J Epidemiol 2010;20(1):30-39
doi:10.2188/jea.JE20090043

Original Article

Standardized Prevalence Ratios for Chronic Hepatitis C Virus Infection Among Adult Japanese Hemodialysis Patients

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Received March 6, 2009; accepted June 11, 2009; released online October 31, 2009

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 ($n = 22472$) 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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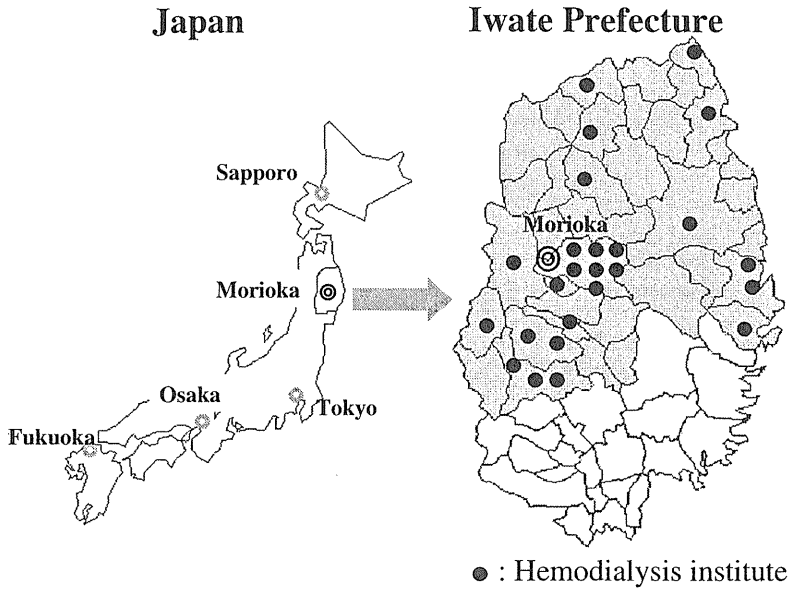

Figure 1. Maps of the KAREN Study area.
The maps show the location of Morioka (the capital of Iwate Prefecture), in northeastern Honshu island. The KAREN Study area (shaded area) covers approximately two-thirds of Iwate Prefecture, and includes 26 hemodialysis facilities; only 1 facility (in which 7 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 22 474 participants (7650 men and 14 824 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

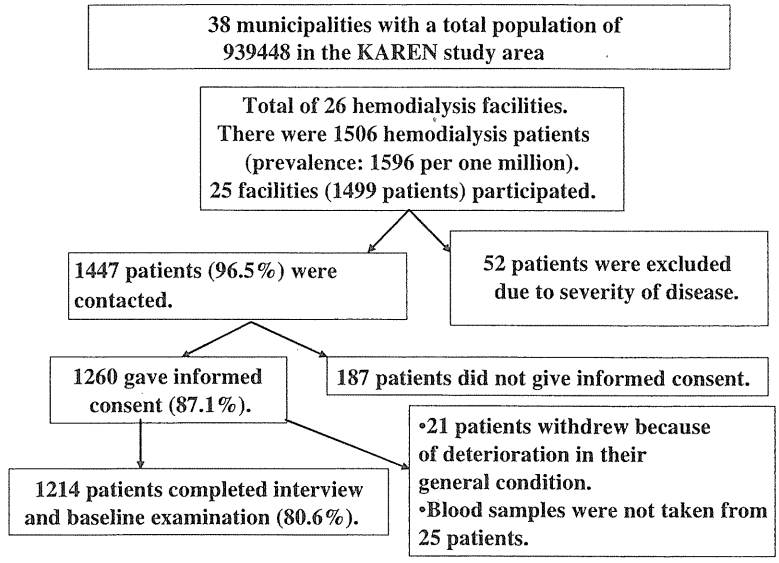

Figure 2. Flow chart for selecting subjects participating in the KAREN Study.
A total of 1506 adult patients were undergoing hemodialysis in 26 institutes in the study area. We were able to contact 1447 patients (96.5%); an additional 52 patients were excluded because of the severity of their condition. 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]

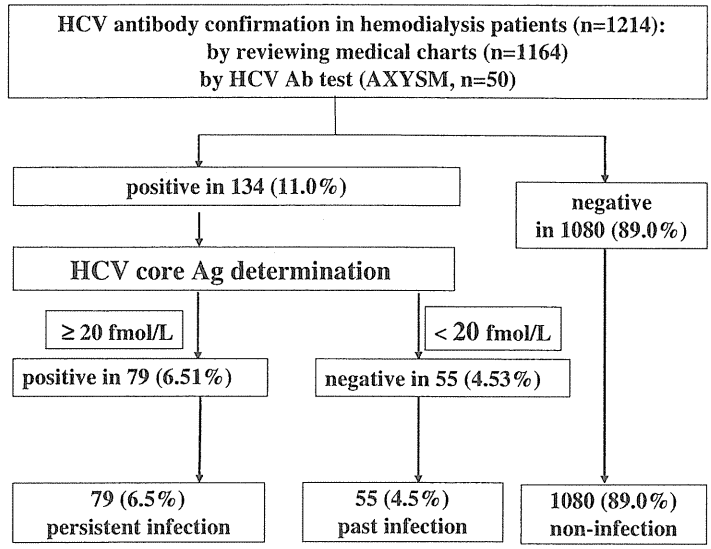

Figure 3. Flow chart of HCV antibody and HCV core antigen screening in hemodialysis patients in the KAREN Study. Information on HCV serology tests was not collected from 50 subjects in the KAREN Study. For those 50 subjects, we defrosted frozen serum samples and performed HCV antibody tests using Architect 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%).

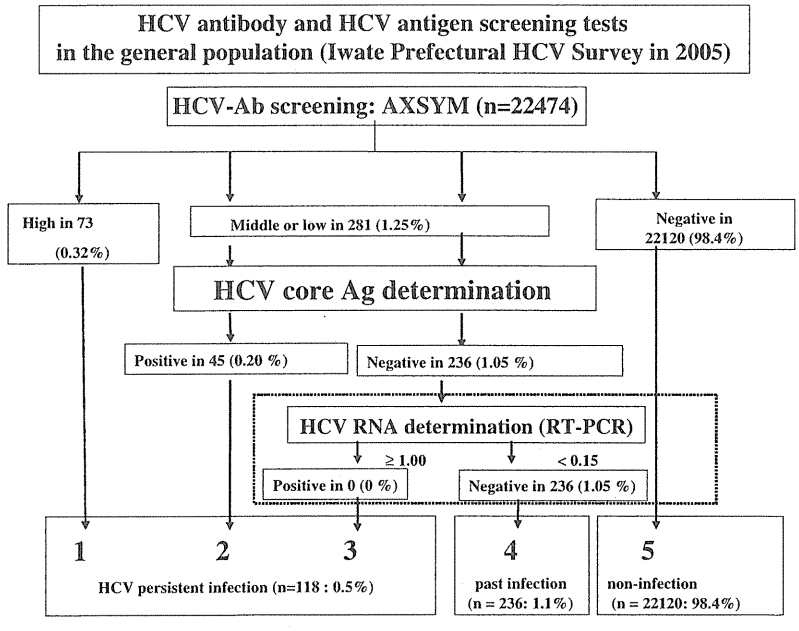

Figure 4. Flow chart of HCV antibody and HCV core antigen screening in population-based controls (Iwate Prefectural HCV survey in 2005).
There were 22 474 participants who underwent annual health check-ups and HCV screening. A total of 354 subjects were positive for HCV antibody (1.57%). HCV core antigen 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

Table 1. Sex- and age-specific prevalences of anti-HCV antibody in hemodialysis patients and a general population

Age group	General population		HD patients	
	Total No.	HCV Ab-positive (%)	Total No.	HCV Ab-positive (%)
Men				
20–39	36	0 (0.0%)	52	4 (7.7%)
40–49	890	16 (1.8%)	96	13 (13.5%)
50–59	1564	14 (0.9%)	191	38 (19.9%)
60–69	3001	43 (1.4%)	233	27 (11.6%)
≥70	2159	50 (2.3%)	207	15 (7.2%)
total	7650	123 (1.6%)	779	97 (12.5%)
Women				
20–39	62	0 (0.0%)	22	0 (0.0%)
40–49	2662	22 (0.8%)	55	5 (9.1%)
50–59	3980	40 (1.0%)	121	5 (4.1%)
60–69	4927	87 (1.8%)	116	1 (0.9%)
≥70	3193	82 (2.6%)	121	11 (9.1%)
total	14824	231 (1.6%)	435	37 (8.5%)

Abbreviations: HCV, hepatitis C virus; HD, hemodialysis; No., number; Ab, antibody.

Table 2. Sex- and age-specific prevalences of HCV core antigen in hemodialysis patients and normal controls

Age group	General population		HD patients	
	Total No.	HCV core Ag-positive (%)	Total No.	HCV core Ag-positive (%)
Men				
20–39	36	0 (0.0%)	52	3 (5.8%)
40–49	890	8 (0.9%)	96	8 (8.3%)
50–59	1564	5 (0.3%)	191	32 (16.8%)
60–69	3001	16 (0.5%)	233	12 (5.2%)
≥70	2159	21 (1.0%)	207	6 (2.9%)
total	7650	50 (0.7%)	779	61 (7.8%)
Women				
20–39	62	0 (0.0%)	22	0 (0.0%)
40–49	2662	5 (0.2%)	55	2 (3.6%)
50–59	3980	5 (0.1%)	121	5 (4.1%)
60–69	4927	28 (0.6%)	116	4 (3.4%)
≥70	3193	30 (0.9%)	121	7 (5.8%)
total	14824	68 (0.5%)	435	18 (4.1%)

Abbreviations: HCV, hepatitis C virus; HD, hemodialysis; No., number; Ag, antigen.

independently associated with chronic HCV infection or past HCV infection, logistic regression analysis was performed using presence of chronic HCV infection or history of HCV infection as the dependent variable and age, sex, and dialysis vintage as explanatory variables. A P value less than 0.05 was considered statistically significant. All statistical analyses were performed using the SPSS software package (SPSS, Japan Inc., Version 14.0).

RESULTS

Table 1 shows sex- and age-specific prevalences of anti-HCV antibody in hemodialysis patients and population-based controls. Among population-based controls, the prevalence of anti-HCV antibody increased with advancing age; however, no such association was observed among hemodialysis patients. A sex difference in the prevalence of anti-HCV antibody was not found in the population-based controls; however, among the hemodialysis patients, the prevalence of anti-HCV antibody was higher in men than in women (12.5% vs 8.5%, $P < 0.05$).

The prevalence of anti-HCV antibody was considerably higher in hemodialysis patients than in controls. The SPR (95% CI) for anti-HCV antibody was 8.39 (6.72–10.1) in male hemodialysis patients and 5.42 (3.67–7.17) in female hemodialysis patients.

Table 2 shows sex- and age-specific prevalences of HCV core antigen in hemodialysis patients and population-based controls. A positive association between the prevalence of HCV core antigen and age was found in controls but not in hemodialysis patients. The prevalence of HCV core antigen was also higher in male hemodialysis patients than in female hemodialysis patients (7.8% vs 4.1%, $P < 0.05$). The SPR (95% CI) for HCV core antigen was 12.9 (9.66–16.1) in male hemodialysis patients and 8.77 (4.72–12.8) in female hemodialysis patients.

Table 3. Prevalences of anti-HCV antibody and HCV core antigen among hemodialysis patients, stratified by hemodialysis vintage

HD vintage	No.	HCV Ab-positive (%)	HCV core Ag-positive (%)
Men			
<6 months	44	4 (9.1%)	3 (6.8%)
6–23 months	158	14 (8.9%)	8 (5.1%)
2–4 yrs	218	18 (8.3%)	10 (4.6%)
5–9 yrs	176	15 (8.5%)	7 (4.0%)
10–14 yrs	75	10 (13.3%)	8 (10.7%)
≥15 yrs	108	36 (33.3%)	25 (23.1%)
total	779	97 (12.5%)	61 (4.6%)
Women			
<6 months	18	1 (5.6%)	1 (5.6%)
6–23 months	74	4 (5.4%)	3 (4.1%)
2–4 yrs	129	8 (6.2%)	4 (3.1%)
5–9 yrs	109	8 (7.3%)	4 (3.7%)
10–14 yrs	49	3 (6.1%)	3 (6.1%)
≥15 yrs	56	13 (23.2%)	3 (5.4%)
total	435	37 (8.5%)	18 (4.1%)

Abbreviations: HCV, hepatitis C virus; HD, hemodialysis; No., number; Ab, antibody; Ag, antigen.

Table 3 shows prevalences of anti-HCV antibody and HCV core antigen by dialysis vintage. Male and female patients with longer hemodialysis vintages (10–14 years or ≥15 years) had high prevalences of anti-HCV antibody than did male and female patients with a dialysis vintage less than 10 years ($P < 0.05$). Male and female patients with a dialysis vintage of 15 years or more had extremely high prevalences of anti-HCV antibody. However, among the dialysis vintage subgroups,

Table 4. Odds ratios for each risk factor for past or chronic HCV infection

Risk factor	Chronic HCV infection			Past HCV infection		
	OR	95%CI	*P*	OR	95%CI	*P*
Age (per 1 year increase)	0.99	(0.97–1.01)	0.484	1.02	(0.99–1.05)	0.107
Male sex	1.99	(1.14–3.44)	0.014	1.06	(0.60–1.89)	0.843
Dialysis vintage (per 1 year increase)	1.09	(1.06–1.12)	<0.001	1.09	(1.06–1.13)	0.006

Odds ratios and their 95% confidence intervals were estimated by logistic regression analysis.
Abbreviations: OR, odds ratio; CI, confidence interval.

Table 5. Prevalences of anti-HCV antibody and HCV core antigen (or RNA) among hemodialysis patients from various countries

Country	Author or name of study	Sample size	HCV Ab-positive (%)	Positive for HCV Ag or RNA (%)	Years tested
Japan	Washio[15]	540	24.3	—	1990
	Nakayama[24]	1470	18.8	—	1993
	DOPPS[8]	not obtained	19.9	—	1997–2001
	Kumagai[6]	1882	—	12.9[a]	1999–2003
	KAREN	1214	11.0	6.5[b]	2003–2004
United States	DOPPS[8]	not obtained	14.4	—	1997–2001
	Da Vita[14]	13664	11.6	—	2001–2004
Belgium	Jadoul[13]	629	6.8	—	2000
France	DOPPS[8]	not obtained	14.7	—	1997–2001
Germany	Hinrichsen[9]	2796	7.0	—	1996–1997
United Kingdom	DOPPS[8]	not obtained	2.7	—	1997–2001
Italy	DOPPS[8]	not obtained	22.2	—	1997–2001
Iran	Shamshirsaz[10]	593	—	8.6[a]	2004[c]
Tunisia	Hmaied[11]	395	20	14[a]	2001–2003
Thailand	Luengrojanakul[12]	221	—	19.9[a]	1994

Abbreviations are the same as those used in Tables 1, 2, and 3. Italics indicate the present study.
Superscript numbers correspond to the reference used in the present study.
[a], determined by HCV-RNA test by the PCR method; [b], determined by HCV core antigen test.
[c], Not clearly described when blood sampling was performed (published in 2004).

male patients with a dialysis vintage of 15 years or more had the highest prevalence of HCV core antigen.

Both male and female patients in the 4 groups with the shortest dialysis vintage (ie, <10 years) had similar prevalences of HCV antibody, regardless of dialysis vintage (approximately 9% in male hemodialysis patients and 5% in female hemodialysis patients in each of the 4 groups).

Table 4 shows the odds ratios attributable to each factor for having chronic HCV infection or past HCV infection. Male sex and dialysis vintage were independently associated with a higher prevalence of chronic HCV infection. The prevalence of chronic HCV infection among male hemodialysis patients was double that of female patients. However, only hemodialysis vintage was independently associated with an increased prevalence of past HCV infection.

DISCUSSION

In this study, we analyzed the prevalences of HCV antibody and HCV core antigen in adult hemodialysis patients. We estimated SPRs for both anti-HCV antibody and HCV core antigen among hemodialysis patients, and compared these estimates to those of the general population living in the same area.

Patients who are positive for HCV core antigen all have chronic HCV infection, whereas patients with anti-HCV antibody include those who have recovered from HCV infection, as well as those with chronic HCV infection. In a general population, patients who have recovered from HCV infection never develop liver cirrhosis or hepatocellular carcinoma (HCC) due to HCV, whereas patients with chronic HCV infection will develop liver cirrhosis or HCC 20 to 30 years after initial infection.[29] Therefore, in a general population, information regarding chronic HCV infection is more important than information on anti-HCV antibody.

In their study of Tunisian hemodialysis patients, Bouzgarrou et al reported that an HCV core antigen assay based on the HCV-RNA test had high sensitivity and high specificity; however, they were unable to provide an accurate estimate of the prevalence of chronic HCV infection and past HCV infection because of the large number of missing cases.[30]

Table 5 shows prevalences of anti-HCV antibody and chronic HCV infection (positivity for HCV core antigen or HCV RNA) in several studies with large sample sizes.[6,8–15,24] Hmaied reported the prevalences of both anti-HCV antibody and HCV-RNA.[11] The proportion of patients with HCV-RNA

among patients with anti-HCV antibody was 70% in their study, and this proportion is similar to that of patients with HCV core antigen among patients with anti-HCV antibody in our study; it is also similar to the proportion of patients with chronic infection among all patients with HCV infection in the general population.[31]

We determined the prevalences of anti-HCV antibody and HCV core antigen in hemodialysis patients who were divided into 6 groups according to hemodialysis vintage. Patients with a hemodialysis vintage of 10 years or more had significantly higher prevalences of anti-HCV antibody and HCV core antigen than did patients with shorter hemodialysis vintages. Furthermore, patients with a hemodialysis vintage of 15 years or more had significantly higher prevalences of anti-HCV antibody than did other groups.

Since 1981, the Japanese Red Cross Blood Transfusion Service has excluded blood samples from donors with high serum ALT levels (≥36 KU/mL) in order to prevent transfusion of blood with non-A non-B hepatitis virus. Erythropoietin has been used clinically for treatment of anemia since 1986. In 1989, the Japanese Red Cross Blood Transfusion Service began using a first generation assay to screen blood donors for anti-HCV antibody.[32] The timing of the introduction of these programs explains the relatively low prevalence of HCV infection among patients with a dialysis vintage less than 10 years and the extremely high prevalence of HCV infection among patients with a dialysis vintage of 15 years or more.

Choo and Kuo first developed a specific assay for HCV in 1989,[33,34] and a second-generation ELISA, which was more sensitive than the first-generation ELISA, was developed in 1992 and became widely used as a clinical diagnostic tool and for epidemiological and other investigative purposes. As a result, the risk of nosocomial HCV infection has dramatically decreased among hemodialysis patients who started hemodialysis treatment after 1992. Our results showing a high prevalence of HCV infection among patients with a hemodialysis vintage of 10 years or more are consistent with the fact that risks for HCV infection have been reduced by the development and widespread use of HCV assays.

However, as compared to the general population, patients with a hemodialysis vintage of less than 10 years had a significantly higher prevalence of HCV infection, even though they would be expected to be at low risk of HCV infection due to blood transfusion and dialysis. This cross-sectional analysis also showed that prevalences were similar among the groups of patients with a dialysis vintage less than 10 years (ie, <6 months, 6–23 months, 2–4 years, 5–9 years), which suggests that most hemodialysis patients with HCV infection became infected before initiation of hemodialysis treatment, and that only a few patients with HCV infection developed the infection after initiation of hemodialysis treatment.

The incidence rate of HCV infection among hemodialysis patients is reported to be lower than 0.5 percent per year,[6,35] indicating that the very high prevalence of HCV infection among hemodialysis patients is not entirely due to the elevated risk of nosocomial infection associated with dialysis therapy. There are several possible pathways for HCV transmission before initiation of hemodialysis. Patients with renal failure may have a high prevalence of HCV infection, regardless of the severity of renal failure, or, alternatively, patients with HCV infection may have a high prevalence of renal failure. It has been shown that HCV is associated with an increased prevalence of renal insufficiency.[36] Renal diseases associated with HCV infection may also contribute to the high prevalence of HCV infection among patients with kidney disease.[37]

Another possible explanation is that patients with mild-to-moderate renal failure (ie, patients with chronic kidney disease) tend to develop ESRD after HCV infection, which may contribute to the high prevalence of HCV among patients with ESRD. Two studies have shown that HCV infection contributed to an increased risk of developing ESRD.[38,39] If HCV infection does indeed contribute greatly to the development of ESRD, better prevention and treatment strategies for HCV infection should not only decrease liver disease-related mortality, they should also decrease the development of ESRD and its related mortality in patients with CKD and in the general population.

Although there was no sex-based difference in the prevalence of HCV infection in the general population, the prevalences of anti-HCV antibody and HCV core antigen were higher in male hemodialysis patients than in female hemodialysis patients. This suggests that male hemodialysis patients are at greater risk for HCV infection, perhaps due to the presence of predisposing factors for HCV infection.

Male hemodialysis patients with a long hemodialysis vintage (≥10 years) had a high rate of chronic HCV infection (70%: the percentage of patients who were positive for HCV core antigen among those were positive for anti-HCV antibody); however, female patients with a similarly long hemodialysis vintage had a lower rate of chronic HCV infection (37.5%). Male sex was independently associated with a high prevalence of HCV core antigen in logistic regression analysis. These data suggest that male hemodialysis patients have a greater risk of HCV infection, and a greater risk of persistent HCV infection, than do female hemodialysis patients.

Thomas et al reported that the spontaneous clearance rate of HCV among female patients was 1.58 times that of male subjects; however, the finding was of only marginal statistical significance.[40] Women are less likely to be regular alcohol drinkers.[27,31] In addition, they have higher levels of serum HDL cholesterol[27,41] and perhaps other unknown protective factors. This may attenuate their risks of initial and chronic HCV infection, and may explain the observed sex-based differences.

Another possible explanation is that women who had recovered from HCV were selectively registered in the study

because of a very high mortality rate for women with chronic HCV infection. However, to our knowledge, no studies have shown that female patients with chronic HCV infection have a higher mortality rate than that of patients who have recovered from HCV infection.

One major feature of this study is the long dialysis vintage of the participants. Mean dialysis vintage of the study participants exceeded 7 years; mean dialysis vintage was only approximately 3 years in reports from the United States and Europe.[42] The generous medical insurance reimbursement system for Japanese dialysis patients and the high quality of hemodialysis treatment, which includes legal controls that strictly restrict re-use of a dialyzer, may have contributed to the longevity of hemodialysis patients. More than 20% of patients in the present study had long dialysis vintage (≥10 years), and long dialysis vintage was associated with a high prevalence of HCV infection in our study.

Since hemodialysis patients have a short life expectancy, there are few cases in which liver cirrhosis or HCC develops long after initiation of hemodialysis. Nakayama and Fabrizi found that hemodialysis patients who were anti-HCV antibody-positive had higher rates of liver disease-related deaths.[24,26] However, the authors did not reveal whether an elevated mortality rate among hemodialysis patients with anti-HCV antibody was totally attributable to the increase in liver disease-related deaths. It is necessary to determine which cause of death contributes to the increase in mortality among hemodialysis patients with HCV infection.

This study was based on data from a population-based study and the sample size was sufficient to satisfy our objectives. Indeed, the large sample size of population-based controls living in the same area is one of the strengths of the study. However, several limitations to our study should be noted. The cross-sectional design of the present study cannot prove causal relationships. In addition, the lack of HCV-RNA data on the hemodialysis subjects who were positive for HCV antibody and negative for HCV core antigen is a major limitation in our study. It is possible that hemodialysis patients who are negative for HCV core antigen nevertheless have very low levels of HCV-RNA; however, the possibility of missing such cases in the present study is very low because, among the population-based controls, none were simultaneously positive for both HCV-RNA and HCV antibody and negative for HCV core antigen (Figure 4). Therefore, we believe that the results of the study were not distorted by lack of data regarding HCV-RNA. A history of blood transfusion is a strong predisposing factor for HCV infection. Thus, lack of information about past history of blood transfusion is also a major limitation. In addition, people who did not participate in the annual health check-ups may have been in poor health and might have had liver disease. This would have resulted in an underestimation of HCV infection in the general population and overestimation of the SPR for HCV among hemodialysis patients.

In conclusion, the prevalences of chronic HCV infection in male and female hemodialysis patients are 13 times and 9 times those of men and women in the general population. Further studies should therefore be carried out to determine the extent of chronic HCV infection in hemodialysis patients in other populations and to determine whether chronic HCV infection contributes to increased mortality in hemodialysis patients.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Bureau of Medical Affairs of the Iwate Prefectural Government, a grant from the Japan Arteriosclerosis Prevention Fund (JAPF), a grant from the Japanese Ministry of Education, Science, Sports and Culture, a Grant-in-Aid for Scientific Research (C), No. 018590568, and a Grant-in-Aid from the Ministry of Health and Welfare, H18-Kan-en-ippan-002, in Japan.

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Clinica Chimica Acta 411 (2010) 1774–1780

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

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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ARTICLE INFO

Article history:
Received 16 March 2010
Received in revised form 26 July 2010
Accepted 26 July 2010
Available online 3 August 2010

Keywords:
Low-density lipoprotein cholesterol
Enzymatic homogeneous LDL-C assay
Friedewald formula
Triglyceride
Japanese
Community-based study

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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doi:10.1016/j.cca.2010.07.034

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

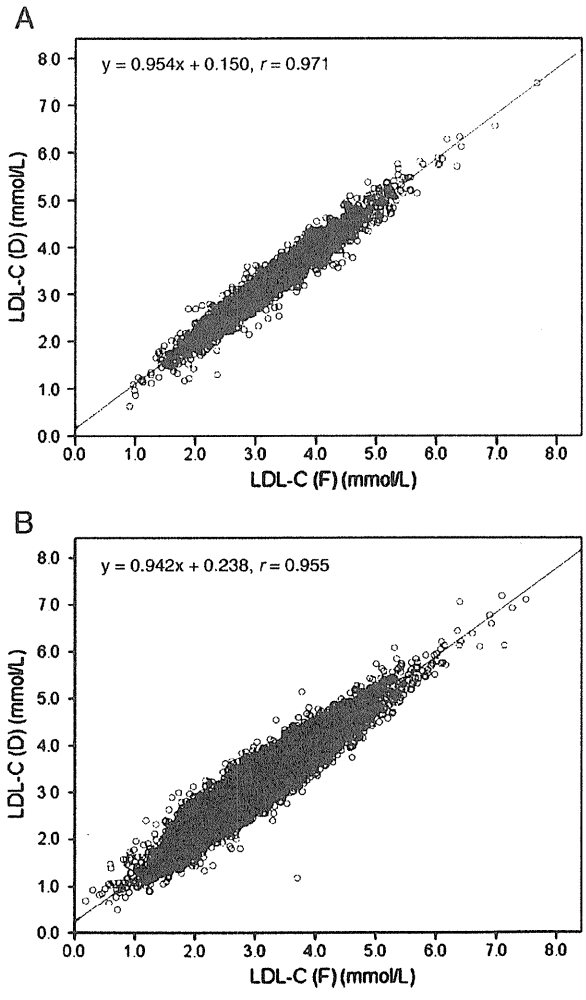

Fig. 1. Correlations between LDL-C (F) and LDL-C (D) in fasting participants (n = 3270) (A) and nonfasting participants (n = 17,294) (B). The solid lines represent regression lines.

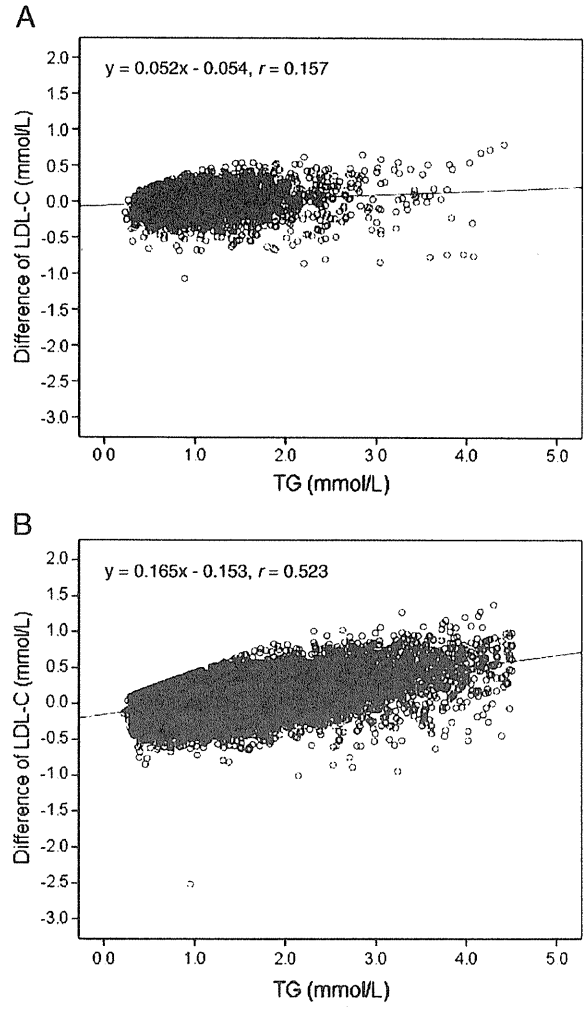

Fig. 2. Effect of increased TG concentrations on the difference in LDL-C concentrations between the homogeneous assay and the Friedewald formula in fasting participants (n = 3270) (A) and nonfasting participants (n = 17,294) (B). The difference in LDL-C, which is calculated by subtracting LDL-C concentrations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

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).

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.

Acknowledgments

This study was supported by grants from the Japan Arteriosclerosis Prevention Fund and the Ministry of Health, Labour and Welfare of Japan (H17-Choju-025 and H19-Choju-030). We would like to thank the staff of the Iwate Health Service Association and the staff in all municipalities (Iwate Prefecture, Ninohe City, Ichinohe Town, Karumai Town, Kunohe Village, Yamada Town, Kawai Village, Miyako City, Niisato Village, Taro Town, Iwaizumi Town, Tanohata Village, Kuji City, Yamagata Village, Fudai Village, Ohno Village, Noda Village, and Taneichi Town).

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Original Article

Serum Low-Density Lipoprotein to High-Density Lipoprotein Ratio as a Predictor of Future Acute Myocardial Infarction Among Men in a 2.7-Year Cohort Study of a Japanese Northern Rural Population

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Aim: To examine and compare the predictive value of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), TC/HDL-C and LDL-C/HDL-C ratios for future cardiovascular outcomes in the general Japanese population.
Methods: A total of 24,566 eligible participants aged 18 years or older, without cardiovascular disease, were enrolled through multiphase health screening and divided into quartile groups based on lipoprotein levels or ratios. Primary endpoints of the study were definitive acute myocardial infarction (AMI) and ischemic stroke, and cases of sudden death with unknown causes were not included. We used Cox proportional hazard models to examine the relationships between the quartiles and incidences of AMI or ischemic stroke, adjusting for traditional risk factors.
Results: Mean age was 63.7 years for males and 60.7 years for females. Mean follow-up period was 2.7 years, and 40 cases of AMI and 182 cases of ischemic stroke were recorded. The hazard ratio (HR) for AMI was significantly higher in the top quartile of the LDL-C/HDL-C ratio and LDL-C levels, and third quartile of TC among male participants. The HR of male participants with a LDL-C/HDL-C ratio of 2.6 or higher was significantly higher than other quartiles. No association between lipoprotein levels or their ratio quartiles and ischemic stroke was seen for either sex after adjusting for risk factors.
Conclusions: Our results indicated that the LDL-C/HDL-C ratio is an independent predictor for AMI, and the importance of better management of cardiovascular risks among people with high LDLC/HDLC ratios for the prevention of future cardiovascular disease.

J Atheroscler Thromb, 2011; 18:89-98.

Key words; Epidemiology, Acute myocardial infarction, Atherosclerosis, Risk factor, Cholesterol

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Received: February 14, 2010
Accepted for publication: September 2, 2010

Introduction

According to the World Health Organization, cardiovascular disease was the most common cause of death worldwide in 2005, accounting for approximately 30% of all deaths[1]. Among individuals 60 years of age or older, the main cause of death was isch-

emic heart disease, followed by cerebrovascular disease[2]. Prevention of cardiovascular disease (CVD), which includes cardiovascular and cerebrovascular disorders, is emphasized in both developed and developing countries[1, 2]

Dyslipidemia is an independent risk factor that contributes to the increase of CVD and death[3, 4]. Epidemiological studies[5, 6] have conclusively linked high levels of low-density lipoprotein cholesterol (LDL-C) and low levels of high-density lipoprotein cholesterol (HDL-C) with CVD incidence and mortality. The positive association between the LDL-C level and risk has been confirmed in a lipid-lowering randomized trial[7]. Current guidelines for the prevention of atherosclerotic disorders recommend specific target levels of lipid profiles to determine CVD risk and to evaluate the effectiveness of lipid-lowering therapies[8, 9]. Accordingly, LDL-C and HDL-C measurements are included in the standard health check-up supported by the Japanese government.

In recent years, additional indicators of CVD based on standard serum lipid profiles have been introduced[5, 10]. For instance, the total cholesterol (TC)/HDLC ratio is a useful and simple index of CVD risk[11]. Furthermore, the LDL-C/HDL-C ratio more accurately predicts CVD risk than LDL-C or HDL-C levels[5, 12]. In a large-scale intervention trial, a change in this ratio was a better indicator of successful CVD risk reduction[13]. Although previous studies have investigated the association between levels and the ratios of various lipoproteins and CVD risk[10, 12-14], only a few reports have evaluated the clinical utility of various lipid measures to predict CVD in Japan.

The aims of our study were to examine and compare the relationships between levels of TC, HDL-C, LDL-C, ratios of TC/HDL-C and LDL-C/HDL-C, and future cardiovascular outcomes among rural Japanese residents.

Methods

Study Participants

This study was part of the large population-based prospective Iwate Kenpoku Cohort study (Iwate-KENCO study) and a government-sponsored, multiphase health check-up program in the northern part of the Japanese main island. Survey methods have been described in detail previously[15, 16].

The baseline survey was conducted from April 2002 to January 2005. Participants were recruited from the community-dwelling population living in the Ninohe, Kuji and Miyako districts of Iwate prefecture, which include 17 municipalities. During the survey period, individuals in these municipalities were invited to participate in multiphase health screening. A total of 31,318 residents (11,003 males and 20,315 females) aged 18 years or older participated in the annual health check-up. We obtained written informed consent from 26,469 of these residents. After excluding 1,903 participants due to a self-reported history of CVD, medical history of CVD confirmed by the Northern Iwate Heart Disease Registry Consortium (NIHDRC) database and the Iwate Stroke Registry (ISR) database[17], taking lipid-lowering medications, and missing data for lipid-related items, we included 24,566 (8,714 males and 15,852 females) in the present analysis.

The study was approved by the Medical Ethics Committee of Iwate Medical University and conducted in accordance with the Declaration of Helsinki[18].

Measurements

We measured height and weight, and calculated body mass index (BMI) using the following equation: BMI=body weight (kg)/height (m)^2. Blood pressure was measured twice in a sitting position after urination and a five-minute rest. Measurements were performed by well-trained staff using automatic devices, and the average of the two measurements was reported for systolic and diastolic blood pressures (SBP and DBP).

Self-administered questionnaires were used in the baseline survey to obtain demographic characteristics, history of cardiovascular disease, cerebrovascular disease, medication use, alcohol consumption, tobacco smoking and exercise habits.

Biochemical Analysis

Fasting or casual blood samples were collected from the antecubital vein, transferred to a laboratory and analyzed the same day. Serum total cholesterol (TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) levels were measured by enzymatic methods. Serum low-density lipoprotein cholesterol (LDL-C) was measured by the enzymatic homogenous assay, Cholestest-LDL (Daiichi Chemicals Co. Ltd, Tokyo, Japan). These lipid profiles were measured by Iwate Health Service Association. These measurements 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 CDC (Centers for Disease Control and Prevention, Atlanta, USA), and have met all criteria for both the precision and accuracy of lipid measurements.

We measured plasma glucose concentrations by the hexokinase ultraviolet method using an automated analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). Glycosylated hemoglobin A1c (HbA1c) levels were determined by high-performance liquid chromatography using an automated analyzer (Tosoh Corporation, Tokyo, Japan).

We determined serum levels of high-sensitivity C-reactive protein (hs-CRP) using the latex-enhanced immunonephelometric method (Dade Behring, Germany). The detection limit of the hs-CRP assay is 0.1 mg/L and results below the limit of detection were reported as 0.1 mg/L.

Outcome Measures

The primary endpoints of this study were the incidence of definitive acute myocardial infarction (AMI) or ischemic stroke, while cases of sudden death of unknown cause were not included as cardiovascular endpoints. Investigators reviewed the population register of each local government and confirmed dates of death and locations to which participants had relocated. Persons known to be alive at the end of follow-up and those who had moved away from the study areas were treated as censored cases[16]. We confirmed that approximately 99.9% of participants who were registered at the baseline were alive. Incidences of AMI among participants were confirmed by assessing the Northern Iwate Heart Disease Registry Consortium (NIHDRC) database, in which definitive AMI cases were determined based on the criteria of the MONICA study. Incidences of ischemic stroke were confirmed by accessing the Iwate Stroke Registry (ISR) database[17].

Statistical Analysis

For each sex, we determined the prevalence (for categorical variables) and mean and standard deviation (for continuous variables). Participants were categorized as follows: LDL-C/HDL-C ratio quartiles (Q1 <1.6, Q2 ≥1.6-<2.1, Q3 ≥2.1-<2.6, Q4 ≥2.6), TC level (mg/dL) quartiles (Q1 <180, Q2 ≥180-<200, Q3 ≥200-<220, Q4 ≥220), LDL-C level (mg/dL) quartiles (Q1 <100, Q2 ≥100-<120, Q3 ≥120-<140, Q4 ≥140), HDL-C level (mg/dL) quartiles (Q1 <50, Q2 ≥50-<60, Q3 ≥60-<70, Q4 ≥70) and TC/HDL-C ratio quartiles (Q1 <2.8, Q2 ≥2.8-<3.4, Q3 ≥3.4-<4.1, Q4 ≥4.1). Significance was estimated using the Kruskal-Wallis test for continuous items and the Chi-square test or Fisher's exact test for categorical items among quartiles.

Disease-free survival curves for AMI or ischemic stroke cases based on lipid profiles and their ratio quartiles were determined using Kaplan-Meier methods. Age- and multivariate-adjusted hazard ratios (HR) and 95% confidence intervals (CI) were computed using Cox proportional hazard models. To estimate adjusted HR, we included age (10 years increase), current smoking status (yes or no), SBP, BMI, uric acid and HbA1c in the multivariate adjusted models from our previous report[15].

All significance tests were two-sided and p values <0.05 were considered significant. All data were analyzed using SPSS version 16 (SPSS Inc., Chicago, USA).

Table 1. Characteristics of participants at baseline

	N (%) or mean (SD)	
	Male (n=8,714)	Female (n=15,852)
Age (years)	63.7 (11.5)	60.7 (11.7)
Body mass index	23.9 (3.0)	24.0 (3.5)
Systolic blood pressure (mmHg)	130.6 (19.6)	125.0 (20.2)
Diastolic blood pressure (mmHg)	78.3 (11.1)	73.6 (11.2)
Total cholesterol (mg/dL)	190.7 (32.5)	204.3 (32.5)
Triglyceride (mg/dL)	124.8 (83.5)	111.1 (66.7)
HDL-C (mg/dL)	55.9 (15.2)	61.2 (14.3)
LDL-C (mg/dL)	113.3 (29.2)	122.9 (28.9)
HbA1c (%)	5.14 (0.73)	5.09 (0.64)
Hypertension (%)	2263 (26.0)	3875 (24.4)
Diabetes mellitus (%)	610 (7.0)	551 (3.5)
Current smoking (%)	2714 (31.1)	471 (3.0)
Regular alcohol consumption (%)	5276 (60.5)	1912 (12.1)

Results

Table 1 shows the characteristics of study participants. Mean age was 63.7 years for males and 60.7 years for females. The proportions of hypertension were 26.0% for males and 24.4% for females, and 7.0% and 3.5% for diabetes mellitus. Mean follow-up period was 2.7 years. During the survey period, 35 males and 5 females suffered from AMI, and 114 males and 68 females suffered from ischemic stroke. We could not estimate a survival curve for AMI for females due to its low incidence.

The LDL-C/HDL-C ratio-specific characteristics at baseline among male participants are shown in **Table 2**. BMI, HbA1c, uric acid, TC, TG, LDL-C and hs-CRP were significantly correlated with higher LDL-C/HDL-C ratio quartiles. In contrast, the proportion of participants who regularly consumed alcohol and HDL-C seemed to be inversely correlated