in 5,721 (25.2%) subjects (2,144 (28.0%) men and 3,577 (23.8%) women). As shown in **Table 1**, there were significant differences in age, BMI, total cholesterol, high-density lipoprotein cholesterol, and hsCRP levels among the three BP categories. DM, atrial fibrillation, renal dysfunction, and current drinking were more frequent in HT subjects, whereas male gender and current smoking was more frequent among PreHT subjects.

Table 1 | Baseline characteristics of blood pressure categories

	NT	PreHT	HT	*P* value
No. of subjects	7,625	5,721	9,330	
Men (%)	26.6	37.5	37.4	<0.001
Age (years)	59 ± 10	62 ± 9*,**	66 ± 8	<0.001
Body mass index (kg/m^2)	23.0 ± 2.9	24.0 ± 3.1*,**	25.0 ± 3.4	<0.001
Systolic blood pressure (mm Hg)	107 ± 8.0	128 ± 6.0*,**	143 ± 18	<0.001
Diastolic blood pressure (mm Hg)	66 ± 6.6	77 ± 6.3*,**	83 ± 11	<0.001
Total cholesterol (mg/dl)	200 ± 33	203 ± 33*	202 ± 32	<0.001
HDL cholesterol (mg/dl)	61 ± 15	60 ± 15	58 ± 15	<0.001
High sensitive CRP (mg/l)	0.96 ± 3.32	1.06 ± 3.53**	1.33 ± 3.98	<0.001
Renal dysfunction (%)	8.3	11.2	15.9	<0.001
Diabetes mellitus (%)	3.1	4.9	7.2	<0.001
Atrial fibrillation (%)	0.9	1.3	1.8	<0.001
Current smoking (%)	12.6	13.1	11.1	0.001
Current drinking (%)	23.7	30.2	31.1	<0.00

Data are presented as mean ± s.d. or percentage.
CRP, C-reactive protein; HDL, high-density lipoprotein; HT, hypertension; NT, normotension; PreHT, prehypertension.
*P < 0.05 vs. NT group. **P < 0.05 vs. HT group.

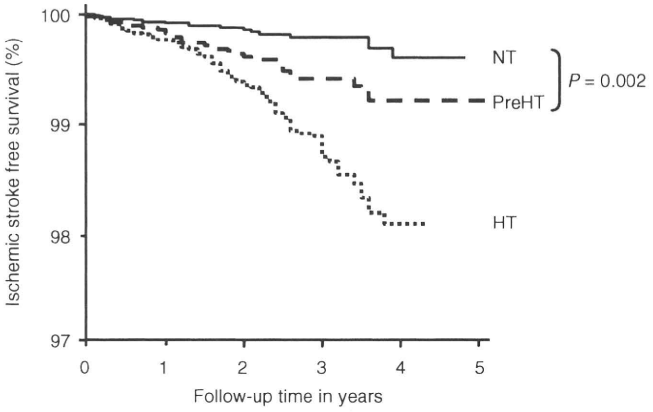

Figure 1 | Cumulative incidence of ischemic stroke in blood pressure categories. HT, hypertension; NT, normotension; PreHT, prehypertension.

Table 2 | HRs for ischemic stroke in blood pressure categories

	No. of subjects	No. of events (%)	HR	95% CI	*P* value
Normotension	7,625	16 (0.2)	1.00	—	—
Prehypertension	5,721	30 (0.5)	1.72	0.93–3.18	0.086
Hypertension	9,330	97 (1.0)	2.86	1.65–4.95	<0.001

HR adjusted for sex, age of 10 years increment, total cholesterol, HDL cholesterol, renal dysfunction, body mass index, diabetes mellitus, smoking habits, alcohol intake, and atrial fibrillation in the Cox proportional hazard model.
CI, confidence interval; HR, hazard ratio.

The median serum hsCRP level was 0.5 mg/l (95% percentile range: 0.1–4.3 mg/l) in men and 0.4 mg/l (95% percentile range: 0.1–3.4 mg/l) in women. A total of 3,281 participants (14.5%) showed hsCRP levels ≤0.1 mg/l. Participants showing hsCRP >10.0 mg/l comprised 1.4% of the study population. However, the presence of an acute infectious condition cannot be judged by CRP levels alone, so establishing a cutoff level for infection is not possible. We therefore performed analyses without any exclusion criteria for high hsCRP levels.

During the average 2.7 years of follow-up, 239 subjects (1.1%) had a first stroke. Of these, 143 (89 men and 54 women) had an ischemic stroke. The cumulative incidence of ischemic stroke was 0.2% in the NT group, 0.5% in the PreHT group, and 1.0% in the HT group. The Kaplan–Meier curves showed that the cumulative incidence of ischemic stroke was higher in the PreHT group than in the NT group (P = 0.002, **Figure 1**). However, in the multivariate Cox regression analysis, an increased HR for ischemic stroke was not significant in the PreHT group (HR = 1.72, 95% CI: 0.93–3.18) compared to the NT group after adjustment for potential confounding factors (**Table 2**).

To compare the risk of future stroke in PreHT stratified by hsCRP level, we divided the NT, PreHT, and HT groups into two subgroups according to median serum hsCRP level (0.5 mg/l in men, 0.4 mg/l in women). As shown in **Table 3**, there were significant differences among the BP subgroups in sex, age, BMI, total and high-density lipoprotein cholesterol, prevalence of DM, renal dysfunction, current smoking, current drinking, and atrial fibrillation. SBP and DBP did not differ between the PreHT subgroups. As shown in **Figure 2**, no significant difference in cumulative incidence of ischemic stroke was found between the NT subgroups stratified by hsCRP levels, whereas a significant difference was found between the PreHT and HT subgroups stratified by hsCRP levels (**Figure 2**). Furthermore, when compared with both NT subgroups, the incidence of ischemic stroke was significantly increased in the PreHT subgroup with higher hsCRP levels, but not in the subgroup with lower hsCRP levels.

For every 1,000 subjects of the present cohort, there were 0.8 ischemic stroke events per year in the NT group, 2.0 events in the total PreHT group, 2.9 events in the PreHT subgroup with higher hsCRP levels, and 4.0 events in the HT group. Thus, the attributable risk to total PreHT was 1.2 excess events of ischemic stroke per year per 1,000 subjects, compared to 2.1 excess events per year attributed to the PreHT subgroup with higher hsCRP levels.

As shown in **Table 4**, after adjustment for potential confounding factors in the Cox model, the risk of future ischemic stroke did not differ between the NT subgroups stratified by hsCRP levels. In contrast, when compared to the NT subgroup with lower hsCRP levels, the HR for ischemic stroke was significantly increased in the PreHT subgroup with higher

Table 3 | Baseline characteristics of blood pressure categories stratified by CRP levels

	NT		Pre HT		HT		
	Lower CRP	Higher CRP	Lower CRP	Higher CRP	Lower CRP	Higher CRP	*P* value
No. of subjects	4,163	3,462	2,656	3,065	3,462	5,868	<0.001
Men (%)	25.9	27.5	40.2	35.1	41.9	34.8	<0.001
Age (years)	58 ± 10	60 ± 10*	62 ± 10	63 ± 9**	65 ± 9	66 ± 8***	<0.001
Body mass index (kg/m^2)	22.3 ± 2.6	23.8 ± 3.1*	23.2 ± 2.8	24.7 ± 3.1**	23.9 ± 3.0	25.6 ± 3.4***	<0.001
Systolic blood pressure (mm Hg)	107 ± 8.2	108 ± 7.8*	128 ± 6.0	128 ± 6.0	142 ± 17	143 ± 18	<0.001
Diastolic blood pressure (mm Hg)	65 ± 6.7	66 ± 6.5*	77 ± 6.3	77 ± 6.3	83 ± 10	83 ± 11	<0.001
Total cholesterol (mg/dl)	198 ± 32	202 ± 34*	201 ± 32	205 ± 34**	199 ± 31	204 ± 33***	<0.001
HDL cholesterol (mg/dl)	64 ± 15	58 ± 14*	62 ± 15	57 ± 14**	61 ± 16	57 ± 14***	<0.001
High sensitive CRP (mg/l)	0.20 ± 0.10	1.87 ± 4.77*	0.22 ± 0.10	1.79 ± 4.70**	0.23 ± 0.10	1.98 ± 4.91***	<0.001
Renal dysfunction (%)	7.3	9.4	9.5	12.8	13.5	17.3	<0.001
Diabetes mellitus (%)	2.1	4.4	4.1	5.6	5.1	8.5	<0.001
Atrial fibrillation (%)	0.6	1.3	0.9	1.7	1.2	2.1	<0.001
Current smoking (%)	11.3	14.1	12.5	13.6	11.2	11.1	<0.001
Current drinking (%)	25	22.1	32.6	28.1	35.2	28.6	<0.001

Data are presented as mean ± s.d. or percentage.
CRP, C-reactive protein; HDL, high-density lipoprotein; HT, hypertension; NT, normotension; PreHT, prehypertension.
*P < 0.05 vs. NT with lower CRP. **P < 0.05 vs. PreHT with lower CRP. ***P < 0.05 vs. HT with lower CRP.

hsCRP levels (HR = 2.63, 95% CI: 1.11–6.24), but not in the subgroup with lower hsCRP levels (HR = 0.91, 95% CI: 0.31–2.73). These findings remained the same also when compared to the total NT group (HR = 2.42, 95% CI: 1.27–4.64 in the PreHT subgroup with higher hsCRP levels). In the HT group, the HR for ischemic stroke was significantly increased in both the higher and lower hsCRP subgroups (HR = 3.47 and 2.64, P = 0.003 and 0.025, respectively) compared to the NT subgroup with lower hsCRP levels. Probability values for the interaction between hsCRP value and BP category on ischemic stroke were 0.182 in the PreHT group and 0.819 in the HT group.

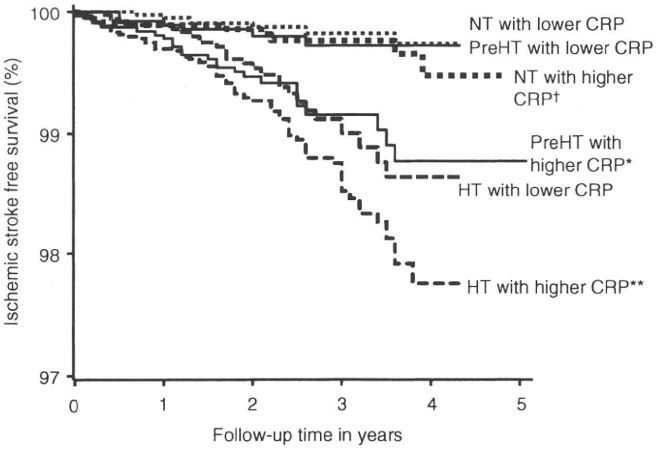

Figure 2 | Cumulative incidence of ischemic stroke in blood pressure categories stratified by CRP level. CRP, C-reactive protein; HT, hypertension; NT, normotension; PreHT, prehypertension. †P = 0.392 vs. NT with lower CRP; *P = 0.040 vs. PreHT with lower CRP; **P = 0.048 vs. HT with lower CRP.

DISCUSSION

In the Iwate-KENCO study, we examined the effect of low-grade inflammation, represented by hsCRP, on the relatively short-term risk of ischemic stroke in PreHT subjects with no cardiovascular history. During the mean follow-up period of 2.7 years, the total PreHT group did not have a significantly higher risk for ischemic stroke independent of established risk factors than the total NT group. In contrast, after stratification of BP category by hsCRP levels, the PreHT subgroup with higher hsCRP levels had a significantly higher risk for ischemic stroke than the total NT group. To the best of our knowledge, this is the first prospective cohort study that has assessed the incidence of ischemic stroke among PreHT individuals stratified by hsCRP levels in the general population.

Previous studies have demonstrated a relationship between PreHT and the incidence of stroke. The Framingham Heart study[17] and the ARIC study[6] reported no significant increase in the risk of ischemic stroke in the PreHT group compared to the optimal BP group (SBP <120 mm Hg and DBP <80 mm Hg). In contrast, recent Japanese studies, such as the Suita study[18] and the Japan Public Health Center-based Prospective study,[19] have shown a significant association between PreHT and the incidence of stroke. The inconsistency between Western and Japanese PreHT subjects in terms of ischemic stroke risk may be derived from differential genetic risk factors and/or dietary pattern, as suggested by a report of Japanese mortality from stroke showing this to be more than double that in the United States.[20] This study has shown no significant increase in the

Table 4 | HRs for ischemic stroke in blood pressure categories stratified by CRP levels

	No. of subjects	No. of events (%)	HR	95% CI	*P* value
NT with lower CRP	4,163	7 (0.2)	1.00	—	—
NT with higher CRP	3,462	9 (0.3)	1.16	0.43–3.13	0.771
PreHT with lower CRP	2,656	6 (0.2)	0.91	0.31–2.73	0.872
PreHT with higher CRP	3,065	24 (0.8)	2.63	1.11–6.24	<0.03
HT with lower CRP	3,462	26 (0.8)	2.64	1.13–6.16	<0.03
HT with higher CRP	5,868	71 (1.2)	3.47	1.54–7.79	<0.01

HR adjusted for sex, age of 10-year increment, total cholesterol, high-density lipoprotein cholesterol, renal dysfunction, body mass index, diabetes mellitus, smoking habits, alcohol intake, and atrial fibrillation in the Cox proportional hazard model.
CI, confidence interval; CRP, C-reactive protein; HR, hazard ratio; HT, hypertension; NT, normotension; PreHT, prehypertension.

risk of ischemic stroke in the PreHT group compared to the NT group. This may be derived from the shorter observation period in our study compared to other studies.

Several epidemiological studies have revealed that hsCRP levels were positively associated with risk of ischemic stroke in the general population.[9–11] The Women's Health Study showed that the prediction effect for the risk of vascular disease by CRP was most striking among women with low cardiovascular risk.[21] In the report from the Honolulu Heart Program, Curb *et al.*[22] suggested that, in the presence of other risk factors such as HT, a high risk of stroke could mask any residual effects of inflammation. In this study with a mean follow-up of 2.7 years, the risk of ischemic stroke was not significantly increased in the total PreHT group, but was increased in the PreHT subgroup with elevated hsCRP levels. These findings indicate that hsCRP is a relatively short-term marker for cerebrovascular risk in PreHT.

Although CRP-related risk stratification for ischemic stroke was shown in the PreHT group but not in the NT group, the mechanisms underlying this phenomenon are uncertain. Several possible explanations are proposed. It has been shown that PreHT is related to subclinical atherosclerosis including increased coronary atherosclerosis,[23] carotid and brachial intima–media thickness[24] and microalbuminuria.[25] Furthermore, it has been reported that elevated CRP reflects the burden of atherosclerosis[26–28] and is directly associated with the development of atherosclerosis.[29] Chrysohoou *et al.* revealed an association between PreHT status and inflammatory markers including CRP in a CVD-free population, and thus suggested that inflammation contributes to the atherosclerotic effects of PreHT.[30] The coexistence of PreHT and elevated hsCRP levels may therefore represent an advanced state of the atherosclerotic process and may thus be associated with an elevated risk of ischemic stroke.

How best to manage PreHT has been the subject of recent debate. The JNC-7 has identified several problems inherent in dealing with large numbers of PreHT individuals. This means that identification of those PreHT individuals at increased risk for future CVD may be warranted. The Strong Heart Study showed that, compared to individuals with NT, the HR for any cardiovascular event was approximately doubled for those with PreHT alone and was 3.7 for those with both PreHT and DM.[7] The ARIC study showed that, compared to the optimal BP group, the HR for cardiovascular events was 2.3 for the high-normal BP only group, 4.1 for the high-normal BP with DM group, and 3.6 for the group with BMI ≥30 kg/m^2 (ref. 6). This study showed that, when compared to the total NT group, the HR for incidence of ischemic stroke was 1.7 ($P = 0.086$) for the total PreHT group and 2.4 ($P = 0.008$) for the PreHT subgroup with elevated hsCRP levels. Our finding of excess risk of ischemic stroke associated with PreHT in conjunction with elevated hsCRP levels may therefore contribute to the development of clinical strategies for the management of PreHT. These could include the adoption of healthy lifestyles and pharmacological treatment including statin therapy, which has recently been reported to be associated with a reduction in CVD risk in subjects with elevated hsCRP levels in the JUPITER trial.[31]

Our study had several limitations. The primary limitation was the relatively short observation period. Hence, it is uncertain whether the present data apply to middle or long-term risk of ischemic stroke. Second, our data are based on a single determination of hsCRP instead of two measures, as currently recommended.[32] This limitation would tend to increase the variability of our hsCRP measurements and could lead to an underestimation of its true effects. Third, our study did not assess the use of drugs that can lower hsCRP levels, such as statins.[33] However, it was unlikely that the frequency of the use of these medications was higher in event-free participants. Fourth, serum hsCRP levels recorded in our study were lower than those previously reported in community based studies in Western countries. When compared to other ethnic groups, several community based studies have demonstrated that hsCRP levels in the general Japanese population are lower[34] and the cutoff point of hsCRP for detecting individuals at high risk for coronary heart disease is also lower.[35] Therefore, the cutoff point of hsCRP for predicting ischemic stroke in our population may not be simply extrapolated to other populations. Finally, of the total number of PreHT subjects who developed ischemic stroke, none had Af in the group with lower CRP levels, while 29% had Af in the group with higher CRP levels. This difference in the frequency of Af between the PreHT subgroups was not statistically significant ($P = 0.290$); however, we cannot completely exclude the possibility that Af had an influence on the association between PreHT stratified by hsCRP level and the incidence of ischemic stroke.

In conclusion, in the PreHT subgroup higher hsCRP levels were associated with an elevated risk of future ischemic stroke compared to NT subjects in the general Japanese population followed up for an average of 2.7 years. HsCRP-related stratification is useful for the estimation of relatively short-term risk of ischemic stroke in PreHT subjects.

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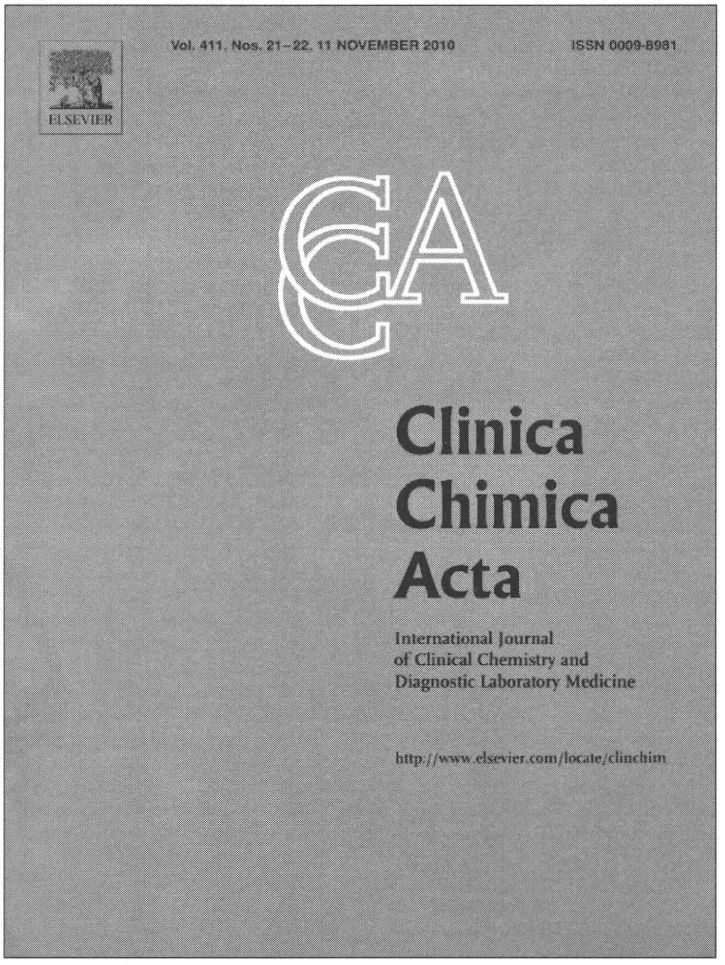

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Comparison of low-density lipoprotein cholesterol concentrations measured by a direct homogeneous assay and by the Friedewald formula in a large community population

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ABSTRACT

Background: We compare the direct homogeneous low-density lipoprotein cholesterol (LDL-C) assay with the Friedewald formula (FF) for determination of LDL-C in a large community-dwelling population.
Methods: A total of 21,194 apparently healthy subjects aged 40 to 79 years with triglyceride (TG) concentrations <4.52 mmol/l were enrolled. LDL-C were directly measured by the enzymatic homogeneous assay (LDL-C (D)) and also estimated by the FF (LDL-C (F)). Paired t-test, Pearson's correlation coefficient and linear regression analysis were performed and the concordances of the National Cholesterol Education Program (NCEP) risk category were estimated.
Results: Both in fasting (n = 3270) and nonfasting samples (n = 17,924), LDL-C (D) highly correlated with LDL-C (F): $r = 0.971$ and 0.955, respectively. Concordant results for NCEP categories were 84.8% for fasting samples and 80.1% for nonfasting samples. However, the bias between the 2 measurements increased in samples with TG concentrations >1.69 mmol/l, especially in nonfasting samples.
Conclusions: The results showing less variability of the direct LDL-C assay than that of the FF in nonfasting samples suggest that epidemiological studies can use LDL-C measured by the direct assay both in fasting and nonfasting samples.

1. Introduction

Many epidemiological studies and clinical trials have shown that elevated low-density lipoprotein cholesterol (LDL-C) concentrations are causally related to an increased risk of coronary artery disease (CAD) [1,2]. The findings from those studies are mainly based on LDL-C concentrations calculated by the Friedewald formula (LDL-C (F)) [3], which derives LDL-C concentrations from total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and triglyceride (TG) concentrations [4–7]. The guidelines for preventing atherosclerotic disease recommend using the Friedewald formula in a fasting state (ideally a 9- to 12-h fast) [1,2]. However, it is impossible to obtain fasting samples from all patients who visit clinics, especially those who visit at night or in the afternoon. In Japan, general screenings for risk factors of cardiovascular disease (CVD) are performed under nonfasting conditions to improve the participation rates. Therefore, a convenient method for determination of LDL-C concentration that is insensitive to postprandial state has been required regardless of whether it is directly obtained or calculated.

Recently, several homogeneous assays have been used as direct measurements for determination of LDL-C concentration. They have become popular in clinics and in health check-ups. Most homogeneous assays have met the National Cholesterol Education Program (NCEP) total error goals for nondiseased individuals in a fasting state compared with β-quantification [8,9]. In addition, no significant difference in LDL-C concentrations measured by the direct homogeneous assay was seen

Abbreviations: LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; LDL-C (F), LDL-C calculated by the Friedewald formula; LDL-C (D), LDL-C measured by the enzymatic homogeneous assay; CAD, coronary artery disease; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; CVD, cardiovascular disease; NCEP, National Cholesterol Education Program; CRMLN, Cholesterol Reference Method Laboratory Network; HbA1c, glycosylated hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; NCEP-ATP III, National Cholesterol Education Program Adult Treatment Panels III; OR, odds ratio; CI, confidence interval.

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between paired fasting and nonfasting samples in the same individuals [10,11], and postprandial changes in LDL-C concentrations measured by the homogeneous assay were similar to those measured by β-quantification [12].

On the other hand, the Friedewald formula is known to underestimate LDL-C concentrations compared with β-quantification even when TG concentrations are <4.52 mmol/l [13–15]. The calculated LDL-C concentrations also have been reported to be significantly decreased in a postprandial state [16–18]. However, in recent large-scale population-based cohort studies, CVD risk has been assessed using LDL-C concentrations calculated by the Friedewald formula in a nonfasting state because of a strong correlation between LDL-C concentrations obtained by the Friedewald formula and β-quantification [19,20] and there are only minimal changes in concentrations of LDL-C in response to normal food intake in a general population [21]. These studies suggest that LDL-C concentrations calculated by the Friedewald formula either in fasting or nonfasting samples could be used in population-based epidemiological studies.

We therefore compared the direct homogeneous LDL-C assay with the Friedewald formula for determination of LDL-C both in fasting and nonfasting samples using baseline data from a large cohort study of community-dwelling residents to clarify whether the direct homogeneous LDL-C assay can be used in population-based epidemiological studies.

2. Materials and methods

2.1. Study population

We analyzed baseline data of the Iwate-Kenpoku cohort (Iwate-KENCO) study, which was designed as a cohort study of community-dwelling residents living in the northern part of the main island of Japan. The methodology of the Iwate-KENCO study was described elsewhere [22–24]. The baseline survey was carried out between 2002 and 2005. Of 24,572 participants (8476 men and 16,096 women) aged 40 to 79 years from whom we obtained written informed consent for participation in this study, 594 subjects with missing data for serum lipids, 212 subjects with TG concentrations >4.52 mmol/l (400 mg/dl) and 54 subjects who did not have complete information were excluded from the analysis. Furthermore, 1697 subjects receiving medication for dyslipidemia and 821 subjects with a history of stroke or myocardial infarction were excluded to examine whether LDL-C concentrations measured by the direct homogeneous assay can be used as baseline data in an epidemiological study assessing the risk of first CVD events in the general community-dwelling population. Therefore, 21,194 participants (7349 men and 13,845 women) were enrolled in the present study. The study was approved by the Medical Ethics Committee of Iwate Medical University and conducted in accordance with the guidelines of the Declaration of Helsinki.

2.2. Measurements of serum lipids

Samples from participants whose last meal was ≥12 h before their blood draw were used as fasting samples (n=3270) and samples from participants who had eaten within 12 h of their blood draw were used as nonfasting samples (n=17,924). Both fasting and nonfasting samples were collected into vacuum tubes containing a serum separator gel. The samples were stored immediately after sampling in an icebox and were transported to a laboratory (Iwate Health Service Association) and analyzed on the same day. Serum TC, TG and HDL-C concentrations were measured by an enzymatic method. Serum LDL-C concentrations were measured by an enzymatic homogenous assay with Cholestest-LDL (Daiichi Chemicals, currently Sekisui Medical, Tokyo). LDL-C was also estimated again using the Friedewald formula. Non-HDL-C was calculated by subtracting HDL-C from TC. Measurements for TC, HDL-C and LDL-C (homogenous assay), except for the TG assay, have been standardized by the Osaka Medical Center for Health Science and Promotion, a member of the Cholesterol Reference Method Laboratory Network (CRMLN) controlled by the Centers for Disease Control and Prevention (Atlanta, USA) [25] and have met all criteria for both precision and accuracy of lipid measurement. During the period of the baseline survey, total coefficient of variations (CVs), mean biases and total errors for LDL-C assay used in this study were 0.2% to 0.4%, 0.7% to 0.8% and 1.2% to 1.5%, respectively. The corresponding values for the TC assay were −2.7% to 0.4%, 0.2% to 0.4% and 1.2% to 3.5%, and the corresponding values for the HDL-C assay were −2.2% to 3.0%, 0.6% to 1.1% and 3.0% to 4.4%. For the TG assay, total CVs at the laboratory were 0.2 to 1.9%. External quality assessment for the TG assay was performed by the Japan Association of Medical Technologists (JAMT) and the analytical performance of the TG assay has met the criteria of quality assessment in the JAMT.

2.3. Measurements of other risk factors

Plasma glucose concentrations were determined by the hexokinase method, and glycosylated hemoglobin (HbA1c) concentrations were determined by HPLC. Diabetes was defined as plasma glucose concentration being ≥7.0 mmol/l in fasting samples or ≥11.1 mmol/l in nonfasting samples, plasma HbA1c concentration being ≥6.5%, use of anti-diabetic agents or a combination of these.

Blood pressures were measured twice in the sitting position after urination and a 5-min rest by well-trained staff using an automatic device. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were each calculated as the mean of 2 measurements. Hypertension was defined as SBP being ≥140 mmHg, DBP being ≥90 mmHg or more, use of antihypertensive agents or a combination of these. Height in stockings and weight in light clothing were measured. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m).

Self-administered questionnaires for past history of stroke and myocardial infarction, medication, alcohol drinking and smoking status were used to collect individual information. To confirm whether participants had had prevalent stroke and myocardial infarction at the baseline survey, data from the Iwate Stroke Registry [26] and Northern Iwate Heart Disease Registry Consortium [27] were systematically reviewed. Smoking status was determined as current, past and never smoking by the questionnaire. Regular alcohol drinking was defined as drinking ≥5 days/week. Presence or absence of medication for dyslipidemia was determined by the answer of whether a participant had used any anti-hyperlipidemia agents.

2.4. Statistical analysis

All analyses, except for a logistic regression analysis, were separately performed in the fasting group and nonfasting group. Participants were also classified into 3 groups according to serum TG concentrations, of which cut-off points were based on the National Cholesterol Education Program Adult Treatment Panels III (NCEP-ATP III) guideline, i.e., normal: <1.69 mmol/l (150 mg/dl), moderate high: 1.69 to 2.26 mmol/l (150 to 199 mg/dl), and high: 2.26 to 4.51 mmol/l (200 to 399 mg/dl) [1]. We calculated the means and proportions of selected variables by TG group. Data for TG were expressed as geometric means. Except for TG, comparisons of selected variables between TG groups were performed using analysis of variance (ANOVA) for continuous variables and the χ^2 test for categorical variables. Concentrations of LDL-C (F) and LDL-C (D) were also compared using the paired t-test.

The correlation between LDL-C (F) and LDL-C (D) and the effect of TG concentrations on the difference in LDL-C by the 2 methods, which was calculated by subtracting LDL-C (F) from LDL-C (D) concentrations, were estimated using Pearson's correlation coefficients and linear regression analysis. To examine concordance of concentrations of

Table 1
Characteristics and serum lipid levels in fasting participants by TG groups.

	Total	TG group, mmol/L			P value[a]
		<1.69	1.69–2.26	2.26–4.51	
Number of fasting participants	3270	2906	224	140	
TG, mmol/L[b]	0.97 (1.6)	0.87 (1.4)	1.89 (1.1)	2.80 (1.2)	<0.001
Men, %	36.8	35.4	42.4	57.1	<0.001
Age, years	63.5 (9.3)	63.5 (9.4)	63.8 (8.9)	62.4 (9.4)	0.342
Body mass index, kg/m^2	24.0 (3.3)	23.8 (3.3)	25.1 (3.4)	25.6 (3.0)	<0.001
Hypertension, %	42.3	41.4	49.1	50.7	0.010
Diabetes, %	9.0	8.4	9.8	20.0	<0.001
Current smokers, %	11.2	10.4	14.3	22.1	<0.001
Regular drinkers, %	19.3	18.9	21.0	25.7	0.110
Serum lipids					
TC, mmol/L	5.31 (0.85)	5.27 (0.83)	5.53 (0.91)	5.73 (0.91)	<0.001
HDL-C, mmol/L	1.58 (0.39)	1.62 (0.39)	1.30 (0.30)	1.20 (0.29)	<0.001
non-HDL-C, mmol/L	3.73 (0.85)	3.65 (0.81)	4.23 (0.88)	4.53 (0.88)	<0.001
LDL-C (F), mmol/L	3.24 (0.77)	3.23 (0.76)	3.36 (0.87)	3.23 (0.88)	0.046
LDL-C (D), mmol/L	3.24 (0.76)	3.22 (0.75)	3.40 (0.83)	3.29 (0.83)	0.003
	$P=0.553$[c]	$P=0.232$[c]	$P=0.019$[c]	$P=0.033$[c]	

TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C (F), LDL-C calculated by the Friedewald formula; LDL-C (D), LDL-C measured by the enzymatic homogeneous assay.
Data are expressed as means (standard deviations) for continuous variables and percentages for categorical variables.
[a] *P* values for comparisons of variables between TG groups by analysis of variance or the chi-squared test.
[b] Data for triglyceride are expressed as geometric means (geometric standard deviations).
[c] *P* values for comparisons between LDL-C (F) levels and LDL-C (D) levels by the paired t-test.

LDL-C (F) and LDL-C (D), participants were classified into four groups according to concentrations of LDL-C (F) or LDL-C (D) based on the LDL-C cut-off points recommended by the NCEP-ATP III guideline, i.e., <2.58 mmol/l (100 mg/dl), 2.58 to 3.35 mmol/l (100 to 129 mg/dl), 3.36 to 4.12 mmol/l (130 to 159 mg/dl), ≥4.13 mmol/l (160 mg/dl) [1]. Cross-tables among the NCEP groups of LDL-C (F) and LDL-C (D) were presented by TG groups.

Logistic regression analysis was performed for the all participants in the fasting and nonfasting groups. In the analysis, the dependent variable was discordance of the groups between LDL-C (F) and LDL-C (D) (coded as 1 for discordance and coded as 0 for concordance) and the independent variables were logarithm-transformed TG (ln TG), age, sex, BMI, hypertension (presence or absence), diabetes (presence or absence), current smokers (or not), regular drinkers (or not) and nonfasting state (or fasting state). In all analyses, 2-sided $P<0.05$ was considered to be statistically significant. The Statistical Package for Social Science (SPSS Japan Inc. ver. 15.0J, Tokyo, Japan) was used for all analyses.

3. Results

Table 1 shows characteristics of fasting participants by TG groups. There were significant differences between TG groups in all variables except for mean age and proportion of regular drinkers: that is, mean BMI and proportions of male participants, participants with hypertension, participants with diabetes and current smokers were higher in the higher TG group. Table 1 also shows fasting serum lipid profiles by TG groups. Mean concentrations of TC, HDL-C, non-HDL-C, LDL-C (F) and LDL-C (D) were significantly different between the TG groups. There was no significant difference between mean fasting concentrations of LDL-C (D) and LDL-C (F) (3.24 mmol/l and 3.24 mmol/l, respectively). In the normal TG group, there was also no significant difference between mean fasting concentrations of LDL-C (D) and LDL-C (F): mean concentrations of LDL-C (D) and LDL-C (F) were 3.22 mmol/l and 3.23 mmol/l, respectively. In the moderate high and high TG groups, mean fasting concentrations of LDL-C (D) were

Table 2
Characteristics and serum lipid levels in nonfasting participants by TG groups.

	Total	TG group, mmol/L			P value[a]
		<1.69	1.69–2.26	2.26–4.51	
Number of nonfasting participants	17,924	13,831	2307	1786	
TG, mmol/L[b]	1.16 (1.7)	0.95 (1.4)	1.92 (1.1)	2.86 (1.2)	<0.001
Men, %	34.3	32.8	36.7	42.8	<0.001
Age, years	62.1 (9.7)	62.1 (9.8)	62.4 (9.4)	61.7 (9.6)	0.071
Body mass index, kg/m^2	24.0 (3.3)	23.7 (3.2)	25.1 (3.2)	25.5 (3.1)	<0.001
Hypertension, %	40.6	38.3	47.9	49.2	<0.001
Diabetes, %	4.9	4.4	5.8	7.8	<0.001
Current smokers, %	12.7	11.6	14.5	18.5	<0.001
Regular drinkers, %	19.0	18.7	19.0	21.8	0.007
Serum lipids					
TC, mmo/L	5.15 (0.83)	5.07 (0.81)	5.36 (0.81)	5.50 (0.84)	<0.001
HDL-C, mmol/L	1.53 (0.38)	1.60 (0.38)	1.37 (0.31)	1.26 (0.29)	<0.001
Non-HDL-C, mmol/L	3.62 (0.82)	3.47 (0.77)	3.99 (0.77)	4.24 (0.81)	<0.001
LDL-C (F), mmol/L	3.01 (0.75)	3.01 (0.73)	3.11 (0.77)	2.91 (0.82)	<0.001
LDL-C (D), mmol/L	3.08 (0.74)	3.03 (0.73)	3.28 (0.73)	3.24 (0.76)	<0.001
	$P<0.001$[c]	$P<0.001$[c]	$P<0.001$[c]	$P<0.001$[c]	

TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C (F), calculated by the Friedewald formula; LDL-C (D), LDL-C measured by the enzymatic homogeneous assay.
Data are expressed as means (standard deviations) for continuous variables and percentages for categorical variables.
[a] *P* values for comparisons of variables between TG groups by analysis of variance or the chi-squared test.
[b] Data for triglyceride are expressed as geometric means (geometric standard deviations).
[c] *P* values for comparisons between LDL-C (F) levels and LDL-C (D) levels by the paired t-test.

significantly higher than those of LDL-C (F): the differences were 0.04 mmol/l and 0.06 mmol/l, respectively.

Table 2 shows characteristics and serum lipid profiles of nonfasting participants by TG groups. The differences in variables and serum lipid profiles between TG groups in nonfasting participants were similar to those in fasting participants. However, there were significant differences between concentrations of LDL-C (D) and LDL-C (F): mean concentrations of LDL-C (D) and LDL-C (F) were 3.08 and 3.01 mmol/l. A difference between them was found even in the normal TG group: mean nonfasting concentrations of LDL-C (D) and LDL-C (F) were 3.03 mmol/l and 3.01 mmol/dl, although the difference was only 0.02 mmol/l. Moreover, mean nonfasting concentrations of LDL-C (D) were significantly higher than those of LDL-C (F) in the moderate and high TG groups: the differences were 0.17 mmol/l and 0.33 mmol/l, respectively.

Both in fasting and nonfasting samples, LDL-C (D) concentrations showed strong linear correlations with LDL-C (F) concentrations: Pearson's coefficient (r) for fasting samples and that for nonfasting samples was 0.971 and 0.955, respectively (both $P<0.001$) (Fig. 1). The effect of TG concentrations on the difference between concentrations of LDL-C (D) and LDL-C (F) differed between fasting and nonfasting samples. In fasting samples, the difference in LDL-C concentrations was positively related to TG concentrations (Pearson's correlation coefficient $r=0.157$, $P<0.001$) and it increased by 0.05 mmol/l with an increment of 1.00 mmol/l in TG concentration (Fig. 2A), whereas in nonfasting samples, the difference in LDL-C concentrations showed a relatively higher positive relation to TG concentrations compared with that in fasting samples (Pearson's correlation coefficient $r=0.523$, $P<0.001$) and increased by 0.17 mmol/l with an increment of 1.00 mmol/l in TG concentration (Fig. 2B).

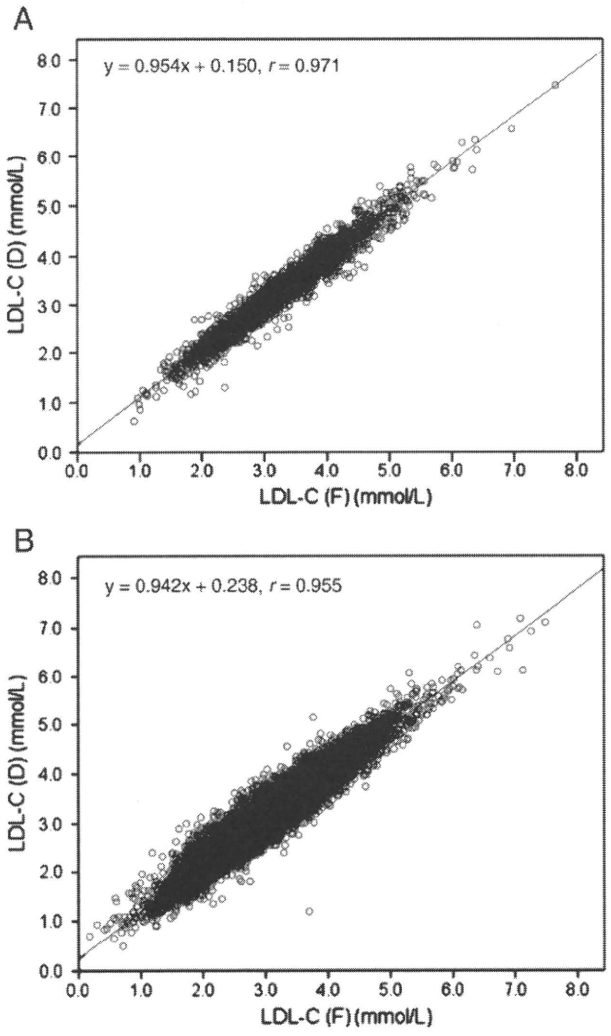

Fig. 1. Correlations between LDL-C (F) and LDL-C (D) in fasting participants (n = 3270) (A) and nonfasting participants (n = 17,294) (B). The solid lines represent regression lines.

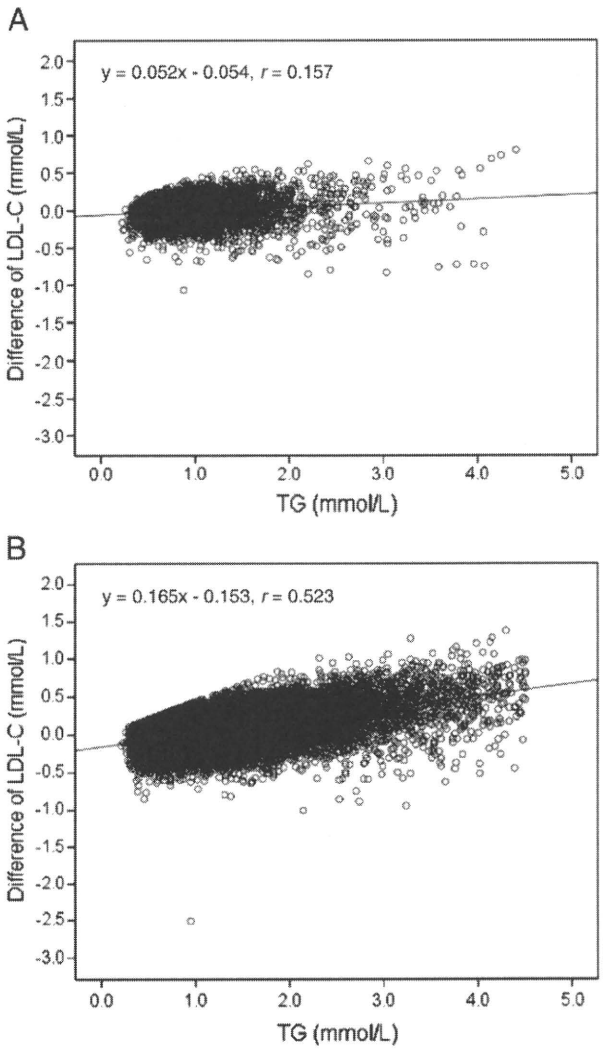

Fig. 2. Effect of increased TG concentrations on the difference in LDL-C concentrations between the homogeneous assay and the Friedewald formula in fasting participants (n = 3270) (A) and nonfasting participants (n = 17,294) (B). The difference in LDL-C, which is calculated by subtracting LDL-C concentrations by the Friedewald formula from those by the homogeneous assay, is plotted as a function of TG concentrations. The solid lines represent regression lines.

Table 3 shows concordance between fasting LDL-C (F) and LDL-C (D) for classifying participants into NCEP categories of risk. Overall, 2772 (84.8%) of the 3270 participants showed concordant results. A total of 497 fasting participants (15.2%) differed by one NCEP group. Of these, the proportions of fasting participants being classified into one upper group by LDL-C (D) concentrations compared with LDL-C (F) concentrations were 54.4% (228 of 420) in the normal TG group, 59.3% (19 of 32) in the moderate high TG group and 62.2% (28 of 45) in the high TG group.

Table 4 shows concordance between nonfasting LDL-C (F) and LDL-C (D) for classifying participants into NCEP categories of risk. Overall, 14,366 (80.1%) of 17,924 participants showed concordant results. A total of 3550 nonfasting participants (19.8%) differed by one NCEP group. Of these, the proportions of nonfasting participants being

Table 3
Concordance of NCEP groups between LDL-C (F) levels and LDL-C (D) levels in fasting participants.

			LDL-C (D), mmol/L			
			<2.59	2.60–3.36	3.36–4.13	4.13+
All fasting participants (n=3270)						
LDL-C (F), mmol/L	<2.59	(n=632)	86.6	13.4	0	0
	2.60–3.36	(n=1273)	4.2	85.6	10.1	0
	3.36–4.13	(n=976)	0.1	10.5	83.2	6.3
	4.13+	(n=389)	0	0	17.0	83.0
Normal TG (<1.69 mmol/L) group (n=2906)						
LDL-C (F), mmol/L	<2.59	(n=571)	87.2	12.8	0	0
	2.60–3.36	(n=1128)	4.1	86.9	9.0	0
	3.36–4.13	(n=882)	0	10.3	83.7	6.0
	4.13+	(n=325)	0	0	16.9	83.1
Moderate high TG (1.69–2.26 mmol/L) group (n=224)						
LDL-C (F), mmol/L	<2.59	(n=33)	87.9	12.1	0	0
	2.60–3.36	(n=87)	2.3	82.8	14.9	0
	3.36–4.13	(n=60)	1.7	8.3	86.7	3.3
	4.13+	(n=44)	0	0	13.6	86.4
High TG (2.26–4.51 mmol/L) group (n=140)						
LDL-C (F), mmol/L	<2.59	(n=28)	71.4	28.6	0	0
	2.60–3.36	(n=58)	10.3	65.5	24.1	0
	3.36–4.13	(n=34)	0	17.6	64.7	17.6
	4.13+	(n=20)	0	0	25.0	75.0

TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; LDL-C (F), LDL-C calculated by the Friedewald formula; LDL-C (D), LDL-C measured by the enzymatic homogeneous assay.
Data are expressed as percentages of the number of participants being classified into each LDL-C (F) group.

classified into one upper group by LDL-C (D) concentrations compared with LDL-C (F) concentrations were 57.8% (1277 of 2210) in the normal TG group, 89.0% (541 of 608) in the moderate high TG group and 93.7% (686 of 732) in the high TG group.

Table 4
Concordance of NCEP groups between LDL-C (F) levels and LDL-C (D) levels in nonfasting participants.

			LDL-C (D), mmol/L			
			<2.59	2.60–3.36	3.36–4.13	4.13+
All nonfasting participants (n=17,924)						
LDL-C (F), mmol/L	<2.59	(n=5134)	79.2	20.7	0.1	0
	2.60–3.36	(n=7294)	5.4	79.8	14.7	0
	3.36–4.13	(n=4241)	0	10.6	80.8	8.7
	4.13+	(n=1255)	0	0	16.0	84.0
Normal TG (<1.69 mmol/L) group (n=13,831)						
LDL-C (F), mmol/L	<2.59	(n=3959)	86.0	14.0	0	0
	2.60–3.36	(n=5698)	6.5	84.2	9.2	0
	3.36–4.13	(n=3240)	0	12.2	81.7	6.0
	4.13+	(n=934)	0	0	17.6	82.4
Moderate high TG (1.69–2.26 mmol/L) group (n=2307)						
LDL-C (F), mmol/L	<2.59	(n=556)	64.2	35.8	0	0
	2.60–3.36	(n=935)	1.3	69.9	28.8	0
	3.36–4.13	(n=609)	0	5.4	82.6	12.0
	4.13+	(n=207)	0	0	10.6	89.4
High TG (2.26–4.51 mmol/L) group (n=1786)						
LDL-C (F), mmol/L	<2.59	(n=619)	49.3	49.9	0.8	0
	2.60–3.36	(n=661)	1.8	55.8	42.1	0.3
	3.36–4.13	(n=392)	0	4.8	69.9	25.3
	4.13+	(n=114)	0	0	13.2	86.8

TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; LDL-C (F), LDL-C calculated by the Friedewald formula; LDL-C (D), LDL-C measured by the enzymatic homogeneous assay.
Data are expressed as percentages of the number of participants being classified into each LDL-C (F) group.

Table 5
Odds ratios for discordance of NCEP groups between LDL-C (F) and LDL-C (D) for each factor.

	OR	(95% CI)	*P* value
ln TG (per 1-ln TG increase)	2.44	(2.26–2.63)	<0.001
Sex (men/women)	1.01	(0.92–1.11)	0.856
Age (per 1-year increase)	1.00	(0.99–1.00)	0.052
Body mass index (per 1-kg/m^2 increase)	1.00	(0.99–1.01)	0.663
Hypertension (presence/absence)	1.01	(0.93–1.09)	0.846
Diabetes (presence/absence)	1.21	(1.05–1.40)	0.009
Current smoking (yes/no)	0.96	(0.85–1.08)	0.473
Regular drinking (yes/no)	0.92	(0.82–1.02)	0.108
Nonfasting state (/fasting state)	1.18	(1.06–1.31)	0.002

LDL-C, low-density lipoprotein cholesterol; LDL-C (F), LDL-C calculated by the Friedewald formula; LDL-C (D), LDL-C measured by the enzymatic homogeneous assay; OR, odds ratio; CI, confidence interval; ln TG, logarithm-transformed triglyceride. The OR was adjusted for ln TG, sex, age, body mass index, hypertension, diabetes, current smoking, regular drinking and nonfasting state.

For all participants in the fasting and nonfasting groups, the logistic regression model revealed that the discordance of NCEP groups between LDL-C (F) and LDL-C (D) was associated with higher TG concentrations: the odds ratio (OR) (95% confidence interval (CI)) was 2.44 (2.26–2.63) with an increment of 1-ln TG. In addition, the presence of diabetes and nonfasting state was associated with the discordance between the two methods: ORs (95% CIs) were 1.21 (1.05–1.40) for the presence of diabetes and 1.18 (1.06–1.31) for the nonfasting state (Table 5).

4. Discussion

We demonstrated that LDL-C (D) concentrations had a significant correlation with LDL-C (F) concentrations and that NCEP categories of LDL-C (D) were highly coincident with those of LDL-C (F) in fasting samples. In addition, even in nonfasting samples, the correlation coefficient between LDL-C (D) and LDL-C (F) concentrations was more than 0.9 and the concordance rate of NCEP categories between the two LDL-C concentrations was approximately 80%. However, the discordance was increased in samples with higher TG concentrations, particularly in nonfasting samples.

Our findings in fasting samples are similar to results of previous studies in Western countries [28–30] and other countries [31–33] showing a strong correlation between concentrations of LDL-C (D) and LDL-C (F). Two studies have shown the concordance rate of calculated LDL-C and directly measured LDL-C for classifying participants into NCEP categories [29,30]. One of those studies used data for LDL-C concentrations measured by an immunoseparation method in 661 primary care patients who had TG concentrations less than 4.52 mmol/l (mean, 1.66 mmol/l) and who were not receiving medication for hyperlipidemia. The other study used data for LDL-C concentrations measured by an enzymatic homogeneous method in 19,777 female healthcare professionals who had TG concentrations <4.52 mmol/l (mean, 1.53 mmol/l) and no history of CVD or cancer. The concordance rates were 48.1% in the former study [29] and 79.3% in the latter study [30]. The concordance rate in the present study (84.8%) was similar to that in the latter study.

On the other hand, we showed that fasting LDL-C (D) concentrations were significantly higher than fasting LDL-C (F) concentrations when TG concentrations were ≥1.69 mmol/l, although the difference between the two LDL-C concentrations was small. Most previous studies [28,29,31–33], except for one study [30], have demonstrated that LDL-C concentrations determined by direct methods tend to be higher than those calculated by the Friedewald formula, especially in subjects with higher TG concentrations.

The Friedewald formula is known to underestimate LDL-C concentrations compared with those measured by β-quantification even in fasting samples with TG concentrations being <4.52 mmol/l [13–15].

The bias increases with increasing TG concentrations, starting at moderate high TG concentrations (1.5 or 2.0 mmol/l) [13,14]. Miller et al. simultaneously compared the Friedewald formula and the enzymatic homogeneous assay, which was the same one as that used in the present study, with β-quantification [12]. They showed that the homogeneous assay had less variability in LDL-C concentrations than did the Friedewald formula in TG concentrations between 3.39 and 6.77 mmol/l (300 and 600 mg/dl) [12]. Therefore, the reason for the higher LDL-C concentrations obtained by the direct assay in fasting samples with TG concentrations being ≥1.69 mmol/l may be due to underestimation of LDL-C concentrations by the Friedewald formula.

A recent cohort study suggested that epidemiological studies could use LDL-C concentrations calculated by the Friedewald formula in participants with nonfasting samples to assess the association of LDL-C with CVD risk [20]. However, some studies have also shown that LDL-C concentrations calculated by the Friedewald formula significantly decrease at the postprandial state [16–18] and that the LDL-C concentrations calculated by the Friedewald formula are also significantly lower than those measured by β-quantification in postprandial samples among the same individuals [16]. On the other hand, there was no significant difference in LDL-C concentrations measured by the same homogeneous assay as that used in our study between paired fasting and nonfasting samples from the same individuals [10,11]. Miller et al. showed that postprandial changes in LDL-C concentrations measured by the assay used in our study were similar to those measured by β-quantification, although LDL-C concentrations measured by the assay in postprandial samples were significantly lower than those in fasting samples [12]. Indeed, we also showed that nonfasting LDL-C concentrations calculated by the Friedewald formula were significantly lower than those measured by the homogeneous assay, particularly in samples with TG being 1.69 mmol/l or greater. The results suggest that the bias between the Friedewald formula and the direct homogeneous assay observed in our study is comparable to the bias between the Friedewald formula and β-quantification in the literature.

We also demonstrated that the discordance of NCEP categories between LDL-C (F) and LDL-C (D) was associated with diabetes as well as higher TG concentrations and nonfasting state. Some studies showed poor validity of the Friedewald formula in diabetic patients [34,35], whereas the homogeneous assay used in our study did not seem to be compromised in diabetic patients [36]. The above-mentioned findings indicate the possibility of less variability of this homogeneous assay than that of the Friedewald formula in nonfasting samples.

The present study has several limitations. First, most participants were in a nonfasting state. However, precisely because all participants were not at a fasting state, we believe that it was significant to perform a direct measurement of LDL-C concentrations. Second, we did not use β-quantification as the standard reference method. Thus, it is not clear whether the homogeneous assay overestimated the LDL-C concentration or whether the Friedewald formula underestimated the LDL-C concentration. Third, our subjects were an apparently healthy population; thus, it is unclear if our results would be applicable to diseased populations, particularly patients with hypertriglycemia or CVD. Finally, it is possible that our results may not be directly applicable to those obtained from other homogeneous assays because the present results were obtained from the direct assay by Daiichi Chemicals (currently Sekisui Medical).

In conclusion, we demonstrated a strong correlation between LDL-C concentrations measured by the direct homogeneous assay and those calculated by the Friedewald formula and high concordance rates of NCEP groups between the two LDL-C concentrations in fasting samples; however, it should be kept in mind that LDL-C concentrations measured by the direct homogeneous assay tend to be slightly higher than calculated LDL-C concentrations when TG concentrations are ≥1.69 mmol/l. We also showed less variability of the direct homogeneous assay than that of the Friedewald formula in large numbers of nonfasting samples. The bias between the Friedewald formula and the direct homogeneous assay observed in our study was comparable to the bias between the Friedewald formula and β-quantification in the literature. The findings suggest that the direct assay for LDL-C measurement can be used in epidemiological studies on the association of LDL-C with risk for CVD both in fasting and nonfasting samples. Future longitudinal studies are needed to clarify the utility of direct nonfasting LDL-C measurements as a predictor of CVD events.

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Original Article

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ABSTRACT

Background: Many studies have estimated the prevalence of anti-hepatitis C virus (HCV) antibody among hemodialysis (HD) patients; however, the prevalence of HCV core antigen—which indicates the presence of chronic HCV infection—is not known.

Methods: Standardized prevalence ratios (SPRs) for anti-HCV antibody and HCV core antigen among HD patients ($n = 1214$) were calculated on the basis of data from the general population ($n = 22\,472$) living in the same area.

Results: The prevalences of anti-HCV antibody and HCV core antigen were 12.5% and 7.8%, respectively, in male hemodialysis patients, and 8.5% and 4.1% in female hemodialysis patients. The SPRs (95% confidence interval) for anti-HCV antibody and HCV core antigen were 8.39 (6.72–10.1) and 12.9 (9.66–16.1), respectively, in males, and 5.42 (3.67–7.17) and 8.77 (4.72–12.8) in females.

Conclusions: The prevalences of chronic HCV infection among male and female HD patients were 13-fold and 9-fold, respectively, those of the population-based controls. Further studies should therefore be conducted to determine the extent of chronic HCV infection among HD patients in other populations and to determine whether chronic HCV infection contributes to increased mortality in HD patients.

Key words: hepatitis C virus infection; hemodialysis; standardized prevalence ratio (SPR); population-based study; cross-sectional analysis

INTRODUCTION

The prevalence of hepatitis C virus (HCV) infection in hemodialysis patients is very high.[1–15] Because hemodialysis patients are vulnerable to HCV infection due to the risk of HCV exposure associated with the dialysis procedure and blood transfusion,[16–18] infection control measures have been established to reduce the risks of HCV infection. Tests for detecting antibodies to HCV were first licensed by the Food and Drug Administration (FDA) in 1990[19] and are now used worldwide. The risk of HCV infection due to dialysis and blood transfusion has therefore dramatically decreased.

The estimated prevalence of HCV infection in hemodialysis patients, although lower than in the past, remains high in developed countries in Europe, despite measures to prevent transmission of HCV.[13,20,21] It has been suggested that HCV infection independently contributes to increased mortality among hemodialysis patients.[14,22–26] In order to reduce mortality associated with HCV infection among hemodialysis patients, the prevalence of HCV infection and the factors that predispose hemodialysis patients to HCV infection require immediate investigation.

The prevalence of anti-HCV antibody among hemodialysis patients has been estimated in many studies, but the prevalence of chronic HCV infection is not known. In

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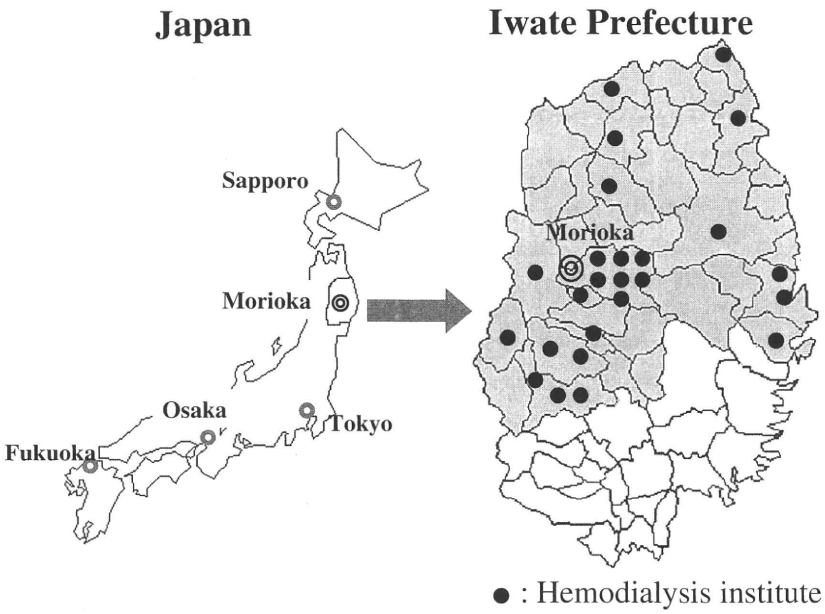

Figure 1. Maps of the KAREN Study area.
The maps show the location of Morioka (the capital of Iwate Prefecture), in northeastern Honshu island. The KAREN Study area (shaded area) covers approximately two-thirds of Iwate Prefecture, and includes 26 hemodialysis facilities; only 1 facility (in which 7 patients were treated) was not included in the study. Closed circles indicate the sites of the hemodialysis facilities.

general, patients who are anti-HCV antibody-positive include those who are chronically infected and those who have recovered from infection. However, all patients who are HCV core antigen-positive are considered chronically infected. Therefore, it is necessary to test for both anti-HCV antibody and HCV core antigen to accurately assess the extent of chronic HCV infection in hemodialysis patients.

We investigated the prevalences of anti-HCV antibody and HCV core antigen in hemodialysis patients. We then compared these prevalences with those of the general population and examined associations between the prevalences and hemodialysis vintage.

SUBJECTS AND METHODS

Subjects

We have conducted the "Kaleidoscopic Approaches to patients with end-stage RENal disease Study" (the KAREN Study) since 2003 in northern Japan (Figure 1). The KAREN Study is a population-based prospective study designed to determine the effects of risk factors on cardiovascular morbidity and mortality in end-stage renal disease (ESRD) patients.[27] A total of 1214 adult hemodialysis patients (80.6% of the total number of hemodialysis patients in the study area; age 22 to 95 years; 779 males and 435 females) are included in the KAREN Study. Figure 2 shows a flow chart of the procedure for selecting subjects participating in the KAREN Study.

Control subjects were recruited from the general population living in the same area, and comprised 22474 participants (7650 men and 14824 women) who underwent annual health check-ups in Iwate Prefecture and HCV screening tests in 2005.

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

Measurements

The initial investigations in the KAREN Study were conducted from June 2003 through March 2004. These consisted of a questionnaire, review of medical records, measurements of blood pressure and anthropometric data, and blood tests. Anthropometrical examinations and blood pressure measurements were performed in a consistent manner. Self-administered questionnaires were used to collect individual information on demographic characteristics, history of cardiovascular disease, use of medication, alcohol consumption, and smoking status.[27]

Two medical doctors and 8 nurses visited 25 medical facilities and reviewed patients' medical records and treatment regimens. They recorded patient characteristics, such as age, sex, past history, family history, date when hemodialysis was initiated, length of hemodialysis sessions, number of hemodialysis sessions per week, prescribed dry weight, interdialysis weight gain at the beginning of the week, cause of ESRD, diabetes status, comorbid conditions, current medications, and history of other hemodialysis treatment.[27]

In the present study, information on anti-HCV antibody serology testing was collected by reviewing medical charts. All anti-HCV antibody serology tests at the 25 medical

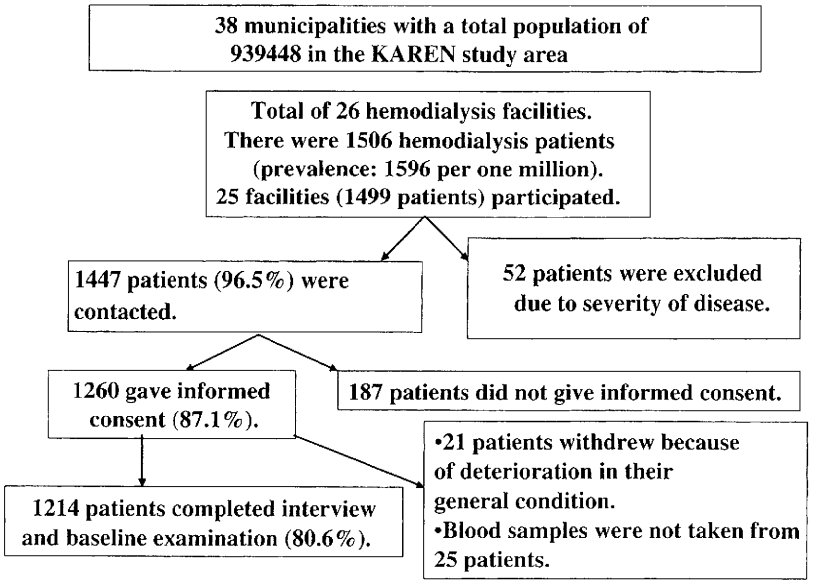

Figure 2. Flow chart for selecting subjects participating in the KAREN Study.
A total of 1506 adult patients were undergoing hemodialysis in 26 institutes in the study area. We were able to contact 1447 patients (96.5%); an additional 52 patients were excluded because of the severity of their condition. A total of 1260 patients (87.1%) gave written informed consent for participation in the study. Of these, 1214 (80.6%) completed the baseline examination.

facilities were performed by using a second- or third-generation assay.

Predialysis blood sampling was performed by dialysis nursing staff immediately before beginning hemodialysis sessions. Blood samples were drawn from arteriovenous fistulae or grafts through hemodialysis cannulae into vacuum tubes. The blood samples were transported to a laboratory (Mitsubishi Kagaku Bio-Clinical Laboratories, Inc., Morioka branch office), and biochemical measurements and combined blood counts were performed on the same day. Residual sera of each sample were collected and stored at −80 °C in our laboratory.

Results of anti-HCV antibody tests could not be obtained from 50 patients upon reviewing their medical charts. Frozen serum samples from those patients were unfrozen and anti-HCV antibody tests were performed using a second-generation assay (Architect HCV, Abbott, Japan). Frozen samples from patients who were positive for anti-HCV antibody (as confirmed by chart review or by HCV antibody determination using frozen samples) were unfrozen and HCV core antigen tests were performed using the Chemiluminescent Enzyme Immunoassay (CLEIA). Quantitative determination of HCV-RNA by reverse transcription polymerase chain reaction (RT-PCR) was not performed in hemodialysis patients who were positive for anti-HCV antibody and negative for HCV core antigen (Figure 3).

The HCV screening survey of the general population was conducted in Iwate Prefecture in 2005. All samples were transported to a laboratory (Iwate Health Service Association), and HCV antibody serology tests were performed by using an enzyme immunoassay (AxSYM HCV Dynapack II, Abbott Japan). Additional HCV core antigen tests were also performed using CLEIA in subjects who were positive for HCV antibody. A total of 236 samples from participants who were positive for anti-HCV antibody and negative for HCV core antigen were then used for qualitative determination of HCV-RNA by RT-PCR (AMPLICOR TM HCV test, Roche, Figure 4).

Statistical analysis

Hemodialysis patients and population-based control subjects were divided into sex- and age-specific groups (20–39, 40–49, 50–59, 60–69, and ≥70 years). Sex- and age-specific prevalences of anti-HCV antibody and HCV core antigen were determined both in hemodialysis patients and controls.

Among hemodialysis patients, the expected number of patients positive for anti-HCV antibody (or HCV core antigen) in each sex- and age-specific group was calculated by using the prevalence of each sex- and age-specific group from the population-based controls. The total number of expected patients positive for anti-HCV antibody (or HCV core antigen) among hemodialysis patients was calculated by summing the numbers of positive individuals expected in all age-specific groups. The ratio of the observed number of hemodialysis patients with anti-HCV antibody (or HCV core antigen) to the expected number was defined as the standardized prevalence ratio (SPR). We assumed that the data would have a Poisson distribution; therefore, the confidence intervals for the SPRs were estimated using standard errors.[28]

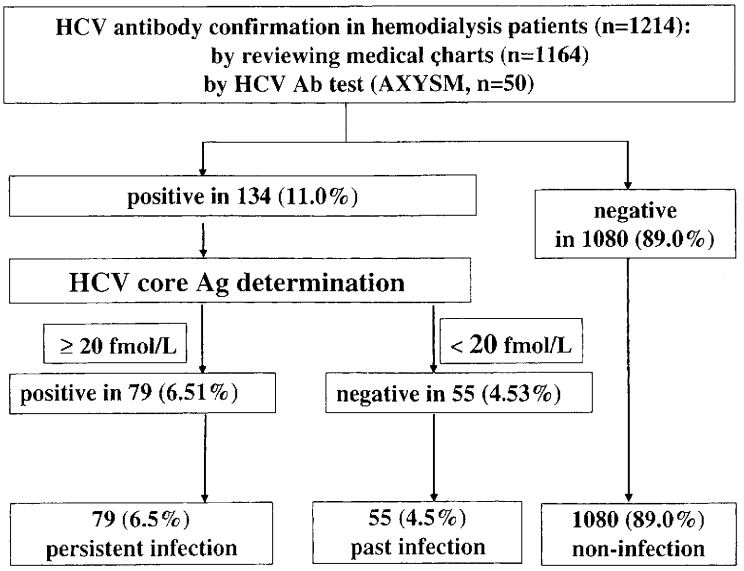

Figure 3. Flow chart of HCV antibody and HCV core antigen screening in hemodialysis patients in the KAREN Study. Information on HCV serology tests was not collected from 50 subjects in the KAREN Study. For those 50 subjects, we defrosted frozen serum samples and performed HCV antibody tests using Architect HCV (Abbott, Japan). A total of 134 subjects (11.0%) were positive for HCV antibody. HCV core antigen tests were then performed for those subjects. A total of 79 were positive for HCV core antigen and were classified with persistent HCV infection (6.0%).

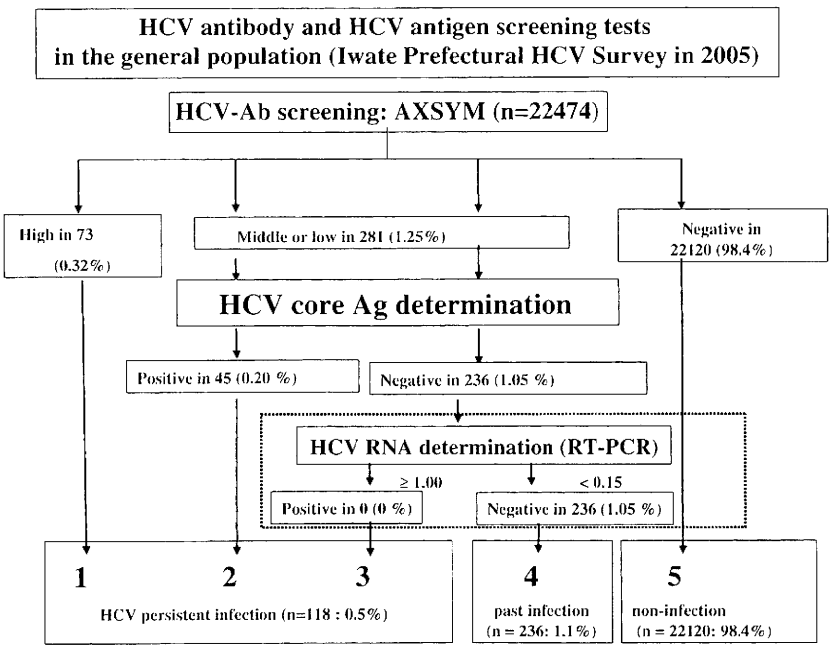

Figure 4. Flow chart of HCV antibody and HCV core antigen screening in population-based controls (Iwate Prefectural HCV survey in 2005).
There were 22474 participants who underwent annual health check-ups and HCV screening. A total of 354 subjects were positive for HCV antibody (1.57%). HCV core antigen tests were performed in subjects with low- or middle-range positivity for HCV antibody. A total of 45 were positive for HCV core antigen. HCV-RNA determination using the RT-PCR method was performed in 236 subjects, but none were positive. Ultimately, 118 subjects were classified with persistent HCV infection (0.53%).

Hemodialysis patients were also divided into 6 groups according to dialysis "vintage" (length of time on dialysis): <6 months, 6 to 23 months, 2 to 4 years, 5 to 9 years, 10 to 14 years, or 15 years or longer. Prevalences of anti-HCV antibody and HCV core antigen in each group were estimated. Differences in prevalences by sex or dialysis vintage (vintage ≥10 years vs <10 years) were tested using the chi-square test. To examine whether each risk factor was