most-two negatively-associated parameters for HCV carriers were serum levels of Total-C and TG, while the most positively-associated parameters were serum aminotransferase levels. Here, a question has arisen whether the hypolipidemia in the HCV carriers was caused by the impaired liver function or not, because the liver is the central organ in lipid metabolism and the decreased level of serum cholesterols has been observed in the patients with liver cirrhosis due to lower ability of cholesterol synthesis and/or malnutrition [20,21]. However, previous studies have not shown whether the hypolipidemia would occur in asymptomatic HCV carriers with normal aminotransferase levels [22-24]. Furthermore, the effects of other factors, including age, gender, nutritional state, and past history of HCV infection, on serum lipid levels have not been studied in HCV carriers.

In the present study, we investigated the relations between the serum lipid profiles and the above host factors in a large cohort in public health examination with over 140,000 participants including significant numbers of asymptomatic HCV carriers without any therapies. The results showed that the hypolipidemia was a characteristic feature in HCV carriers irrespective of aminotransferase levels or nutritional states.

METHOD

Cohort Study and Population

The HCV-testing was conducted during the annual public health examination for community residents, based in part on a project for urgent comprehensive countermeasures against hepatitis and hepatocellular carcinoma at the ages of 40, 45, 50, 55, 60, 65, or 70 years, from 2002 to 2006, and was supported by the Japanese Ministry of Health, Labour and Welfare. Additionally, the Ibaraki prefecture extended the project of HCV-testing for an additional year to 2007, and the present study used data from a 6-year period. The present cohort study used the data from a total of 146,857 individuals (50,399 males; 34%, 96,458 females; 66%) who participated in the annual public health examinations from 2002 to 2007 in Ibaraki Prefecture. The HCV-test was conducted with HCV-antibody, antigen, and/or RNA testing in accordance with the guideline for the medical HCV examination, as summarized in our previous report [19]. In the flow chart for the determination of HCV infection, using a cut off index (COI) of the HCV-antibody titer obtained with the HCV-antibody test (Lumipulse®, Fujirebio, Inc, Tokyo, Japan), subjects were initially divided into the HCV-negative with COI < 1, the HCV-positive candidates with $1 \leq COI < 50$, and the HCV-positive with $COI \geq 50$. The HCV-positive candidates were finally determined to be HCV-negative and -positive based upon the HCV-antigen test for the HCV core protein and the nucleic acid amplification test (NAT) for HCV-RNA.

The health examination involved measurements of serum lipid levels, including Total-C, HDL-cholesterol (HDL-C), and TG, as well as age, height, weight, and

serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). According the general health examination, serum was collected on fasting. Serum LDL-C levels were calculated using the Friedewald formula, as follows: LDL-C (mg/dL) = Total-C (mg/dL) – HDL-C (mg/dL) - 0.2 × TG (mg/dL) [25]. Over 802mg/dL (8.8mmol/L) of TG level was excluded from the calculation of LDL-C [26]. The lipid levels were diagnosed as indicating normal, hypolipidemia, or hyperlipidemia based on the respective reference value for Japanese clinical laboratory examination [27,28]. Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters [29]. All of the health examinations, including HCV-tests and serum biochemical analyses, were conducted in the Ibaraki Health Service Association and Ibaraki Prefectural Institute of Public Health (Mito, Japan), and the data of health examination were analyzed anonymously, after informed consent was obtained from community representatives to conduct an epidemiological study based on the guidelines of the Council for International Organizations of Medical Science [30].

Classification by factors

In the present study, both HCV-negative and -positive subjects were further divided into subgroups based upon different factors; 1) gender, 2) age, 3) serum HCV-antibody titer, 4) serum markers of liver damage, and 5) nutritional state. The age classification was established by the age range, and was divided into 5-year increments. In the classification by serum HCV-antibody titer, the HCV-negative subjects were divided into two subgroups; HCV-antibody titer COI<1 and COI≥1, and the subjects with

COI≥1 were finally decided as being HCV-negative by the HCV-antigen test and the NAT [19]. For classification by liver damage, the HCV-negative and -positive subjects were further divided into the two groups, based upon the healthy limits of serum aminotransferases (ALT and AST); normal (NORMAL) was less than 30 IU of both, and abnormal (ABNORMAL) was over 30 IU of either or both aminotransferases. In Japan, the healthy limits of both serum aminotransferase levels for diagnosis of liver damage in public health examinations were re-established to be under 30 IU, based on the recent guidance for antivirus therapy for HCV [31]. The nutritional status was evaluated by BMI, and the classification was conducted along with the WHO-defined BMI class; under weight (Wt) was BMI<18.5, Normal Wt of 18.5≤BMI<25, Over Wt o 25≤BMI<30, and Obese class (Cls) according to obese classes 1~3 (BMI>30).

Statistical analysis

Data are expressed as the mean ± SEM, or percentage. Significant differences between the two groups were determined by unpaired student's *t*-test or Mann-Whitney *U*-test depending upon the number of subjects and variations in the groups compared. Comparison of the percent distribution between the two groups was estimated by Pearson's $\chi 2$-analysis. Multivariate logistic regression analysis was performed to determine factors including HCV-positive, age, BMI, ALT, and AST associated with serum level of each lipid diagnosed as the hypolipidemia (Total-C≤119 mg/dL, HDL-C≤39 mg/dL in male and ≤44 mg/dL in female, LDL-C≤64 mg/dL, TG≤49 mg/dL). The strength of association was described with an odds ratio with 95% confidence

intervals and *p*-value. The statistical analysis was performed using SPSS II software version 11.0 (SPSS Inc., Chicago, IL).

RESULT

HCV-positive rate and profile of serum lipids between HCV-positive and negative

Among the 146,857 individuals who participated in the health examination from 2002 to 2007, the HCV-positive rates were 0.90%, 1.37%, and 0.67% in all (sum of the genders), males and females, respectively. There were no significant differences in BMI between the HCV-negative (male; 23.9±0.01, female; 23.1±0.01) and positive (male; 23.3±0.1, female; 23.1±0.1) subjects. **Table 1** shows the average serum lipid levels (Total-C, HDL-C, LDL-C, and TG) by gender between the HCV-positive and negative subjects. Among all subjects, all serum lipids in the HCV-positive subjects were significantly lower than in the HCV-negative subjects, regardless of gender.

The lipid levels in both HCV-negative and -positive subjects were divided into hypolipidemia, normal lipid, and hyperlipidemia, based upon whether they were below, within, and above the normal ranges of the respective reference values for Japanese (**Fig.1**). Among both genders, the proportion that were above the normal range for all examined lipids was significantly lower in the HCV-positive compared to those in the HCV-negative subjects (χ^2 analysis $p<0.0001$ in all: Total-C; 29% in the HCV-negative vs. 6% in the HCV-positive for males, 41% vs. 21% in females, HDL-C; 3% vs. 1% in males, 6% vs. 4% in females, LDL-C; 24% vs. 7% in males, 34% vs. 20% in females, TG; 35% vs. 18% in males, 21% vs. 14% in females).

The HCV-negative subjects were also divided into those with HCV-antibody titer ≥ 1 and <1, and the former and latter were considered as having a prior infection and never infected [17]. The percentages of HCV-negative subjects with prior infection were

0.91%, 1.28%, and 0.72%, for all, males, and females, respectively, and the number of subjects was similar to the HCV-positive subjects for each gender. Significant differences in the serum lipids were observed when the HCV-positive subjects were compared regarding the presence or absence of a prior infection (**Table 1**). Among the HCV-negative subjects, the examined lipids tended to be lower in those with prior infection compared with those who had never been infected, particularly in males, but there were no statistically significant differences.

Table 2 shows the multivariate logistic regression analysis of risk factors for lower level of serum lipids. In the parameters including HCV-positive, age, ALT, AST, and BMI, the significances were recognized in almost of analyses for the respective lower level of serum lipids in both genders, while there were no significances in age for Total-C in male, ALT and BMI for Total-C in female, and both aminotransferases for LDL-C in female. In the HCV-positive parameter of both genders, the odds ratios in all examined lipids were remarkably higher than other analyzed parameters in all examined lipids. Although this analysis implied that the influence of HCV infection was the strongest risk factor for the lower level of serum lipids, the further analyses by matching gender, age, ALT, AST, and BMI were carried out to exclude these factors.

Composition of serum lipids between the HCV-positive and negative subjects

Figure 2 shows the balance of serum lipid composition by gender between HCV-positive and negative subjects. In both genders, there was no significant differences in the balance of serum lipid composition between HCV-positive and negative subjects.

Among males, the rates of TG and HDL-C in the HCV-positive subjects tended to be lower and higher, respectively, compared with the HCV-negative subjects (TG: 42.4±0.1% vs. 40.5±0.5%, HDL-C: 18.7±0.03% vs. 20.2±0.3%, in the HCV-negative vs. -positive subjects), but they were not statistically significant. The serum lipid balance in females was almost the same between the HCV-negative and positive subjects. The results show that the all serum lipids were reduced equally in subjects with HCV infection.

Serum levels of lipids classified by healthy levels of aminotransferases

The HCV-negative and -positive subjects were classified into the NORMAL (ALT≤30 and AST≤30) and ABNORMAL (ALT>30 and/or AST>30) populations based upon the healthy serum aminotransferase levels. The HCV-positive rates were 0.36% and 3.30% in the NORMAL and ABNORMAL populations, respectively. In the HCV-negative subjects, 82.1% were in the NORMAL compared with 17.9% that were in the ABNORMAL population (χ^2 analysis $p<0.0001$). In contrast, the NORMAL and ABNORMAL populations in the HCV-positive were 33.1% and 66.9% ($p<0.0001$), respectively. Serum lipid levels classified by the aminotransferases are shown in **Fig.3**. There were significant differences in the lipid levels between the HCV-negative and -positive subjects in the NORMAL population. In both genders, all examined lipid levels in the NORMAL population were significantly lower in the HCV-positive compared with the negative subjects, except for TG in females. The significantly lower levels of all examined lipids in the HCV-positive were also observed in the ABNORMAL population.

The results indicate that the lower levels of serum lipids were associated with the infection of HCV rather than the condition of liver damage.

Differences of lipids among age ranges

Figure 4 shows the differences in the age-range of serum lipid levels in 5-year increments in the HCV-negative and -positive subjects by gender. In both genders, lower levels of all examined lipids in the HCV-positive subjects were observed for all age-ranges, except for some younger age-ranges for TG levels. Among the age-ranges under 50 years, the Total-C and LDL-C levels in the HCV-negative subjects were lower in females than in males, but the lower levels were reversed in those aged above 50 years. In the HCV-positive subjects, however, both levels were weakly influenced by age for both genders, and therefore, the lower levels in males remained unchanged throughout all age-ranges.

Serum levels of lipids classified by BMI

Figure 5 shows the serum lipid levels classified by the WHO-defined classification of BMI. In all BMI classes for both genders, except for the Under Wt class in females, Total-C and LDL-C levels in the HCV-positive subjects were significantly lower than in the negative subjects. Similarly, a significant decrease of HDL-C levels was observed in the BMI classes for both genders in the HCV-positive group, except for in the obese class who also showed lower levels; however, this finding was not significant. In TG, lower levels were observed in the HCV-positive subjects for all BMI classes in both

genders, and significant differences were found in the Normal Wt and Over Wt classes for both genders and for the Obese class in males. Accompanied with the higher class of BMI, the typical dislipidaemic patterns of higher TG and lower HDL-C levels were observed in both HCV-positive and negative subjects, but the effects of BMI were smaller in HCV-positive than in negative subjects.

DISCUSSION

Among over 140,000 participants undergoing public health examinations, we evaluated the serum lipid profiles in the HCV-positive subjects by various host factors including gender, age, nutritional state, hepatic damage, and HCV-antibody titer. In contrast to HCV hepatitis patients in hospitals, this cohort included a significant large number of asymptomatic HCV-positive subjects with normal aminotransferase levels. In generally, serum lipid levels are influenced by some factors including gender, age, diseases, and/or nutritional states. As shown in Table 2, the multivariate analysis showed that most factors were significantly associated with hypolipidemia, and especially, the HCV-positive was the strongest factor. In comparison by matching the respective factor, serum levels of all examined lipids (Total-C, HDL-C, LDL-C, and TG) were significantly decreased in the HCV-positive compared to those in the HCV-negative subjects, regardless of gender, age, BMI, or serum aminotransferase levels. Furthermore, the significant hypolipidemia was observed in the HCV-positive subjects when compared to those of the HCV-negative subjects with a prior infection. Particularly, to our knowledge, the hypolipidemia in the HCV-positive subjects with normal serum aminotransferase levels have never reported.

It has been well known that the hypolipidemia caused by impaired liver function is observed in chronic liver diseases including liver cirrhosis [20,21]. Therefore, there is an apprehension whether some cirrhotic patients with lower aminotransferase levels were included in the NORMAL population in the present study or not. In active hepatitis infected with HCV shifting to cirrhosis, both aminotrasferase levels trend to

decline, but are still above the normal range [32-34]. Accordingly, we assumed that there might be few chronic cirrhotic patients with HCV in the NORMAL population. In addition, the malnutrition is generally found in the chronic cirrhotic patients, and consequently, BMI would be lower. However, in the present study, the lower lipid levels were observed in all BMI classes among the HCV-positive subjects. These results support the idea that the lipid abnormalities in the HCV-positive subjects are directly caused by HCV infection itself rather than by the secondary effects of HCV infection, *i.e.*, hepatic damage or nutritional disorder.

Previously, some studies showed that serum LDL-C level was significantly decreased in the patients infected with HCV compared with that in the uninfected subjects [12,14,17,35]. However, serum HDL-C level was unchanged in the HCV-positive subjects [12,14,17,35]. In contrast, Siagris *et al.* [15], as well as ourselves, showed that both LDL-C and HDL-C levels were significantly reduced in the HCV-positive subjects. The different findings about serum HDL-C level in the HCV-positive subjects should be due to the difference in the compared control levels. In the aforementioned studies [12,17,35], the HDL-C levels of controls were 45~47 mg/dL, which was considerably lower than those in the studies of ourselves and Siagris *et al.* (53~54 mg/dL) [15]. The difference in the HDL-C level in the controls may be due to the population characteristics, including race, dietary culture, and lifestyle. Thus, both HDL-C and LDL-C levels would be decreased in the HCV-positive subjects who had relatively higher level of HDL-C.

In comparison between with the presence and absence of HCV infection,

different results in serum TG level have been reported. Dai *et al.* showed the significant decrease of TG level in the HCV-positive subjects in a large cohort study [13]. In contrast, there are no significant differences in serum TG level between the HCV infected patients and healthy controls in a relatively younger population (42.0±14.6 years of age) [15]. In the present study, we also observed the significant decreases in the TG level, but there was no difference in case of comparison in the relatively younger populations (35~44 years in males; 35~49 years in females, **Fig.4**). It is not clear why serum TG level in younger ages would hardly be affected by HCV infection, and further studies are needed.

Furthermore, there are findings that genotypes of HCV are related to the reduction of hepatic lipid metabolisms. In the American, Greek, Austrian, African, and French patients with HCV genotype 3a, hypocholesterolemia was more remarkable than other genotypes [14,15,36-38]. Furthermore, in Egyptian, significantly lower level of lipids has been also reported in HCV patients predominantly infected with genotype 4 [17]. Although the HCV genotype was not determined in the present study because of cohort study in the public health examination, the most common genotypes in Japanese population are 1b and 2a, while genotypes 3a and 4 are very rare [39]. This genotype population in Japanese is similar to the genotype populations in Taiwan where lower level of lipids in the HCV carriers has also been reported in a cohort study [40]. Therefore, the abnormalities of serum lipids in the HCV carriers would not depend on the virus genotype.

Several previous studies have reported a relationship between lipid levels and the Sustained Viral Response (SVR) of anti-virus therapy in the HCV patients. Corey *et*

al. observed that serum Total-C and LDL-C levels were significantly higher after treatment of peginterferon and ribavirin for about 7 months in the HCV patients with SVR compared to those in the nonresponder/relapsers whose serum lipid levels did not differ from responder before the initiation of the HCV therapy [14]. Furthermore, Gopal *et al.* showed that HCV patients with higher LDL-C level before HCV therapy were associated with greater odds of achieving an SVR [41]. Therefore, focusing on the lipid prolife in the HCV patients should have important implications in the anti-virus therapy including interferon and ribavirin.

Although the exact reason for the significant decrease of serum lipid levels in the HCV-positive subjects is still unclear, previous studies showed HCV impaired assembly and secretion of very low-density lipoprotein from hepatocytes [42], and reduced transport of lipids by HCV-induced oxidative stress and PPARα inability [43,44]. In addition, a study of cholesterol metabolism by comprehensive analyzing serum biomarker sterols [45] has suggested that endogenous cholesterol biosynthesis is down-regulated while intestinal cholesterol absorption is not reduced in patients with HCV infection [46]. Because lower serum cholesterol concentrations in the HCV patients could not be explained by hepatic damage or malnutrition, HCV itself might down-regulate cholesterol biosynthesis in the human body.

In conclusion, the present study demonstrated that the serum levels of lipids including Total-C, LDL-C, HDL-C, and TG were significantly lower in the HCV-positive subjects than in the negative ones, irrespective of host factors including aminotransferase levels and nutritional states. Therefore, HCV infection itself might directly cause

abnormalities of lipid metabolism.

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