Table 2. Prevalence of *ENPP1* K121Q genotype or rs7566605 genotype in subjects with positive for anti-HCV, according to the presence of hepatitis C viremia

	HCV carrier[a]	HCV RNA-negative[b]	*P* value[c]
K121Q genotype	*n* = 342	*n* = 116	
AA	289 (84.5%)	88 (75.9%)	
AC	53 (15.5%)	26 (22.4%)	
CC	0	2 (1.7%)	0.01[d]
rs 7566605 genotype	*n* = 341	*n* = 116	
GG	159 (46.6%)	52 (44.8%)	
GC	141 (41.3%)	52 (44.8%)	
CC	41 (12.0%)	12 (10.3%)	0.75

[a]Positive for HCV RNA or HCV core antigen
[b]Negative for HCV RNA and HCV core antigen
[c]Data were analyzed by χ^2 test
[d]*P* value was 0.048 evaluated by subclasses of AA or AC + CC genotype

Table 3. Prevalence of *ENPP1* K121Q genotypes or rs7566605 genotype in HCV carriers, according to the body mass index (BMI)

	Normal weight (BMI <25)	Overweight (BMI ≥25 and <30)	Obesity (BMI ≥30)	*P* value[a]
K121Q genotype	*n* = 216	*n* = 76	*n* = 4 (%)	
AA	182 (84.3%)	66 (86.8%)	3 (75.0%)	
AC	34 (15.7%)	10 (13.2%)	1 (25.0%)	0.75[b]
CC	0	0	0	
rs 7566605 genotype	*n* = 216	*n* = 75	*n* = 4	
GG	107 (49.5%)	30 (40.0%)	2 (50.0%)	
GC	83 (38.4%)	35 (46.7%)	2 (50.0%)	
CC	26 (12.0%)	10 (13.3%)	0	0.36

[a]Data were evaluated by χ^2 test
[b]Data were analyzed excluding CC genotype

tions of age, sex, history of alcohol consumption, BMI, plasma glucose levels, and HbA1c levels between the groups, AST, ALT, γ-GTP, and insulin levels were significantly higher and triglycerides, total cholesterol, and platelet counts were significantly lower in the HCV carrier group than in the HCV RNA-negative group.

Differential distributions of the ENPP1 *K121Q SNP or rs7566605 genotypes and the clinical characteristics*

We successfully genotyped 458 and 457 subjects for the *ENPP1* K121Q SNP and rs7566605, respectively. The *ENPP1* K121Q SNP was differentially distributed between the HCV carrier group and the HCV RNA-negative groups ($P < 0.01$), whereas the rs7566605 genotype was not (Table 2). In univariate analysis, the *ENPP1* K121Q genotypes AC and CC were significantly more prevalent in the HCV RNA-negative group than in the HCV carrier group [odds ratio (OR), 1.74; 95% confidence interval (CI), 1.04–2.91; $P = 0.04$]. No other factors, including age, sex, BMI, history of alcohol consumption, past history of blood transfusion, and the rs7566605 genotype, were significantly different between the groups (data not shown). In multivariate analysis using four factors (age, sex, *ENPP1* K121Q genotype, and rs7566605 genotype), only the *ENPP1* K121Q genotypes AC and CC were associated with being negative for HCV RNA (OR, 1.78; 95% CI, 1.05–2.99; $P = 0.03$).

Relationships between the ENPP1 *K121Q or rs7566605 genotypes and BMI or insulin resistance*

We examined the relationships between the SNPs and available BMI values in HCV carriers: the subjects were classified as overweight (BMI ≥25 and <30 kg/m^2), obese (BMI ≥30 kg/m^2), or normal (BMI <25 kg/m^2). The distributions of the *ENPP1* K121Q and rs7566605 genotypes were similar in all three BMI subgroups (Table 3). In addition, there was no association between these two SNPs and fasting plasma glucose levels greater than 126 mg/dl or a history of diabetes (data not shown). Then, subjects with fasting plasma glucose levels less than 126 mg/dl were selected, and the relationship between the SNPs and insulin resistance was studied after classifying the subjects as insulin resistant (HOMA-IR value ≥2) or not (HOMA-IR value <2). The distributions of the *ENPP1* K121Q and rs7566605

Table 4. Prevalence of *ENPP1* genotypes or rs7566605 genotypes in HCV carriers, according to insulin resistance

	Lower HOMA-IR index (<2)	High HOMA-IR index (≥2)	*P* value[a]
K121Q genotype	*n* = 130	*n* = 106	
AA	106 (81.5%)	94 (88.7%)	
AC	24 (18.5%)	12 (11.3%)	0.13[b]
CC	0	0	
rs 7566605 genotype	*n* = 131	*n* = 105	
GG	68 (51.9%)	48 (45.7%)	
GC	47 (35.9%)	48 (45.7%)	
CC	16 (12.2%)	9 (8.6%)	0.27

HOMA, homeostasis model assessment of insulin resistance
[a]Data were valuated by χ^2 test
[b]Data were analyzed excluding CC genotype

Table 5. Clinical and virological characteristics in individuals who are HCV carriers, according to the *ENPP1* K121Q genotype

Characteristics	*ENPP1* K121Q genotype[a]		*P* value[b]
	AA (*n* = 289)	AC (*n* = 53)	
Age (years)	70.9 ± 9.5	69.7 ± 10.5	0.43
Sex (male/female)	101/188	15/38	0.35
Body mass index	23.1 ± 3.0 (251)	22.8 ± 3.1 (45)	0.44
Alcohol consumption (daily/occasionally/none)[c]	100/22/157	18/4/30	0.98
Past history of blood transfusion (yes/no)[c]	39/234	11/38	0.15
HCV core antigen (fmol/l)[d]	5358.3 ± 4906.7 (272)	4001.8 ± 4526.4 (53)	0.04
HCV core antigen (<1000/≥1000)[e]	73/216	18/35	0.19
HCV serotype (I/II)[f]	182/107	42/11	0.02
AST (IU/l)	49.9 ± 34.4	46.7 ± 23.4	0.83
ALT (IU/l)	45.9 ± 40.5	40.2 ± 21.7	0.86
γ-GTP (IU/l)	36.2 ± 55.0 (210)	28.1 ± 32.5 (38)	0.75
PLT ($\times 10^4$)	19 ± 6.1 (288)	20.0 ± 6.7	0.30
TG (mg/dl)	110.1 ± 57.1 (210)	110.6 ± 58.6 (38)	0.92
Total cholesterol (mg/dl)	170.0 ± 35.0 (210)	172.3 ± 33.2 (38)	0.66
HbA1c (%)	5.3 ± 0.7 (210)	5.4 ± 0.9 (38)	0.67
Glucose (mg/dl)	98.0 ± 35.4 (230)	93.7 ± 28.9 (42)	0.20
Insulin (μU/ml)	11.6 ± 11.7 (230)	10.9 ± 10.2 (42)	0.59
Ferritin (mg/dl)	151.0 ± 215.5	138.5 ± 182.3	0.33
HA (ng/ml)	196.9 ± 365.9 (287)	236.4 ± 391.8	0.58
Type IV collagen 7S (ng/ml)	5.0 ± 1.8 (287)	5.0 ± 2.0	0.39

Data are shown as means ± SD (number of subjects examined)
[a]There was no subject with CC genotype in persistent HCV infection group
[b]Data were evaluated by χ^2 test, Fischer's exact test, or Mann–Whitney test, as appropriate
[c]Excluding subjects whose history was not available
[d]Excluding subjects whose HCV core antigen level was below the cutoff value
[e]Including subjects whose HCV core antigen level was below the cutoff values
[f]Including subjects whose HCV genotype was determined even if serotype was undetermined

genotypes were also similar in the HOMA-IR subgroups (Table 4).

Clinical and biochemical characteristics of the HCV carriers classified based on the ENPP1 *K121Q or rs7566605 genotype*

In the HCV carrier group, biochemical markers from the subjects with AA and AC genotypes at the *ENPP1* K121Q SNP were compared (Table 5). We did not identify any subjects in the HCV carrier group with a CC genotype at this locus. The levels of HCV core antigen in subjects with an AA genotype were higher than in subjects with an AC genotype. The frequency of serotype II was also higher in subjects with an AA genotype than in subjects with an AC genotype. No other clinical or biochemical characteristics were different between the subjects with the different K121Q genotypes.

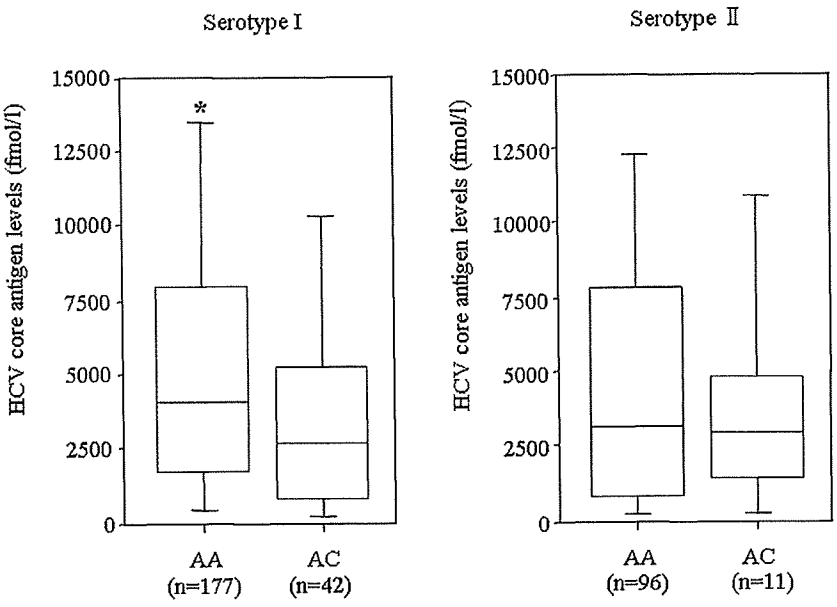

Fig. 1. The association between the K121Q genotype in *ENPP1* and the hepatitis C viral (HCV) load. The box-and-whisker plot shows the HCV core antigen level in the HCV carrier group according to the genotypes. The *boxes* indicate the 25th, 50th (median), and 75th percentiles. The *whiskers* indicate the 10th and 90th percentiles. The *asterisk* refers to a statically significant difference between the HCV core antigen levels in patients with the AA or AC genotype (Mann–Whitney *U* test, $*P = 0.04$)

We then further analyzed the association between the *ENPP1* K121Q variant and HCV core antigen levels according to the HCV serotype (Fig. 1). In the subgroup of subjects classified as HCV serotype I, the hepatitis C viral load was significantly higher in the subjects with the AA genotype (the wild-type genotype) than in those with the AC genotype ($P = 0.04$). Five subjects with the AA genotype were not included in this comparison because their levels of HCV core antigen were below the threshold. In any case, the percentage of subjects with HCV core antigen levels below the cutoff value of 1000 fmol/l was lower in the AA genotype subgroup than in the AC genotype subgroup (23.0% vs. 61.5%, $P < 0.01$ calculated using Fisher's exact test; OR, 2.68; 95% CI, 1.30–5.54; $P < 0.01$). Although a past history of blood transfusion was also associated with HCV core antigen levels (OR, 2.75; 95% CI, 1.25–6.06; $P = 0.01$), no other factors were associated with this variable. In multivariate analysis using the *ENPP1* K121Q variant and past history of blood transfusion, these two factors were independently associated with low HCV core antigen levels (OR, 2.44; 95% CI, 1.12–5.32; $P = 0.03$ and OR, 2.56; 95% CI 1.14–5.72; $P = 0.02$, respectively). This correlation between the HCV core antigen levels and the K121Q genotype, however, was not observed in the subgroup of subjects classified as HCV serotype II (Fig. 1).

In addition, we compared the biochemical markers from the subjects with the GG, GC, and CC genotypes at rs7566605. There were no significant differences among the clinical or biochemical characteristics of the subjects from these three groups, including the viral load (data not shown).

Discussion

Obesity and insulin resistance, which are caused by a combination of genetic and environmental factors, affect the clinical course of CHC infection.[5,6] The K121Q polymorphisms in the *ENPP1* gene and the rs7566605 genotype have been shown to be significantly associated with obesity and insulin resistance.[7–12] Whether polymorphisms in genes associated with obesity or insulin resistance affect persistent HCV infection or HCV-induced liver injury, however, has yet to be determined. We sought to examine the relationship between polymorphisms in these types of genes and viremia or the clinical course of liver injury in subjects positive for anti-HCV antibodies in a community-based HCV hyperendemic area in Japan. Our study, which shows that polymorphisms associated with the K121Q variant and the rs7566605 genotype are prevalent in Japan, suggests that these genotypes are not associated with obesity or insulin resistance in the examined HCV hyperendemic area. In addition, these polymorphisms were not associated with HCV-induced liver injury. In contrast, the frequencies of the K121Q polymorphism in subjects with hepatitis C viremia and those without viremia were different. Moreover, the K121Q polymorphism was associated with HCV viral load in a subgroup of HCV carriers (serotype I).

ENPP1 is the best characterized of the five human ectoenzyme *ENPP* proteins. *ENPP1* is expressed in many tissues, including muscle, fat, and liver, and overexpression of *ENPP1* in various cell lines inhibits insulin receptor tyrosine kinase activity and causes insulin resistance.[20] It was also reported that the K121Q variant

of *ENPP1* is associated with insulin resistance.[21,22] Compared to the *ENPP1* K121 protein, the *ENPP1* Q121 variant interacts more strongly with the insulin receptor and more effectively inhibits insulin-stimulated insulin receptor autophosphorylation and insulin receptor substrate-1 phosphorylation in vitro.[23] In our study, however, there was no association between the *ENPP1* K121Q variant and insulin resistance in HCV carriers. Keshavarz et al. also failed to find evidence of an association between the *ENPP1* K121Q variant and type 2 diabetes in a Japanese population.[24] The overall frequency of the 121Q allele (9.1%; 83/916) in our study was similar to that in the Japanese population, as previously reported (10.5%; 375/3562).[24] These results indicate that our study population represented the rest of Japan and that the K121Q variant does not influence insulin resistance in Japanese subjects, in particular in subjects with HCV infections.

rs7566605 is upstream of the transcription start site of *INSIG2*, the protein product of which inhibits the synthesis of fatty acids and cholesterol.[25] Overexpression of *INSIG2* in the liver reduced plasma triglyceride levels in obese Zucker diabetic fatty rats, and linkage between this gene and obesity phenotypes was observed in the mice.[26,27] Association testing in nine cohorts produced evidence that individuals with the CC genotype at rs7566605 have higher BMI values and a higher risk of obesity than those with the GG or GC genotype.[28] More recently, however, no association was reported between this genotype and obesity.[29,30] In addition, the rs7566605 genotype was not associated with the clinical or biochemical characteristics of subjects positive for anti-HCV antibodies, obesity, or insulin resistance in our study. These conflicting results about the relationship between the rs7566605 genotype and BMI may have resulted from the heterogeneous population samples. Future studies should enroll a large number of patients with HCV infections and control subjects from throughout the Japanese population.

False-positive results for the HCV antibody test may have occurred in the HCV RNA-negative group in our study. Several studies have shown that samples with readings just slightly above the cutoff value of the anti-HCV test have a greater likelihood to be false-positives compared with those with higher values.[31,32] HCV-positive patients may also show reactivity to nuclear and smooth muscle antigens.[33,34] There was, however, no difference in the distributions of the *ENPP1* K121Q genotypes (AA, AC, or CC) among patients with low titers (≥1 and <5), intermediate titers (≥5 and <30), and high titers (≥30) of anti-HCV antibodies in our study (data not shown). In addition, although there was no evidence of spontaneous clearance of HCV infection in this study, Micallef et al. systematically reviewed 31 longitudinal studies with a total of 675 subjects and reported that spontaneous viral clearance occurs in approximately one in four people with acute hepatitis C, which was similar to the size of the HCV RNA-negative group (25%).[35] Although autoantibody data and evidence of spontaneous HCV clearance in the clinical courses are not available, these results indicate that many subjects in the HCV RNA-negative group in our study population may have cleared their HCV infection spontaneously without false-positive results for the HCV antibody test.

Spontaneous HCV clearance typically occurs within the first 6 months after acute infection,[36] and spontaneous elimination of HCV in subjects with chronic HCV infection is rare.[16] These results suggest that *ENPP1* may influence the spontaneous clearance of HCV during the acute phase of infection in our population. Furthermore, sex is known to be an important factor for HCV clearance,[37–39] although a sex-based difference was not observed in our study (see Table 1). Studies based on polymorphisms have been widely used to identify host genetic factors that influence disease occurrence, progression, and outcome.[40] However, it is unclear whether *ENPP1* and sex are associated in HCV clearance. Another potential confounding variable is alcohol use, which is known to be negatively associated with HCV clearance.[41] Alcohol use, however, is limited in this community, and thus was unlikely to be a confounder. Further studies are needed to clarify the associations between host factors and *ENPP1* and their roles in HCV clearance.

Analysis of the *ENPP1* gene in 6147 subjects showed an association between a three-allele risk haplotype (K121Q, IVS20delT-11, and A→G+1044TGA) and obesity and type 2 diabetes.[42] In that report, it was shown that the presence of at least one copy each of the Gln121(121Q), IVS20delT-11, and G+1044TGA variants was associated with a significant increase in serum *ENPP1* protein levels. In addition, serum levels of osteopontin were lower in *ENPP1*-deficient mice than in wild-type mice, suggesting that *ENPP1* affects osteopontin expression.[43] Osteopontin-deficient mice also suffered from prolonged rotavirus-induced diarrhea.[44] SNPs in the promoter region of the osteopontin gene have been identified as markers that predict the efficacy of interferon-based therapies in patients with CHC.[45] Although our studies do not directly identify increased serum levels of *ENPP1* or osteopontin, *ENPP1* may induce nonproductive binding of HCV to cells, blockade of HCV attachment, or inhibition of penetration into cells through osteopontin expression.

The precise roles that host factors play in HCV replication have not been well characterized. Although Woitas et al. reported that anti-HCV-antibody-seropositive patients who were homozygous for the HIV-protective CC chemokine receptor (CCR) 5-Δ32

showed a markedly increased viral load compared with CCR5 wild-type or CCR5-Δ32 heterozygous patients,[46] the authors did not show results based on the HCV genotype or serotype. Hepatitis C viral load was found to be significantly higher in patients infected with HCV genotype 1 compared to patients infected with HCV genotype 2 or 3.[47] Our study indicates that the AC genotype at the K121Q SNP of *ENPP1* is linked to lower HCV core antigen levels, which correlated with hepatitis C viral load in the HCV serotype I subgroup, but not in the serotype II subgroup. The mechanisms contributing to the relationship between the K121Q polymorphism and the hepatitis C viral load are unclear. HCV replication in the cytoplasm, however, is highly dependent on the functions of nonstructural HCV proteins together with those of host factors.[48,49] Thus, functional studies about the molecular mechanisms underlying *ENPP1* signaling in HCV replication should be conducted in the future.

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Original Article

Association between changes in body composition and the increasing prevalence of fatty liver in Japanese men

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Aim: Prevalence of fatty liver is increasing. In this study, to elucidate the factor that contributes most to recent increases in prevalence of fatty liver, we determined the independent predictors for the onset of fatty liver and compared these predictors between 2000 and 2005.

Methods: Japanese persons, aged 30–74 years, who participated in regular health checks at Kagoshima Kouseiren Medical Health Care Center (10 336 persons in 2000 and 11 011 persons in 2005) were enrolled in the study. Diagnosis of fatty liver was performed by ultrasonography. Body fat percentage (BFP) was determined using a bipedal bioimpedance instrument.

Results: The prevalence of fatty liver has increased between 2000 and 2005 in men (33.3 vs 38.5% in 2000 vs 2005, respectively, $P < 0.0001$), but not in women (21.3 vs 21.0%, $P = 0.8101$). Logistic regression analysis revealed that both body mass index (BMI) and BFP are independent predictors of fatty liver in both men and women. BMI did not change in either men (23.4 ± 2.9 vs 23.8 ± 3.0 kg/m^2, $P = 0.0528$) or women (22.8 ± 3.1 vs 22.8 ± 3.3 kg/m^2, $P = 0.9862$) during the survey period. In contrast, BFP increased in men (20.6 ± 4.7 vs 22.3 ± 5.0 kg/m^2, $P = 0.0003$), but not in women (27.4 ± 5.5 vs 28.4 ± 5.9 kg/m^2, $P = 0.3993$). There was no significant change in triglycerides and glucose levels.

Conclusion: These results suggest that altered body composition, particularly increased BFP without an increase in BMI, has developed in men and is strongly associated with the increasing prevalence of fatty live amongst Japanese men.

Key words: fatty liver, body fat percentage, body mass index, body composition, life-style, metabolic syndrome

1. INTRODUCTION

FATTY LIVER HAS become a significant problem on a worldwide scale, including in Japan, because the prevalence of fatty liver is increasing.[1–3] Although body mass index (BMI) is considered to be a major risk factor for fatty liver, BMI is only slightly increased amongst Japanese men, and is slightly decreased in women according to national surveys.[4] Although the increase in the prevalence of fatty liver cannot be explained simply by the increase in the prevalence of obesity, the underlying factors have yet to be fully clarified.

In this study, to elucidate the factors that contribute to the recent increase in the prevalence of fatty liver, we determined the predictors of fatty liver in participants who underwent health checks and compared these predictors between the participants in 2000 and 2005.

2. METHODS AND MATERIALS

THE SUBJECTS IN this study were Japanese persons aged 30–74 years, who participated in regular health checks at Kagoshima Kouseiren Medical Health Care Center: 10336 persons (6484 men, 3852 women) from April 2000 to March 2001 (2000 group) and 11011

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Table 1 Comparison of variables between participants with and without fatty liver

Fatty liver	Men			Women		
	With	Without	*P* value	With	Without	*P* value
Age (years)	51 ± 9	54 ± 10	<0.0001	56 ± 9	54 ± 10	0.0015
BMI (kg/m^2)	25.6 ± 2.8	22.6 ± 2.5	<0.0001*	25.9 ± 3.4	21.9 ± 2.7	<0.0001*
BFP (%)	25.4 ± 4.4	20.3 ± 4.3	<0.0001*	33.9 ± 5.5	26.9 ± 5.0	<0.0001*
ALT (IU/L)	38 ± 24	24 ± 29	<0.0001*	28 ± 19	18 ± 8	<0.0001*
γ-GTP (IU/L)	58 ± 60	40 ± 49	0.0012*	24 ± 21	16 ± 14	<0.0001*
TC (mg/dL)	218 ± 36	206 ± 33	<0.0001*	224 ± 35	215 ± 33	<0.0001*
TG (mg/dL)	179 ± 148	118 ± 106	<0.0001*	221 ± 62	81 ± 41	<0.0001*
HDL-C (mg/dL)	51 ± 12	59 ± 15	<0.0001*	57 ± 12	67 ± 14	<0.0001*
BG (mg/dL)	113 ± 26	105 ± 19	0.0002*	108 ± 27	97 ± 11	0.0004*
S-BP (mmHg)	125 ± 17	121 ± 17	0.0003*	122 ± 17	114 ± 18	<0.0001*
D-BP (mmHg)	82 ± 11	77 ± 11	<0.0001*	77 ± 10	72 ± 10	<0.0001*

Data are expressed as mean ± SD. ALT, alanine aminotransferase; BFP, body fat percentage; BG, blood glucose; BMI, body mass index; D-BP, diastolic blood pressure; HDL-C, high density lipoprotein-cholesterol; S-BP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; γ-GTP, γ-glutamyl transpeptidase. *P value after adjusting for age (ANCOVA).

persons (6829 men and 4182 women) from April 2005 to March 2006 (2005 group).

Diagnosis of fatty liver was carried out using ultrasonography (SSA-250A and SSA-750A, Toshiba, Japan; Logic 400, GE Yokogawa, Japan), which was based on the findings of bright liver (increased echogenity) with liver-kidney contrast (increased echo level of the liver compared with the right kidney). BMI was calculated as follows: body weight (kg)/height^2 (m^2). Body fat percentage (BFP) was determined using a bipedal bioimpedance instrument (Model TBF-220; Tanita, Japan). Venous blood samples were taken from all subjects before 09.00 hours after overnight fasting and analyzed immediately. Alanine aminotransferase, γ-glutamyl transpeptidase, total cholesterol, triglycerides and glucose were measured by standard laboratory procedures. High-density lipoprotein (HDL) cholesterol level was determined by direct homogeneous assay of the serum using detergents (Daiichi Chemicals, Japan). History of alcohol intake was determined by questionnaire in which subjects reported a rough approximation of their daily intake.

Differences between groups were examined for statistical significance using the χ^2 test test, unpaired t-test and analysis of covariance (ANCOVA). Multivariate analysis was performed using logistic regression analysis. Correlations were examined by linear regression analysis using the coefficient of correlation. All data analyses were performed using Statview software version J-5.0 (Abacus Concepts, CA, USA). A P-value less than 0.05 was considered significant.

3. RESULTS

3.1. Independent predictors of fatty liver in the 2005 group

TO IDENTIFY FACTORS that associated with the pathogenesis of fatty liver, we compared the clinical and laboratory features between persons with and without fatty liver (Table 1) and performed logistic regression analysis (Table 2).

In both men and women, there was a significant difference in age between the fatty liver and non-fatty liver groups. In men, the age of the fatty liver group was lower than that of the non-fatty liver group; in contrast, the age of women in the fatty liver group was higher. This may reflect the gender difference in incidence of fatty liver, which has been reported elsewhere.[3] Only in men was age found to be an independent predictor.

Markers of obesity, BMI and BFP, were significantly higher in the fatty liver group. In addition, both BMI and BFP were found to be independent predictors of fatty liver in both men and women.

Triglycerides, HDL-cholesterol, glucose and ALT were also independent predictors in both sexes. Total cholesterol and diastolic blood pressure were independent predictors only in men.

As for alcohol, we compared the proportion of persons who drink more than 20 g/day between those with and without fatty liver, and found no difference between the groups (37.4 vs. 39.8%, $P = 0.0569$ in men; 1.1 vs. 1.8%, $P = 0.1463$ in women).

Table 2 Independent predictors of fatty liver by logistic regression analysis

	Regression Coefficient	Standard	Error	*P* value	Odds ratio	95% CI
Men	Age	−0.011	0.003	<0.0001	0.989	0.983–0.995
	BMI	0.207	0.019	<0.0001	1.227	1.183–1.273
	BFP	0.126	0.011	<0.0001	1.135	1.110–1.160
	ALT	0.017	0.002	<0.0001	1.017	1.017–1.021
	TC	0.006	0.001	<0.0001	1.006	1.004–1.008
	TG	0.001	0.000	0.0035	1.001	1.000–1.008
	HDL-C	−0.026	0.003	<0.0001	0.974	0.968–0.999
	FBS	0.011	0.001	<0.0001	1.011	1.008–1.014
	D-BP	0.009	0.003	0.0015	1.009	1.003–1.015
Women	BMI	0.161	0.034	<0.0001	1.175	1.098–1.256
	BFP	0.136	0.020	<0.0001	1.146	1.102–1.191
	ALT	0.049	0.005	<0.0001	1.051	1.041–1.061
	TG	0.009	0.001	<0.0001	1.009	1.007–1.011
	HDL-C	−0.022	0.004	<0.0001	0.978	0.971–0.987
	FBS	0.025	0.003	<0.0001	1.025	1.019–1.031

ALT, alanine aminotransferase; BFP, body fat percentage; BG, blood glucose; BMI, body mass index; CI, confidence interval; D-BP, diastolic blood pressure; HDL-C, high density lipoprotein-cholesterol; TC, total cholesterol; TG, triglycerides.

3.2. Comparison between 2000 and 2005 groups (Table 3)

The prevalence of fatty liver increased between 2000 and 2005 in men (from 33.3 to 38.5%), but not in women (from 21.3 to 21.0%).

Age was significantly higher in the male 2005 group. Age might not be involved in the higher prevalence of fatty liver in 2005 group, because the age of men was lower in the fatty liver group (Table 1).

There was no significant difference in BMI of both sexes. BFP increased significantly in men, but not in women.

Total cholesterol level was significantly elevated in both men and women. HDL-cholesterol levels decreased significantly in men. There was no significant difference in triglycerides and glucose levels. There was a significant difference in men's diastolic blood pressure.

4. DISCUSSION

FATTY LIVER IS an increasingly recognized condition, linked to the metabolic syndrome associated with obesity and insulin resistance. BMI has been considered to be the most important risk factor for fatty liver.[3,5]

Table 3 Comparison between the 2000 and 2005 groups

	Men			Women		
	2000	2005	*P* value	2000	2005	*P* value
Fatty liver (%)	33.3	38.5	<0.0001*	21.3	21.0	NS*
Age (years)	52 ± 10	53 ± 10	<0.0001**	54 ± 10	55 ± 10	0.0243**
BMI (kg/m^2)	23.4 ± 2.9	23.8 ± 3.0	NS***	22.8 ± 3.1	22.8 ± 3.3	NS***
BFP (%)	20.6 ± 4.7	22.3 ± 5.0	0.0003***	27.4 ± 5.5	28.4 ± 5.9	NS***
ALT (IU/L)	28 ± 28	29 ± 28	NS***	19 ± 13	20 ± 12	NS***
TC (mg/dl)	204 ± 33	211 ± 35	0.0058***	209 ± 34	217 ± 34	<0.0001***
TG (mg/dl)	151 ± 126	142 ± 127	NS***	100 ± 57	89 ± 42	NS***
HDL-C (mg/dl)	60 ± 16	56 ± 14	0.0230***	69 ± 16	65 ± 14	NS***
BG (mg/dl)	109 ± 21	108 ± 23	NS***	102 ± 16	97 ± 17	NS***
D-BP (mmHg)	78 ± 11	79 ± 23	<0.0001***	73 ± 16	73 ± 16	NS***

Data except fatty liver prevalence were expressed as mean ± SD. ALT, alanine aminotransferase; BFP, body fat percentage; BG, blood glucose; BMI, body mass index; D-BP, diastolic blood pressure; HDL-C, high density lipoprotein-cholesterol; TC, total cholesterol; TG, triglycerides. *χ^2 test; **unpaired *t*-test; ***analysis of covariance (ANCOVA, adjusted for age).

However, the recent increase in prevalence has not necessarily involved increasing BMI;[3] factors other than an increase in BMI are concerned. In the present study, as shown in Table 3, the increasing prevalence of fatty liver amongst men was associated with changes in BFP, and total cholesterol and HDL-cholesterol levels. It is significant that the prevalence of fatty liver increased with increasing BFP, even without an increase in BMI.

The increase in BFP without a corresponding increase in BMI may indicate changes in body composition; that is, an increase in body fat deposits and corresponding decrease in the fat-free component. As for fat deposits, Eguchi *et al.* report that the severity of fatty liver is positively correlated with visceral fat accumulation regardless of BMI.[6] It is possible that the increase in BFP corresponds to an increased accumulation of visceral fat. On the other hand, Caprosto *et al.* report that the resting metabolic rate (RMR) is lower in patients with non-alcoholic steatohepatitis than in controls.[7] Because of the correlation between RMR and muscle mass, lower RMR possibly reflects a decrease in muscle mass. Thus, the decrease in the fat-free component may correspond to a decrease in muscle mass. The literature suggests that Asian populations have a high level of abdominal fat at a lower BMI relative to Caucasians.[8,9] Therefore, Asians are at a higher risk for obesity-associated disorders even without obesity, and this is the rationale behind the World Health Organization's Regional Office for the Western Pacific criteria (overweight at risk) for Asians.[9] It is considered that the characteristic body composition of the Asian population has been further impacted upon by the present-day lifestyle in Japan, resulting in an increased prevalence of fatty liver without an increase in BMI. Since visceral fat obesity strongly associates with the metabolic syndrome, our study underscores the importance of monitoring visceral fat accumulation using representative indicators such as waist circumference or waist/hip ratio, or monitoring visceral fat volume by CT scan, during regular health checks. Table 3 shows that the prevalence of fatty liver has increased with increases in total cholesterol and decreases in HDL-cholesterol, possibly suggesting an association with insulin resistance.[10] Additional studies are required to further clarify these associations.

The changes in body composition in the present study should be distinguished from obesity. Obesity may bring about increases in both BFP and BMI. We consider that inadequate dieting (and rebound), irregular eating habits (e.g. fasting at breakfast) and a lack of exercise are the probable causes of the reported changes in body composition. As described above, it seems difficult to prevent the increasing prevalence of fatty liver only by weight (BMI) control. Thus, we must emphasize the need for a new strategy to reduce risk of fatty liver, in which relevant nutritional support and exercise are employed to reduce body fat deposits and develop muscle mass.

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Endoscopic characterization of the small bowel in patients with portal hypertension evaluated by double balloon endoscopy

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Background. The endoscopic abnormalities present in the small bowel (SB) of patients with portal hypertension (PH) are not well understood. This study sought to evaluate endoscopic findings of the SB in patients with PH by double balloon endoscopy (DBE). ***Methods.*** We evaluated the endoscopic findings of SB in 15 patients with PH and 49 controls without liver disease or PH. A total of 24 and 90 procedures were performed for PH patients and control patients, respectively, through oral and/or anal approaches. ***Results.*** Fourteen of the 15 patients exhibited villous abnormalities, including edema (73%), atrophy (40%), and reddening (47%) of villi. Vascular lesions, such as angiodysplasia-like abnormalities (67%), dilated/proliferated vessels (93%), and varices (7%), were observed in all patients with PH. Although they were associated with ascites, these abnormalities did not correlate with any laboratory findings. None of these abnormalities was observed in controls. Definitive or suspected bleeding sources were identified in 9 of 13 patients with both PH and obscure gastrointestinal bleeding (OGIB), which was similar to the incidence in controls with OGIB. Although the frequency of postprocedure fever (>37.5°C) was higher in patients with PH in comparison to controls (29% vs. 2%, $P < 0.01$), endoscopic treatment under DBE was performed on 3 PH patients without serious complications. ***Conclusions.*** Endoscopic abnormalities of the SB may be prevalent in patients with PH. Although postprocedure fever of DBE may occur more commonly in patients with PH, DBE is useful as both a diagnostic and therapeutic tool to evaluate the SB.

Key words: portal hypertensive enteropathy, double balloon endoscopy, portal hypertension, liver cirrhosis, small bowel

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Introduction

Portal hypertension (PH) can be caused by hepatic fibrosis or obstruction of the portal vein. Hepatic fibrosis, of which liver cirrhosis is an advanced form, results from chronic liver disease. PH has numerous complications bearing high morbidity and mortality, including variceal bleeding. Splanchnic blood flow is significantly altered by PH.[1,2] Varices develop in the esophagus, stomach, duodenum, colon, or rectum. Portal hypertensive gastropathy (PHG) or colopathy follow the development of PH and can lead to gastrointestinal bleeding. There is a subset of cases, however, in which the bleeding source remains unclear following upper and lower gastrointestinal endoscopies in patients with PH.[3–6]

The changes in the gastrointestinal mucosa of the esophagus, stomach, colon, and rectum are well described in patients with PH. The majority of studies have focused on the involvement of the gastric and colonic mucosa in patients with PH; however, it is likely that the small bowel (SB), including the duodenum and ileum, would also undergo mucosal changes as a result of PH, which is defined as portal hypertensive enteropathy (PHE).[7,8] As the SB is distal to both the mouth and the anus, it is difficult to evaluate the entire SB by endoscopic diagnosis using upper and lower gastrointestinal endoscopy; regions of the small intestine will always lie beyond the limits of the endoscope. Therefore, the endoscopic abnormalities in the SB of patients with PH have not been well characterized.

Recently, new endoscopic methods, video capsule endoscopy (VCE) and double balloon endoscopy (DBE), have been developed for examination of the entire SB.[9,10] VCE permits direct visualization of the SB mucosa. Although VCE is both easy and painless, this technique usually does not allow visualization in real time. In addition, the technology is limited by the inability to take biopsies for histology or perform therapeutic

endoscopic interventions using VCE. In contrast, DBE provides higher-resolution imaging with improved visualization because of the capability to insufflate air, irrigate, and suction obscuring mucus/material and the ability to perform a focused examination of any abnormality visualized. This technique also allows clinicians to obtain tissue samples, making treatment of the entire SB possible in a clinical setting.[11–13]

It has also a high diagnostic yield for occult gastrointestinal bleeding (OGIB), when the SB is suggested to be the source of bleeding by VCE or DBE.[14] However, only a few descriptions of the endoscopic findings or specific bleeding sources discovered in the SBs of patients with PH are available.[6,15] None of these studies has utilized DBE to assess the incidence and characteristics of the SB abnormalities seen in patients with PH. We sought to use DBE to define the endoscopic findings present in the SB of patients with PH and to determine if these findings are associated with specific clinical characteristics. We also evaluated the availability of DBE for endoscopic therapy and the associated complications.

Patients and methods

Patients

This study was a nonrandomized, retrospective analysis of patients with PH caused by cirrhosis or extrahepatic portal vein obstruction (EHO) who were examined by DBE at Miyazaki Medical Center Hospital between September 2004 and March 2007. We confirmed the presence of liver cirrhosis by compatible physical examination, laboratory findings, histology, or radiographic features. EHO was diagnosed in patients with PH who had normal liver function tests, no clinical signs of cirrhosis, and compatible radiographic findings. PH was diagnosed by endoscopic or radiographic evidence of esophageal, gastric, or intraabdominal varices with or without splenomegaly. The severity of cirrhosis was graded using the Child-Pugh classification.

A total of 24 procedures in 15 consecutive patients with PH (12 men, 3 women; mean age, 65.8 ± 8.7 years; age range, 48–75 years) were performed. Oral, anal, and combined approaches were performed in 2, 8, and 5 patients, respectively. One patient required 5 procedures; the anal approach had to be repeated in 1 patient. We compared these results to those for 90 DBE procedures in 49 control patients (39 men and 10 women; mean age, 48.8 ± 21.1 years; age range, 16–85 years). In 49 control patients, 14 patients underwent DBE for OGIB, 10 for abdominal pain, 8 for ileus, 7 for inflammatory bowel disease, 3 for diarrhea, 2 for suspicion of tumor, 2 for fever of unknown etiology, 2 for inability to perform an endoscopic retrograde cholangiopancreatography because of previously manipulated intestines, and 1 for suspicion of infection. Patients who did not have chronic liver disease or PH who were treated at our hospital served as controls. Oral, anal, and combined approaches were performed for 7, 22, and 20 of the control patients, respectively, which includes several who were subjected to repeated procedures. Written informed consent for examination by DBE was obtained from all patients.

Methods of double balloon endoscopy

The double balloon endoscopic system (Fujinon EN-450T5/W; Fujinon, Saitama, Japan) utilizes a video endoscope with a working length of 200 cm and a flexible single-use overtube with a length of 145 cm (including the balloon). The double balloon technique has been described previously.[10] During withdrawal, administration of hyoscine butylbromide or glucagon reduces peristalsis in the SB, optimizing visualization. Sodium picosulfate is given 1 day before examination; no other specific preparation is required for an oral approach. For retrograde enteroscopy from an anal approach, bowel cleansing was performed as for colonoscopy. Therapeutic procedures were performed through a working channel. Argon plasma beam-directed coagulation (APC; 1.2 l/min/max, 35 W; ERBE 300 series, Tubigen, Germany) was used in the subset of cases in which bleeding sources were identified.

Classification of endoscopic abnormalities in the small bowel in patients with portal hypertension

The data collected for each patient included age, gender, etiology of cirrhosis, Child-Pugh class, and gastrointestinal tract abnormalities identified by upper and lower endoscopy. We evaluated each patient for any evidence of varices in the esophagus, stomach, colon, or anorectum and for changes indicative of PHG or portal hypertensive colopathy (PHC). PHG was diagnosed following recognition of elementary lesions, such as a mosaic-like pattern, red-point lesions, cherry-red spots, or black-brown spots.[7] The colonic abnormalities seen endoscopically in PHC are similar to those seen in PHG, including diffuse hyperemia and edema resembling chronic colitis, angiodysplasia-like lesions, patchy hyperemic lesions, a severe colitis-like appearance, and spontaneous bleeding from the mucosa.[7,8] The abnormal endoscopic findings seen by DBE in patients with PH, which were definitive for PHE, were divided into two categories: villous abnormalities and vascular lesions. Villous abnormalities included edema, atrophy, and reddening

of villi. Angiodysplasia-like lesions, dilated/proliferated vessels, and varices comprised the vascular lesions. A finding of each of these lesions was scored as a point, to provide a final score with a maximum of six points. The angiodysplasia-like lesions were subclassified as red spots, vascular spiders, and lymphoid follicles with dilated vessels. Dilated/proliferated vessels were further subclassified into tree-like dilated vessels and coil-like fine vessels.

Diagnosis for source of occult gastrointestinal bleeding

Patients with positive fecal occult blood and/or iron deficiency anemia with negative upper endoscopy and colonoscopy were defined as having OGIB.[16] Before DBE, all patients with OGIB were evaluated within 1 month by upper endoscopy and colonoscopy. For patients with OGIB, endoscopic findings by DBE were classified as positive (diagnostic), suspicious, or negative (failed).[17] Findings were classified as positive if the observed findings could explain the signs/symptoms of the patient. These findings typically helped to determine further management or were confirmed by other modalities. Findings were considered suspicious if an observed finding failed to explain completely the signs/symptoms of the patient, necessitating further investigation to evaluate its clinical relevance. When no abnormality could be detected despite clinical indication of an existing lesion, findings were considered to be negative.

Clinical characteristics and endoscopic abnormalities that we defined as portal hypertensive enteropathy

We compared the clinical characteristics and prevalence of PHE-defining endoscopic abnormalities between patients with PH and those without chronic liver disease (control patients). We also calculated the number of abnormal findings in 13 patients with liver cirrhosis. We compared patients with four or more findings of PHE to those with fewer than four findings to determine if this calculated score correlated with the severity of liver disease, the presence of esophagogastric varices (EGV), PHG, PHC, or other clinical characteristics.

Statistical analysis

All statistical analyses were performed using Statview J-4.5 software (Abacus Concepts, Berkeley, CA, USA) or SPSS (Chicago, IL, USA). Data are shown as the means (± SD). Comparisons were performed using the Mann–Whitney U test, Fisher's exact test, or the χ^2 test, as appropriate. Differences were considered statistically significant when the P value was less than 0.05.

Results

Prevalence and endoscopic findings of portal hypertensive enteropathy

The characteristics of 15 patients with PH and 49 control patients evaluated by DBE are detailed in Table 1. The average age of patients and the frequency of OGIB as an indication for DBE in patients with PH were higher than those for control patients. Several laboratory values, including platelet counts, serum albumin, and bilirubin, were also significantly different between the two groups. In contrast, the levels of serum alanine transferase (ALT) did not differ between the two groups.

Fourteen of the 15 patients exhibited villous abnormalities, including edema (Fig. 1A), atrophy (Fig. 1B), or reddening (Fig. 1C) of villi. All 15 patients with PH displayed vascular lesions, including angiodysplasia-like abnormalities [Fig. 2A-(1), -(2), or -(3)], dilated/proliferating vessels [Fig. 2B-(1) or -(2)], or varices (Fig. 2C). Thus, although endoscopic abnormalities were observed in the SB of all patients with PH, there were no villous abnormalities or vascular lesions in control patients.

The association between portal hypertensive enteropathy-defining abnormal findings and clinical characteristics

The etiology of the PH was liver cirrhosis in 13 patients and EHO without cirrhosis in 2 patients (see Table 1). By DBE, 14 of the 15 patients with PH exhibited villous abnormalities, while vascular lesions were observed in all (Table 2). We sought to evaluate the correlation between these endoscopic abnormalities, which we considered to be associated with PH, and clinical parameters in the 13 patients with PH caused by cirrhosis. We compared patients with four or more positive findings of PHE to those with fewer than four positive findings to determine if PHE was associated with liver disease severity or with specific endoscopic findings of the upper or lower gastrointestinal tract (Table 3). PHE was unrelated to patient age, the presence of PHG or PHC, or severity of EGV. In addition, PHE did not correlate with any laboratory findings. The frequency of ascites in patients with high PHE scores, however, was significantly higher than that seen in those with low scores ($P = 0.02$).

Diagnostic findings in small bowel by double balloon endoscopy

We assessed the number of PHE-determining findings, diagnostic rates of small intestinal bleeding, and complications of DBE (Table 4). The frequency of

Table 1. Demographic, clinical, and endoscopic parameters of patients

Parameter	Patients with portal hypertension	Control patients	*P* value*
Patients (procedures)	15 (24)	49 (90)	–
Age (mean ± SD; years)	65.8 ± 8.7	48.8 ± 21.1	<0.01
Sex (male/female)	12/3	39/10	>0.99
Indications for double balloon endoscopy			
OGIB/ileus/other	13/1/1	14/8/27	<0.001
Etiology of portal hypertension			
Cirrhosis	13	0	–
Etiology (HBV/HCV/alcohol/unknown)	1/5/4/3	—	–
Child-Pugh class (A/B/C)	1/12/0	—	–
Extrahepatic portal vein obstruction	2	0	–
Presence of esophagogastric varices	9	0	–
Presence of portal hypertensive gastropathy	10	0	–
Presence of portal hypertensive colopathy	9	0	–
Presence of anorectal varices	8	0	–
Ascites	5	0	–
Platelet ($\times 10^4/mm^3$)	12.7 ± 11.7	24.8 ± 8.9	<0.001
Serum albumin (g/dl)	3.0 ± 0.6	3.9 ± 0.6	<0.001
Total bilirubin (mg/dl)	0.9 ± 0.4	0.6 ± 0.4	<0.01
ALT (IU/l)	28.3 ± 18.9	28.8 ± 22.1	0.91

OGIB, obscure gastrointestinal bleeding
*Comparisons were performed with the Mann–Whitney *U* test, Fisher's exact test, or the χ^2 test, as appropriate

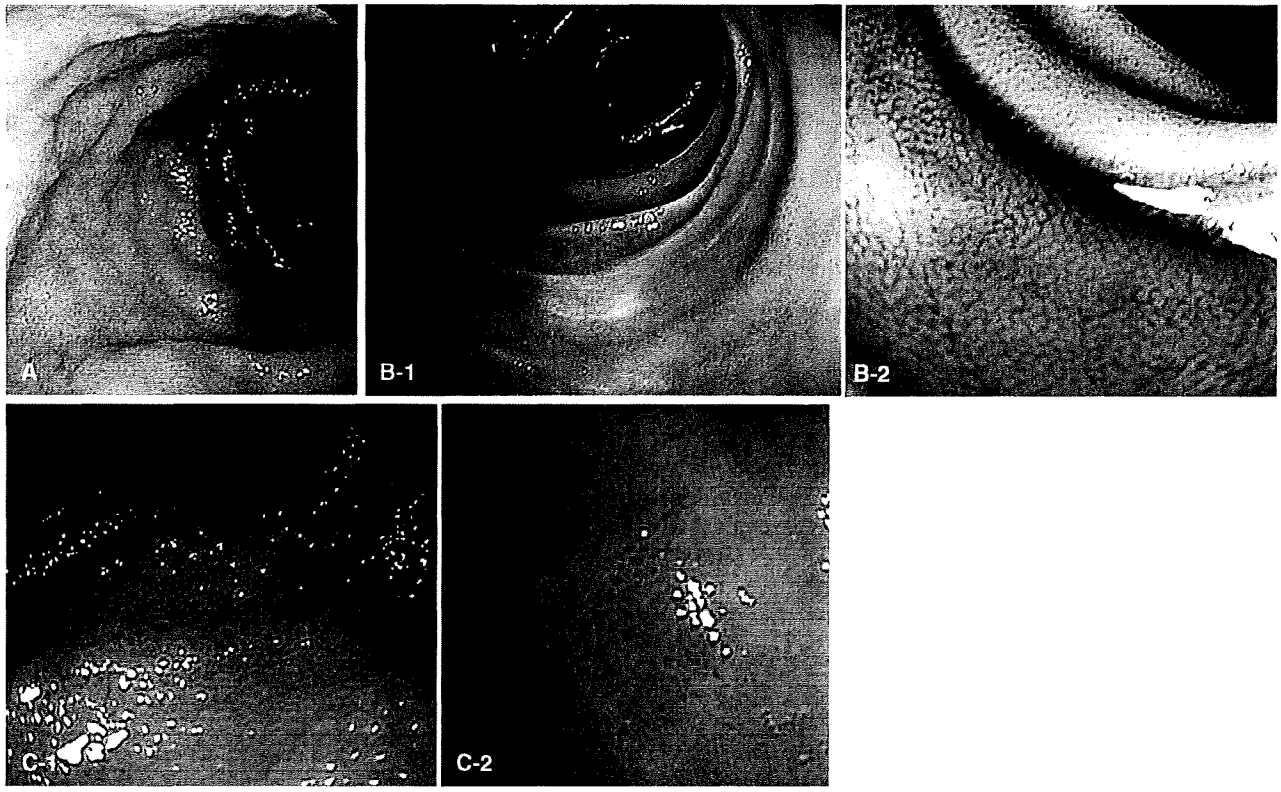

Fig. 1. Three different types of villous abnormalities were seen in the small bowel of patients with portal hypertension: edema of villi (**A**); atrophy of villi (**B-1**, **B-2**); reddening of villi (**C-1**, **C-2**)

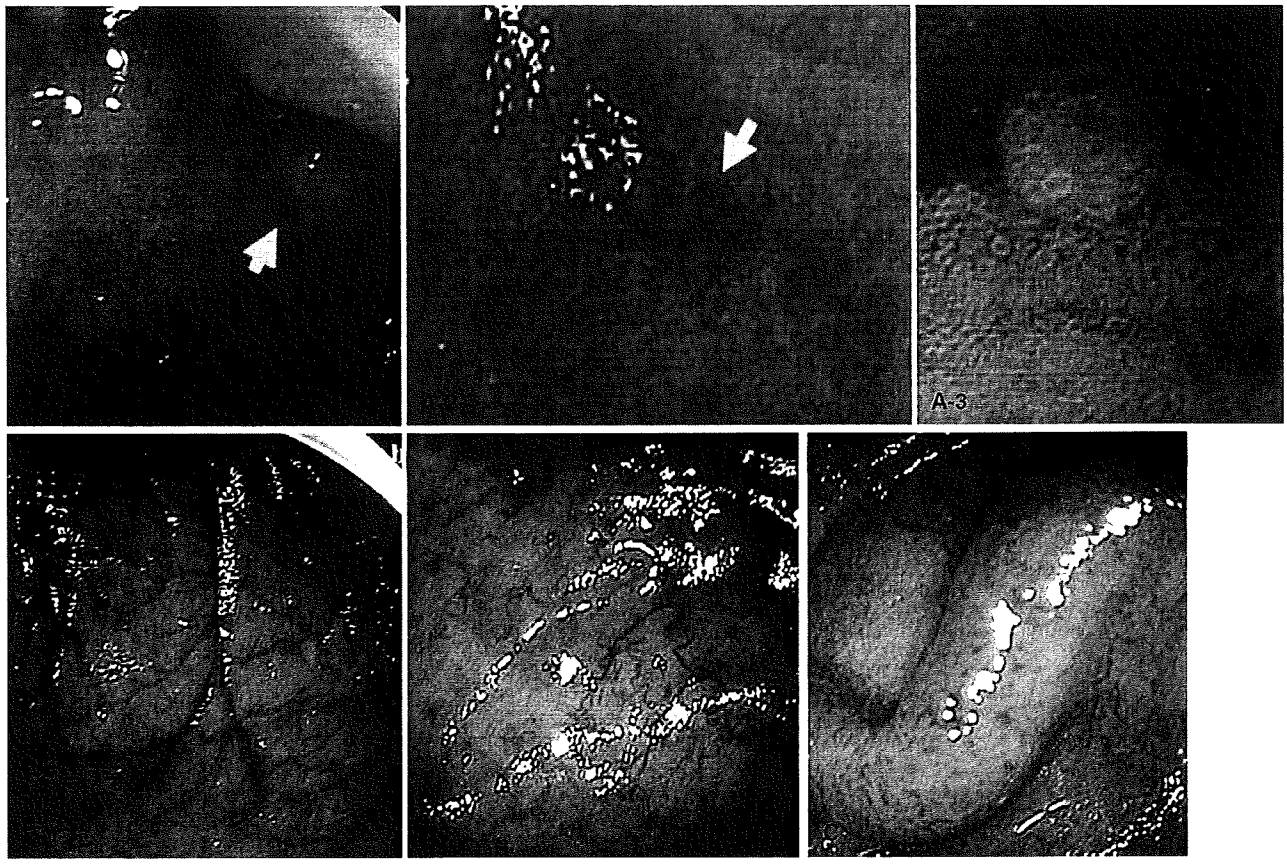

Fig. 2. Three different types of vascular lesions, including angiodysplasia-like lesions (**A**), dilated/proliferated vessels (**B**), and varices (**C**), were seen in the small bowel of patients with portal hypertension. **A-1**, red spots (*arrow*); **A-2**, vascular spiders (*arrow*); **A-3**, lymphoid follicles with dilated vessels; **B-1**, tree-like dilated vessels; **B-2**, coil-like fine vessels; **C**, varices

Table 2. Classification and frequency of the endoscopic findings of portal hypertensive enteropathy

Endoscopic findings	$n = 15$ (%)
1. Villous abnormalities	14 (93%)
A. Edema of villi	11 (73%)
B. Atrophy of villi	6 (40%)
C. Reddening of villi	7 (47%)
2. Vascular lesions	15 (100%)
A. Angiodysplasia-like lesions	10 (67%)
(1) Red spots	9 (60%)
(2) Vascular spiders	2 (13%)
(3) Lymphoid follicles with dilated vessels	2 (13%)
B. Dilated/proliferated vessels	14 (93%)
(1) Tree-like dilated vessels	12 (80%)
(2) Coil-like fine vessels	2 (13%)
C. Varices	1 (7%)

endoscopic abnormalities in the SB, which were diagnostic of PHE, was significantly higher in patients with PH than that seen in control patients. Definitive or suspicious bleeding sources, however, were observed in 69% (9/13) of patients with PH and 50% (7/14) of control patients; this diagnostic rate was not significantly different between the two groups of patients with OGIB. Bleeding sources identified included angiodysplasia-like lesions in the SB in 5 patients with PH, in whom 3 were definitive and 2 were suspicious. We identified

Table 3. The association of the number of positive portal hypertensive enteropathy-associated findings in patients with cirrhosis and other clinical features

	Number of positive findings of portal hypertensive enteropathy		
	4–6	0–3	*P* value*
Patients	6	7	
Age (mean ± SD; years)	71.0 ± 3.8	66.3 ± 7.1	0.28
Sex (male/female)	5/1	6/1	>0.99
Etiology (HBV/HCV/alcohol/uknown)	0/2/2/2	1/3/2/1	0.69
Child-Pugh class (A/B/C)	1/5/0	0/7/0	0.46
Presence of esophagogastric varices	2 (33%)	5 (71%)	0.29
Presence of portal hypertensive gastropathy	5 (83%)	5 (71%)	>0.99
Presence of portal hypertensive colopathy	2 (33%)	6 (86%)	0.10
Presence of anorectal varices	2 (33%)	4 (57%)	0.59
Presence of ascites	4 (67%)	0 (0%)	0.02
Prothrombin time (%)	72.7 ± 17.0	65.3 ± 15.3	0.32
Platelet ($\times 10^4/mm^3$)	11.1 ± 8.5	7.0 ± 2.7	0.32
Serum albumin (g/dl)	2.8 ± 0.5	2.8 ± 0.3	0.67
Total bilirubin (mg/dl)	0.9 ± 0.5	0.8 ± 0.4	>0.99
Alanine aminotransferase (IU/l)	34.3 ± 23.4	26.4 ± 17.2	0.62
Complication associated with double balloon endoscopy	3 (50%)	3 (43%)	>0.99

*Comparisons were performed with the Mann–Whitney *U* test, Fisher's exact test, or the χ^2 test, as appropriate

Table 4. Comparison of patients with and without portal hypertension

	Patients with portal hypertension	Control patients	*P* value*
Patients (procedures)	15 (24)	49 (90)	
Presence of portal hypertensive enteropathy	15 (100%)	0 (0%)	<0.001
Diagnostic rates of small intestinal bleeding (positive/suspicious/negative)[a]	7/2/4	5/2/7	0.57
Complications	7/24 (29%)	2/90 (2%)	<0.001

[a] In patients with obscure gastrointestinal bleeding
*Comparisons were performed with the Mann–Whitney *U* test, Fisher's exact test, or the χ^2 test, as appropriate

jejunal varices in 1 patient, a SB ulcer in 1, a SB diverticulum in 1, and duodenal varices in 1. Of these abnormalities, the varices are likely associated with PH, whereas the SB ulcer and diverticulum may not be associated. The duodenal varices were excluded from the findings of PHE in this study (see Table 2). The bleeding sources in control patients included a duodenal ulcer in 1 patient, SB ulcers in 5 patients, and SB angiodysplasia in 1 patient.

Treatment in small bowel by double balloon endoscopy or complications associated with its use

Endoscopic treatments using DBE were performed in three patients with endoscopic abnormalities in the SB. One patient received APC treatment for angiodysplasia-like lesions, one patient was treated with clipping and APC for angiodysplasia-like lesions, and a third was treated with clipping of lymphoid follicles with dilated vessels. Seven of 24 or 2 of 90 procedures in patients with or without PH, respectively, developed fevers (temperatures higher than 37.5°C) in the first 24 h after procedure (Table 4). The difference in frequency was statistically significant between the two groups ($P < 0.001$). Although aspiration pneumonia was suspected to occur in one of the patients with PH, the causes of fever in the other patients were not clear. Antibiotic therapy, however, was not necessary except for the one patient with pneumonia. There were no severe complications, excluding pneumonia, in either group with or without endoscopic treatment.

Discussion

Currently, there is no classification system with which to grade the severity of endoscopic abnormalities in cirrhotic patients with PHE. De Palma et al. proposed that PHE lesions be classified into two categories, mucosal inflammatory-like abnormalities (edema, ery-

thema, granularity, and friability) and vascular lesions (cherry-red spots, telangiectasias, angiodysplasia-like lesions, and varices).[15] Rana et al. defined the diagnosis of ileopathy as the presence of lesions similar in appearance to spider angioma, diffuse or patchy regions of hyperemia, cherry-red spots, and prominent veins.[8] Although we did not investigate the histology of mucosal lesions in this study, we classified the endoscopic findings in the SB of patients with PH into two categories, villous abnormalities and vascular lesions. We also subclassified the findings in these two categories and calculated the total number of positive findings in these six subcategories (see Table 2). Although it is unclear if these findings were specific for PHE, our observations indicated their prominence in patients with PH. Further studies are required to improve the classification and scoring system proposed in this report.

De Palma et al. reported that 68% of cirrhotic patients with PH were found to have PHE evaluated by VCE.[15] Endoscopic abnormalities in the ileum were noted in 13 of 38 patients examined (34%).[8] In contrast, we report that all patients with PH were observed to have at least one abnormal finding in the SB considered to be associated with PH. The high percentage likely correlates with the finding that 13 of the 15 patients with PH had evidence of OGIB with negative findings on upper and lower endoscopies. Previously reported prevalences of PHC vary from 37% to 70%, likely because of the heterogeneity of patients.[18–20] The limitations of studies, including our report, examining patients with PHE are the small number of patients with PH. In addition, although it would be better to compare the results of VCE and DBE in this study, we unfortunately did not have data for VCE. Further studies with larger numbers of patients will be needed to determine accurately the frequency of PHE as assessed by DBE and VCE.

One of the main causes of death for patients with PH is gastrointestinal bleeding. Portal hypertensive gastrointestinal vasculopathy, which can occur throughout the esophagus, stomach, and colon, is typically the origin of bleeding in patients with PH. PHE secondary to PH, especially the presence of varices in the SB, may also be a common source of bleeding.[15,21,22] There are no data, however, detailing that abnormal findings in the SB has any impact on the clinical treatment of PH, with the exception of cases with OGIB. Prospective observation should reveal the impact of PHE in cases without OGIB.

De Palma et al. initially demonstrated that 25 of 37 (68%) patients with cirrhosis and PH also had PHE and that the prevalence of PHE increased with worsening Child-Pugh class; 32% of patients with PHE were Child-Pugh class C, while only 9% of those without PHE were Child-Pugh class C.[15] PHE was also significantly associated with 2+ or larger esophageal varices, PHG, and PHC. Repici et al., however, found no correlation between the presence of PHE and Child-Pugh score, the size of varices, or the presence of PHG or PHC.[23] In this study, all patients with PH had evidence of PHE. We attempted to correlate the number of positive PHE-associated findings with laboratory findings. Our comparison of cirrhosis patients with at least four positive findings of PHE with those exhibiting fewer than four such findings demonstrated that laboratory findings, such as serum albumin, were not significantly different between the two groups. In addition, neither esophagogastric varices, PHG, PHC, nor anorectal varices correlated with PHE. The number of positive findings of PH, however, was associated with presence of ascites (see Table 3). The mechanism by which ascites develops in patients with cirrhosis is multifactorial, with the largest contribution from severe sinusoidal PH. Thus, the pathophysiology supports the association of PH with our PHE scoring system. Large prospective studies are required to evaluate the clinical significance of SB mucosal changes in patients with PH in the presence or absence of cirrhosis.

The complication rate of DBE is approximately 1%.[24,25] While perforation is a rare complication associated with DBE, no severe complications occurred in this study. Our study indicates, however, that postprocedure fever induced by DBE was more common in patients with PH than in those without PH. This complication may be associated with bacterial translocation, which typically occurs in patients with liver cirrhosis.[26,27] Comparison of cirrhotic patients with and without complications did not reveal any association of the incidence of complications with the severity of liver damage. In addition, the median time of the DBE procedure did not correlate with the incidence of complications (data not shown). In contrast, no complications were observed in the two EHO patients with PH. As the small number of EHO patients is insufficient to reveal any association, further examination will be required.

As DBE is contraindicated in patients with esophageal varices, because of an increased risk of rupture, VCE is the first diagnostic step for PHE in patients with PH. Performing DBE, however, is reasonable to examine the SB in patients with PH, as it provides a high diagnostic yield and the capability to perform therapeutic interventions. The small vascular lesions characteristic of PHE may only rarely be the sources of OGIB in patients with PH. After the bleeding has stopped, it is difficult to identify these sources of bleeding in patients with PHE as these vascular lesions are tiny. In cases in which bleeding is suspected in the SB, the diagnostic rate identifying the source of bleeding was higher in cases that underwent DBE within three days of the bleeding episode than in those who were evaluated after 1 to 2 weeks (unpublished data). Thus,

we propose that DBE should be used to examine the SB in OGIB patients with PH, especially within the first 3 days after bleeding.

Currently, there are no data evaluating the treatment of the SB using DBE in patients with PHE. The thin intestinal wall of the SB makes it difficult to perform sclerotherapy and ligation, the standard treatments for esophageal, gastric, and rectal varices. DBE, in contrast to VCE, does facilitate concurrent diagnosis and treatment. As clinical use of DBE for diagnosis and treatment of PHE is still new, future studies will be needed to define the role of DBE in this disease.

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Alanine aminotransferase flare-up in hepatitis C virus carriers with persistently normal alanine aminotransferase levels in a hyperendemic area of Japan

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Background. The clinical features of hepatitis C virus (HCV) carriers with persistently normal alanine aminotransferase (PNALT) levels (ALT ≤ 34 IU/l) have not been fully elucidated. We investigated clinical factors associated with ALT flare-up in PNALT individuals in a HCV hyperendemic area of Japan. ***Methods.*** We analyzed 101 HCV carriers who had PNALT between 1993 and 2000. The first occurrence of ALT flare-up (ALT ≥ 35 IU/l) between 2001 and 2005 was evaluated by the Kaplan-Meier method. Multivariate analysis of factors predicting ALT flare-up were conducted using Cox proportional hazards models. ***Results.*** The mean follow-up period was 2.8 years, and the 5-year cumulative incidence of ALT flare-up was estimated to be 31.8%. In multivariate analysis, an ALT level of 20–34 IU/l and a high serum ferritin level (≥90 ng/ml) in the most recently available data up to the year 2000, as well as H63D heterozygosity in the *HFE* gene, were independently and strongly associated with the incidence of ALT flare-up (Hazard ratios = 5.6, 3.1, and 4.8, respectively). In addition, *HFE* H63D heterozygosity was significantly associated with higher serum ferritin levels in subjects with PNALT (153.8 ± 73.3 ng/ml in subjects with the 63HD genotype vs. 89.4 ± 51.3 ng/ml in subjects with the 63HH genotype, $P = 0.043$). ***Conclusions.*** HCV carriers with PNALT in this population were at risk for ALT flare-up. Basal ALT levels, serum ferritin levels, and *HFE* polymorphism are potentially important predictors of ALT flare-up.

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Key words: hepatitis C virus, persistent normal ALT, community-based population, ferritin, ALT flare-up

Introduction

Persistent hepatitis C virus (HCV) infection is one of the most common causes of chronic liver disease, liver cirrhosis, and hepatocellular carcinoma (HCC).[1–3] The progression of HCV infection to hepatic fibrosis and HCC is associated with several factors, including elevated levels of alanine aminotransferase (ALT), duration of infection, age, and sex.[4–7] Short-term studies have shown that 20%–30% of patients with persistent HCV infection have persistently normal serum ALT levels and minimal necroinflammatory changes in the liver. Liver damage in these HCV carriers does not appear to progress to severe hepatitis or HCC.[8–10] For this reason, HCV-infected patients with persistently normal ALT (PNALT) are typically not treated for infection or examined by liver biopsy.[11,12] However, there have also been reports indicating that hepatic fibrosis can progress slowly even when serum ALT levels remain normal,[13,14] suggesting that PNALT patients should be treated and biopsied.[15] Recently, Tanaka et al.[6] reported that individuals with normal ALT levels are still at risk for developing HCC. These contrasting findings may result from differing clinical definitions of ALT abnormality, the time frame for defining persistence, and patient age at the time of infection or liver biopsy. Because of these ambiguities, the clinical features and disease progression in HCV carriers with PNALT remain unclear and warrant investigation.

ALT reactivation can occur many years after infection in some PNALT patients,[10,16,17] potentially leading to progressive liver damage. Although the efficacy of combination therapy with interferon and ribavirin or interferon monotherapy in PNALT patients may be similar to that in patients with abnormal ALT levels,[18,19] these therapies are expensive, effective in only 50% of patients, poorly tolerated, and unsuitable for some patient populations, especially older individuals. Because of this variability, it is important to define the clinical features of HCV carriers with PNALT, especially in older patients. This information will help identify HCV carriers at risk for fibrosis and HCC and help determine the best treatment options.

Since 1993, we have been following HCV-seropositive residents in a hyperendemic area of Japan. Our previous studies of this community-based population showed that abnormal ALT levels (≥35 IU/l) were associated with a fourfold increased risk of HCC.[20] Because of reports that HCV patients with normal ALT levels are also at risk for HCC, we decided to elucidate the clinical and virological features of HCV carriers with PNALT. The present analysis focuses on ALT flare-up in HCV carriers with PNALT. In addition, the average age of subjects in this study was 71.4 years, which is older than the average age of HCV carriers in the United States. Because it is estimated that the age of HCV carriers in the United States and Europe will increase over the next two or three decades, becoming more similar to the situation in Japan,[21] this seminal study provides important clinical information applicable to other HCV patient populations.

Methods

Study population

Between 1993 and 1995, we examined 1151 residents who tested positive for anti-HCV antibodies in a hyperendemic area (Town C) of Japan.[22] The overall prevalence of anti-HCV antibodies was higher (20.6%) in this region than in the surrounding area. As part of a collaborative effort between the University of Miyazaki, the local government, and the public health service, an ultrasonography screening program was started in 1994 to detect HCC in HCV seropositive residents of Town C. In 2001, a clinical research study was initiated in conjunction with the liver disease screening program.

Of these residents, 440 HCV carriers with at least four annual ALT measurements between 1993 and 2000 were included in the present analysis. These subjects tested positive for HCV core antigen (HCVcAg) or HCV RNA at least 6 months after their initial anti-HCV screening and were diagnosed as having persistent HCV infection (HCV carriers) in 1995. Although these subjects included HCV carriers who had taken oral or intravenous medical herbs or other palliative therapies, we excluded those subjects who had received interferon therapy or were diagnosed with HCC before 2000. Subjects with normal ALT levels between 1993 and 2000 were considered to have PNALT in 2000.

Serological studies and viral markers

Between 1993 and 1995, HCV-specific antibodies were detected using a second-generation enzyme immunoassay kit (Immunocheck F-HCV Ab, International Reagents, Kobe, Japan). Biochemical tests were also performed to measure levels of ALT (normal value, <35 IU/l), aspartate aminotransferase (normal value, <40 IU/l), and γ-glutamyl transpeptidase (normal values: males, <70 IU/l; females, <30 IU/l) annually from 1993 to 2000. ALT levels in HCV-infected patients can be affected by the progression of liver fibrosis, and platelet counts correlate with the progression of liver fibrosis. However, platelet counts were not obtained before 2001 and could not be included in this study. Serum levels of HCVcAg were determined by a fluorescence enzyme immunoassay (Immunocheck F-HCV Ag Core, International Reagents),[23] with a detection threshold of 8 pg/ml of HCVcAg. For anti-HCV antibody-positive residents with HCVcAg levels below 8 pg/ml, HCV RNA was examined in 1995 by a qualitative reverse transcription polymerase chain reaction (PCR) assay (Amplicore HCV, Roche Diagnostics, Tokyo, Japan). The serologically defined genotype (serotype) of HCV was determined using a serological genotyping assay kit (Immunocheck F-HCV Grouping, International Reagents). We also examined patient ferritin levels (normal values: males, ≥24 and ≤286 ng/ml; females, ≥7 and ≤110 ng/ml) using serum stored from 1996 to 2000.

Mutational analysis of the HFE gene

Mild to moderate iron overload is associated with liver injury in patients with chronic hepatitis C. *HFE* mutations could be associated with excess iron loading in patients with chronic hepatitis C. We determined whether *HFE* mutations were associated with ALT flare-up in subjects with PNALT. The following three major point mutations in *HFE* have been associated with hereditary hemochromatosis: cysteine to tyrosine at amino acid 282 (C282Y), serine to cysteine at amino acid 65 (S65C), and histidine to aspartic acid at amino acid 63 (H63D). To test for these mutations in PNALT HCV carriers, genomic DNA was extracted using a MagExtractor System MFX-2000 (Toyobo, Osaka, Japan), according to the manufacturer's protocols.